

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

NEVAKAR, INC.,
Petitioner,

v.

SYDNEXIS, INC.,
Patent Owner.

IPR2021-00439 (Patent 9,421,199 B2)
IPR2021-00440 (Patent 9,770,447 B2)
IPR2021-00441 (Patent 10,076,515 B2)
IPR2021-00442 (Patent 10,201,534 B2)¹

Before ERICA A. FRANKLIN, SUSAN L. C. MITCHELL, and
JAMIE T. WISZ, *Administrative Patent Judges*.

FRANKLIN, *Administrative Patent Judge*.

ORDER

Granting Joint Motion to Terminate the Proceedings Before Institution
37 C.F.R. §§ 42.71; 42.74

¹ This order addresses issues common to each of the captioned cases. Thus, we issue a single order for entry in each case.

IPR2021-00439 (Patent 9,421,199 B2)
IPR2021-00440 (Patent 9,770,447 B2)
IPR2021-00441 (Patent 10,076,515 B2)
IPR2021-00442 (Patent 10,201,534 B2)

On April 22, 2021, in each of the above-captioned cases, with authorization of the Board, the parties filed a joint motion to terminate the proceeding. Paper 7.² The parties also filed a true copy of their written settlement agreement. Ex. 1090. Additionally, citing to 35 U.S.C § 317 (b) and 37 C.F.R. § 42.74 (c), the parties filed a joint request that the settlement agreement be treated as business confidential information, maintained separate from the publicly available files of the involved patent, and made available only to Federal Government agencies on written request, or to persons showing good cause on written request. Paper 8, 2.

In each joint motion, the parties explain that termination of the proceeding is appropriate because they have reached an agreement settling their dispute with respect to the involved patents. Paper 7, 1.

These cases are in the preliminary proceeding stage; a decision whether to institute trial in each case has not been entered. Under these circumstances, we determine that it is appropriate to enter judgment terminating the proceedings. Additionally, the parties' request for the settlement agreement to be treated as business confidential information and kept separate from the file of the involved patents is *granted*.

² Citations to paper and exhibit numbers in this Order refer to filings in IPR2021-00439. Similar documents were filed in each of the above-captioned cases.

IPR2021-00439 (Patent 9,421,199 B2)
IPR2021-00440 (Patent 9,770,447 B2)
IPR2021-00441 (Patent 10,076,515 B2)
IPR2021-00442 (Patent 10,201,534 B2)

Accordingly, it is hereby

ORDERED that, in each of the above-captioned cases, the joint motion to terminate the captioned proceeding is *granted*;

FURTHER ORDERED that, in each of the above-captioned cases, the joint request for the settlement agreement to be treated as business confidential information and kept separate from the file of the involved patent under the provisions of 35 U.S.C. § 317(b) and 37 C.F.R. § 42.74(c) is *granted*; and

FURTHER ORDERED that the proceeding in each of the above-captioned cases is *terminated*.

IPR2021-00439 (Patent 9,421,199 B2)
IPR2021-00440 (Patent 9,770,447 B2)
IPR2021-00441 (Patent 10,076,515 B2)
IPR2021-00442 (Patent 10,201,534 B2)

PETITIONER:

James R. Barney
Mark J. Feldstein
Joshua L. Goldberg
Yieyie Yang
Drew D. Christie
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PATENT OWNER:

Michael Rosato
Lora Green
Jad Mills
WILSON SONSINI GOODRICH & ROSATI
mrosato@wsgr.com
lgreen@wsgr.com
jmills@wsgr.com



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United States Patent and Trademark Office
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Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY.DOCKET NO./TITLE	REQUEST ID
14/726,139	05/29/2015	Sydnexis, Inc.	46682-701.201	50382

Acknowledgement of Loss of Entitlement to Entity Status Discount

The entity status change request below filed through Private PAIR on 11/01/2017 has been accepted.

CERTIFICATIONS:

Change of Entity Status:

✕ Applicant changing to regular undiscounted fee status.

NOTE: Checking this box will be taken to be notification of loss of entitlement to small or micro entity status, as applicable.

This portion must be completed by the signatory or signatories making the entity status change in accordance with 37 CFR 1.4(d)(4).

Signature:	/Xiaofan Yang/
Name:	Xiaofan Yang
Registration Number:	61459



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APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/726,139	08/23/2016	9421199	46682-701.201	7389

21971 7590 08/03/2016
WILSON, SONSINI, GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304-1050

ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b) (application filed on or after May 29, 2000)

The Patent Term Adjustment is 0 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (<http://pair.uspto.gov>).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Data Management (ODM) at (571)-272-4200.

APPLICANT(s) (Please see PAIR WEB site <http://pair.uspto.gov> for additional applicants):

Gregory I. Ostrow, San Diego, CA;
Sydnexis, Inc., San Diego, CA;
Kenneth J. Widder, Rancho Santa Fe, CA;
David S. Baker, Carlsbad, CA;

The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The USA offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to encourage and facilitate business investment. To learn more about why the USA is the best country in the world to develop technology, manufacture products, and grow your business, visit SelectUSA.gov.

EXAMINER'S AMENDMENT

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in a telephone interview with Xiaofan Yang on May 25, 2016.

In the claims:

In claim 1, line 2, "0.05" has been changed to "0.03".

In claim 9, line 2, "from about 0.001^{wt%} to about" has been cancelled.

In claim 9, line 3, "0.04 wt%, from about 0.001wt% to about 0.03wt%," has been canceled.

In claim 24, line 2, "0.05" has been changed to "0.03".

In claim 28, line 1, "or preventing" has been cancelled.

In claim 28, line 1, "development" has been cancelled.

In claim 28, line ¹2 "progression" has been added before ", comprising".

In claim 28, line 3, "0.05" has been changed to "0.03".

In claim 28, line 4 "." after "7.9" has been cancelled.

In claim 28, line 4, ", wherein the muscarinic antagonist is atropine, or atropine sulfate" has been added after "7.9".

The following is an examiner's statement of reasons for allowance: the amendments set forth in the examiner's amendment and the data presented to the

Change(s) applied
to document,
/P.A.P./
7/20/2016



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UNITED STATES DEPARTMENT OF COMMERCE
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/726,139	05/29/2015	Gregory I. Ostrow	46682-701.201	7389

21971 7590 07/21/2016
WILSON, SONSINI, GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304-1050

EXAMINER

FAY, ZOHREH A

ART UNIT	PAPER NUMBER

1621

NOTIFICATION DATE	DELIVERY MODE

07/21/2016

ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

patentdocket@wsgr.com

**UNITED STATES DEPARTMENT OF COMMERCE****U.S. Patent and Trademark Office**

Address : COMMISSIONER FOR PATENTS

P.O. Box 1450

Alexandria, Virginia 22313-1450

APPLICATION NO./ CONTROL NO.	FILING DATE	FIRST NAMED INVENTOR / PATENT IN REEXAMINATION	ATTORNEY DOCKET NO.
14/726,139	29 May, 2015	OSTROW ET AL.	46682-701.201

WILSON, SONSINI, GOODRICH & ROSATI 650 PAGE MILL ROAD PALO ALTO, CA 94304-1050		EXAMINER	
		ZOHREH FAY	
		ART UNIT	PAPER
		1621	20160712

DATE MAILED:

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner for Patents

Attached please find the corrected Examiner's Amendment.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ZOHREH FAY whose telephone number is (571)272-0573. The examiner can normally be reached on Monday to Friday 9:30-6:00. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Winston Shen can be reached on (571) 272-3157. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300. Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/ZOHREH FAY/
Primary Examiner, Art Unit 1621

EXAMINER'S AMENDMENT

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in a telephone interview with Xiaofan Yang on May 25, 2016.

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In claim 28, line 4 "." after "7.9" has been cancelled.

In claim 28, line 4, ", wherein the muscarinic antagonist is atropine, or atropine sulfate" has been added after "7.9".

The following is an examiner's statement of reasons for allowance: the amendments set forth in the examiner's amendment and the data presented to the

advantages of the combination of atropine and deuterated water in comparison to the combination of atropine and water has placed the application in condition for allowance.

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ZOHREH FAY whose telephone number is (571)272-0573. The examiner can normally be reached on Monday to Friday 9:30-6:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Winston Shen can be reached on (571) 272-3157. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Application/Control Number: 14/726,139
Art Unit: 1621

Page 4

ZF
/ZOHREH FAY/
Primary Examiner, Art Unit 1621

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: **Mail** **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
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or Fax **(571)-273-2885**

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

21971 7590 06/13/2016
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PALO ALTO, CA 94304-1050

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/726,139	05/29/2015	Gregory I. Ostrow	46682-701.201	7389

TITLE OF INVENTION: OPHTHALMIC COMPOSITION

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	\$480	\$0	\$0	\$480	09/13/2016

EXAMINER	ART UNIT	CLASS-SUBCLASS
FAY, ZOHREH A	1621	514-304000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363). ☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached. ☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. Use of a Customer Number is required.	2. For printing on the patent front page, list (1) The names of up to 3 registered patent attorneys or agents OR, alternatively, (2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed. 1 <u>Wilson, Sonsini,</u> 2 <u>Goodrich & Rosati</u> 3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE

(B) RESIDENCE: (CITY AND STATE OR COUNTRY)

SYDNEXIS, INC.

San Diego, California

Please check the appropriate assignee category or categories (will not be printed on the patent): ☐ Individual ☒ Corporation or other private group entity ☐ Government

4a. The following fee(s) are submitted:

- ☒ Issue Fee
☐ Publication Fee (No small entity discount permitted)
☐ Advance Order - # of Copies _____

4b. Payment of Fee(s): (Please first reapply any previously paid issue fee shown above)

- ☐ A check is enclosed.
☐ Payment by credit card. Form PTO-2038 is attached.
☒ The director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number 23-2415 (enclose an extra copy of this form).

5. Change in Entity Status (from status indicated above)

- ☐ Applicant certifying micro entity status. See 37 CFR 1.29
☐ Applicant asserting small entity status. See 37 CFR 1.27
☐ Applicant changing to regular undiscounted fee status.

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature Xiaofan Yang
Typed or printed name Xiaofan Yang

Date 06/15/2016
Registration No. 61459

Electronic Patent Application Fee Transmittal

Application Number:	14726139			
Filing Date:	29-May-2015			
Title of Invention:	OPHTHALMIC COMPOSITION			
First Named Inventor/Applicant Name:	Gregory I. Ostrow			
Filer:	Michael J. Hostetler/FY/Jennifer Vail			
Attorney Docket Number:	46682-701.201			
Filed as Small Entity				
Filing Fees for Utility under 35 USC 111(a)				
Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Utility Appl Issue Fee	2501	1	480	480

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Extension-of-Time:				
Miscellaneous:				
Total in USD (\$)				480

Electronic Acknowledgement Receipt

EFS ID:	26078723
Application Number:	14726139
International Application Number:	
Confirmation Number:	7389
Title of Invention:	OPHTHALMIC COMPOSITION
First Named Inventor/Applicant Name:	Gregory I. Ostrow
Customer Number:	21971
Filer:	Michael J. Hostetler/FY/Jennifer Vail
Filer Authorized By:	Michael J. Hostetler
Attorney Docket Number:	46682-701.201
Receipt Date:	15-JUN-2016
Filing Date:	29-MAY-2015
Time Stamp:	18:31:15
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	\$ 480
RAM confirmation Number	5634
Deposit Account	232415
Authorized User	YANG, XIAOFAN

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

File Listing:					
Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Issue Fee Payment (PTO-85B)	46682-701-201_IssueFee.pdf	84166	no	1
			f5404cccbdf1ce79354b8bb07978f7573f3dd75		
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	30651	no	2
			6f4af6686552e0ae6e26b068376aae8edc8ae134		
Warnings:					
Information:					
Total Files Size (in bytes):			114817		
This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503. <u>New Applications Under 35 U.S.C. 111</u> If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application. <u>National Stage of an International Application under 35 U.S.C. 371</u> If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course. <u>New International Application Filed with the USPTO as a Receiving Office</u> If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.					



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NOTICE OF ALLOWANCE AND FEE(S) DUE

21971 7590 06/13/2016
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EXAMINER

FAY, ZOHREH A

ART UNIT

PAPER NUMBER

1621

DATE MAILED: 06/13/2016

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.

14/726,139

05/29/2015

Gregory I. Ostrow

46682-701.201

7389

TITLE OF INVENTION: OPHTHALMIC COMPOSITION

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	\$480	\$0	\$0	\$480	09/13/2016

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: **Mail** **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax **(571)-273-2885**

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

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21971 7590 06/13/2016
WILSON, SONSINI, GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304-1050

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/726,139	05/29/2015	Gregory I. Ostrow	46682-701.201	7389

TITLE OF INVENTION: OPTHALMIC COMPOSITION

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	\$480	\$0	\$0	\$480	09/13/2016

EXAMINER	ART UNIT	CLASS-SUBCLASS
FAY, ZOHREH A	1621	514-304000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

- ☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
- ☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list

- (1) The names of up to 3 registered patent attorneys or agents OR, alternatively, 1 _____
- (2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed. 2 _____
- 3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE

(B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent): ☐ Individual ☐ Corporation or other private group entity ☐ Government

4a. The following fee(s) are submitted:

- ☐ Issue Fee
- ☐ Publication Fee (No small entity discount permitted)
- ☐ Advance Order - # of Copies _____

4b. Payment of Fee(s): (Please first reapply any previously paid issue fee shown above)

- ☐ A check is enclosed.
- ☐ Payment by credit card. Form PTO-2038 is attached.
- ☐ The director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. Change in Entity Status (from status indicated above)

- ☐ Applicant certifying micro entity status. See 37 CFR 1.29
- ☐ Applicant asserting small entity status. See 37 CFR 1.27
- ☐ Applicant changing to regular undiscounted fee status.

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____

Date _____

Typed or printed name _____

Registration No. _____



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/726,139	05/29/2015	Gregory I. Ostrow	46682-701.201	7389

21971 7590 06/13/2016
WILSON, SONSINI, GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304-1050

EXAMINER

FAY, ZOHREH A

ART UNIT PAPER NUMBER

1621

DATE MAILED: 06/13/2016

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b) (Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Notice of Allowability	Application No. 14/726,139	Applicant(s) OSTROW ET AL.	
	Examiner ZOHREH FAY	Art Unit 1621	AIA (First Inventor to File) Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to the amendments and remarks filed on 4/5/2016.
☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.
2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.
3. ☒ The allowed claim(s) is/are 1, 4-25 and 27-30. As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.
4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

- a) ☐ All b) ☐ Some *c) ☐ None of the:
1. ☐ Certified copies of the priority documents have been received.
 2. ☐ Certified copies of the priority documents have been received in Application No. _____.
 3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

* Certified copies not received: _____.

Applicant has **THREE MONTHS FROM THE "MAILING DATE"** of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.

THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.
Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).
6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)	5. ☒ Examiner's Amendment/Comment
2. ☒ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date <u>3/29/2016</u>	6. ☒ Examiner's Statement of Reasons for Allowance
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material	7. ☐ Other _____.
4. ☐ Interview Summary (PTO-413), Paper No./Mail Date _____.	

/ZOHREH FAY/
Primary Examiner, Art Unit 1621

EXAMINER'S AMENDMENT

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in a telephone interview with Xiaofan Yang on May 25, 2016.

In the claims:

In claim 1, line 2, "0.05" has been changed to "0.03".

In claim 9, line 2, "from about 0.001" has been cancelled.

In claim 9, line 3, "wt% to about 0.01wt%, from about 0.001wt% to about 0.03wt%," has been canceled.

In claim 24, line 2, "0.05" has been changed to "0.03".

In claim 28, line 1, "or preventing" has been cancelled.

In claim 28, line 1, "development" has been cancelled.

In claim 28, line 2 "progression" has been added before ", comprising".

In claim 28, line 4, "0.05" has been changed to "0.03".

In claim 28, line 5, "." after "7.9" has been cancelled.

In claim 28, line 5, ", wherein the muscarinic antagonist is atropine, or atropine sulfate" has been added after "7.9".

The following is an examiner's statement of reasons for allowance: the amendments set forth in the examiner's amendment and the data presented to the

Art Unit: 1621

advantages of the combination of atropine and deuterated water in comparison to the combination of atropine and water has placed the application in condition for allowance.

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

The application has been amended as follows: Any inquiry concerning this communication or earlier communications from the examiner should be directed to ZOHREH FAY whose telephone number is (571)272-0573. The examiner can normally be reached on Monday to Friday 9:30-6:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Melenie McCormick can be reached on (571) 272-8037. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Art Unit: 1621

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

ZF
/ZOHREH FAY/
Primary Examiner, Art Unit 1621

Substitute for form 1449/PTO INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use as many sheets as necessary)				Complete if Known	
				Application Number	14726139
				Filing Date	05-29-2015
				First Named Inventor	OSTROW; Gregory I.
				Art Unit	1621
				Examiner Name	Zohreh A. Fay
				Attorney Docket Number	46682-701.201
Sheet	1	of	1		

U. S. PATENT DOCUMENTS					
Examiner Initials*	Cite No.	Document Number	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		Number-Kind Code ² (if known)			
		None			

FOREIGN PATENT DOCUMENTS						
Examiner Initials*	Cite No.	Foreign Patent Document	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T ⁶
		Country Code ³ -Number ⁴ -Kind Code ⁵ (if known)				
		None				

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No. ¹	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T ²
	001	CRIADO et al. Scavenging of photogenerated oxidative species by antimuscarinic drugs: atropine and derivatives. Redox Rep 7(6):385-394 (2002).	☐

Examiner Signature	/ZOHREH A FAY/	Date Considered	05/26/2016

EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. ¹ Applicant's unique citation designation number (optional). ² See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. ³ Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). ⁴ For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. ⁵ Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. ⁶ Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.



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BIB DATA SHEET

CONFIRMATION NO. 7389

SERIAL NUMBER	FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.		
14/726,139	05/29/2015	514	1621	46682-701.201		
RULE						
APPLICANTS Sydnexis, Inc., San Diego, CA;						
INVENTORS Gregory I. Ostrow, San Diego, CA; Kenneth J. Widder, Rancho Santa Fe, CA; David S. Baker, Carlsbad, CA;						
** CONTINUING DATA ***** This appln claims benefit of 62/151,926 04/23/2015 and claims benefit of 62/096,433 12/23/2014 and claims benefit of 62/016,502 06/24/2014						
** FOREIGN APPLICATIONS *****						
** IF REQUIRED, FOREIGN FILING LICENSE GRANTED ** ** SMALL ENTITY ** 06/08/2015						
Foreign Priority claimed ☐ Yes ☒ No 35 USC 119(a-d) conditions met ☐ Yes ☒ No Verified and Acknowledged <u>/ZOHREH A FAY/</u> Examiner's Signature		☐ Met after Allowance Initials	STATE OR COUNTRY CA	SHEETS DRAWINGS 10	TOTAL CLAIMS 30	INDEPENDENT CLAIMS 3
ADDRESS WILSON, SONSINI, GOODRICH & ROSATI 650 PAGE MILL ROAD PALO ALTO, CA 94304-1050 UNITED STATES						
TITLE OPHTHALMIC COMPOSITION						
FILING FEE RECEIVED 1200	FEES: Authority has been given in Paper No. _____ to charge/credit DEPOSIT ACCOUNT No. _____ for following:			☐ All Fees ☐ 1.16 Fees (Filing) ☐ 1.17 Fees (Processing Ext. of time) ☐ 1.18 Fees (Issue) ☐ Other _____ ☐ Credit		

Issue Classification 	Application/Control No. 14726139	Applicant(s)/Patent Under Reexamination OSTROW ET AL.	
	Examiner ZOHREH FAY	Art Unit 1621	

CPC					
Symbol				Type	Version
A61K	31	46		F	2013-01-01
A61K	9	0048		I	2013-01-01

CPC Combination Sets				
Symbol	Type	Set	Ranking	Version

NONE (Assistant Examiner) _____ (Date) _____		Total Claims Allowed: 27	
/ZOHREH FAY/ Primary Examiner.Art Unit 1621 (Primary Examiner) _____ (Date) _____		O.G. Print Claim(s) 1	O.G. Print Figure none

Issue Classification 	Application/Control No. 14726139	Applicant(s)/Patent Under Reexamination OSTROW ET AL.
	Examiner ZOHREH FAY	Art Unit 1621

☐ Claims renumbered in the same order as presented by applicant															
☐ CPA															
☒ T.D.															
☐ R.1.47															
Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original
1	1	17	19												
2	4	18	20												
3	5	19	21												
4	6	20	22												
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6	8	22	24												
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10	12	26	29												
11	13	27	30												
12	14														
13	15														
14	16														
15	17														
16	18														

NONE	Total Claims Allowed: 27	
(Assistant Examiner)	(Date)	
/ZOHREH FAY/ Primary Examiner.Art Unit 1621	5/26/2016	O.G. Print Claim(s) 1
(Primary Examiner)	(Date)	O.G. Print Figure none

Search Notes 	Application/Control No. 14726139	Applicant(s)/Patent Under Reexamination OSTROW ET AL.
	Examiner ZOHREH FAY	Art Unit 1621

CPC- SEARCHED		
Symbol	Date	Examiner
A61K 31/46	5/26/2016	ZF

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
West	2/29/2016	ZF
Inventor search	2/29/2016	ZF
Updated	5/26/2016	ZF

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner
A61K	31/46	6/25/2016	ZF

Doc Code: DIST.E.FILE Document Description: Electronic Terminal Disclaimer - Filed		PTO/SB/25 U.S. Patent and Trademark Office Department of Commerce	
Electronic Petition Request	TERMINAL DISCLAIMER TO OBIATE A PROVISIONAL DOUBLE PATENTING REJECTION OVER A PENDING "REFERENCE" APPLICATION		
Application Number	14726139		
Filing Date	29-May-2015		
First Named Inventor	Gregory Ostrow		
Attorney Docket Number	46682-701.201		
Title of Invention	OPHTHALMIC COMPOSITION		
☒ Filing of terminal disclaimer does not obviate requirement for response under 37 CFR 1.111 to outstanding Office Action ☒ This electronic Terminal Disclaimer is not being used for a Joint Research Agreement.			
Owner	Percent Interest		
Sydnexis, Inc.	100%		
The owner(s) of percent interest listed above in the instant application hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application which would extend beyond the expiration date of the full statutory term of any patent granted on pending reference Application Number(s)			
14859042 filed on 09/18/2015 as the term of any patent granted on said reference application may be shortened by any terminal disclaimer filed prior to the grant of any patent on the pending reference application. The owner hereby agrees that any patent so granted on the instant application shall be enforceable only for and during such period that it and any patent granted on the reference application are commonly owned. This agreement runs with any patent granted on the instant application and is binding upon the grantee, its successors or assigns. In making the above disclaimer, the owner does not disclaim the terminal part of any patent granted on the instant application that would extend to the expiration date of the full statutory term of any patent granted on said reference application, "as the term of any patent granted on said reference application may be shortened by any terminal disclaimer filed prior to the grant of any patent on the pending reference application," in the event that any such patent granted on the pending reference application: expires for failure to pay a maintenance fee, is held unenforceable, is found invalid by a court of competent jurisdiction, is statutorily disclaimed in whole or terminally disclaimed under 37 CFR 1.321, has all claims canceled by a reexamination certificate, is reissued, or is in any manner terminated prior to the expiration of its full statutory term as shortened by any terminal disclaimer filed prior to its grant.			
☒ Terminal disclaimer fee under 37 CFR 1.20(d) is included with Electronic Terminal Disclaimer request.			

☐ I certify, in accordance with 37 CFR 1.4(d)(4), that the terminal disclaimer fee under 37 CFR 1.20(d) required for this terminal disclaimer has already been paid in the above-identified application.	
Applicant claims the following fee status: ☒ Small Entity ☐ Micro Entity ☐ Regular Undiscounted	
I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.	
THIS PORTION MUST BE COMPLETED BY THE SIGNATORY OR SIGNATORIES I certify, in accordance with 37 CFR 1.4(d)(4) that I am: ☒ An attorney or agent registered to practice before the Patent and Trademark Office who is of record in this application Registration Number <u>72796</u> ☐ A sole inventor ☐ A joint inventor; I certify that I am authorized to sign this submission on behalf of all of the inventors as evidenced by the power of attorney in the application ☐ A joint inventor; all of whom are signing this request	
Signature	/Adrianna Zhang/
Name	Adrianna Zhang

*Statement under 37 CFR 3.73(b) is required if terminal disclaimer is signed by the assignee (owner).
 Form PTO/SB/96 may be used for making this certification. See MPEP § 324.

Electronic Patent Application Fee Transmittal

Application Number:	14726139			
Filing Date:	29-May-2015			
Title of Invention:	OPHTHALMIC COMPOSITION			
First Named Inventor/Applicant Name:	Gregory I. Ostrow			
Filer:	Adrianna P.P. Zhang/Linda Anders			
Attorney Docket Number:	46682-701.201			
Filed as Small Entity				
Filing Fees for Utility under 35 USC 111(a)				
Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
Statutory or Terminal Disclaimer	2814	1	160	160
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Extension-of-Time:				
Miscellaneous:				
Total in USD (\$)				160

Doc Code: DISQ.E.FILE

Document Description: Electronic Terminal Disclaimer – Approved

Application No.: 14726139

Filing Date: 29-May-2015

Applicant/Patent under Reexamination: Ostrow et al.

Electronic Terminal Disclaimer filed on May 24, 2016

☒ APPROVED

This patent is subject to a terminal disclaimer

☐ DISAPPROVED

Approved/Disapproved by: Electronic Terminal Disclaimer automatically approved by EFS-Web

U.S. Patent and Trademark Office

Electronic Acknowledgement Receipt

EFS ID:	25874712
Application Number:	14726139
International Application Number:	
Confirmation Number:	7389
Title of Invention:	OPHTHALMIC COMPOSITION
First Named Inventor/Applicant Name:	Gregory I. Ostrow
Customer Number:	21971
Filer:	Adrianna P.P. Zhang/Linda Anders
Filer Authorized By:	Adrianna P.P. Zhang
Attorney Docket Number:	46682-701.201
Receipt Date:	24-MAY-2016
Filing Date:	29-MAY-2015
Time Stamp:	18:51:59
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	\$ 160
RAM confirmation Number	4984
Deposit Account	232415
Authorized User	ANDERS, LINDA

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2	Fee Worksheet (SB06)	fee-info.pdf	30308	no	2
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/726,139	05/29/2015	Gregory I. Ostrow	46682-701.201	7389

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EXAMINER

FAY, ZOHREH A

ART UNIT	PAPER NUMBER

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NOTIFICATION DATE	DELIVERY MODE

04/13/2016

ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

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Summary of Record of Interview Requirements

Manual of Patent Examining Procedure (MPEP), Section 713.04, Substance of Interview Must be Made of Record

A complete written statement as to the substance of any face-to-face, video conference, or telephone interview with regard to an application must be made of record in the application whether or not an agreement with the examiner was reached at the interview.

Title 37 Code of Federal Regulations (CFR) § 1.133 Interviews

Paragraph (b)

In every instance where reconsideration is requested in view of an interview with an examiner, a complete written statement of the reasons presented at the interview as warranting favorable action must be filed by the applicant. An interview does not remove the necessity for reply to Office action as specified in §§ 1.111, 1.135. (35 U.S.C. 132)

37 CFR §1.2 Business to be transacted in writing.

All business with the Patent or Trademark Office should be transacted in writing. The personal attendance of applicants or their attorneys or agents at the Patent and Trademark Office is unnecessary. The action of the Patent and Trademark Office will be based exclusively on the written record in the Office. No attention will be paid to any alleged oral promise, stipulation, or understanding in relation to which there is disagreement or doubt.

The action of the Patent and Trademark Office cannot be based exclusively on the written record in the Office if that record is itself incomplete through the failure to record the substance of interviews.

It is the responsibility of the applicant or the attorney or agent to make the substance of an interview of record in the application file, unless the examiner indicates he or she will do so. It is the examiner's responsibility to see that such a record is made and to correct material inaccuracies which bear directly on the question of patentability.

Examiners must complete an Interview Summary Form for each interview held where a matter of substance has been discussed during the interview by checking the appropriate boxes and filling in the blanks. Discussions regarding only procedural matters, directed solely to restriction requirements for which interview recordation is otherwise provided for in Section 812.01 of the Manual of Patent Examining Procedure, or pointing out typographical errors or unreadable script in Office actions or the like, are excluded from the interview recordation procedures below. Where the substance of an interview is completely recorded in an Examiners Amendment, no separate Interview Summary Record is required.

The Interview Summary Form shall be given an appropriate Paper No., placed in the right hand portion of the file, and listed on the "Contents" section of the file wrapper. In a personal interview, a duplicate of the Form is given to the applicant (or attorney or agent) at the conclusion of the interview. In the case of a telephone or video-conference interview, the copy is mailed to the applicant's correspondence address either with or prior to the next official communication. If additional correspondence from the examiner is not likely before an allowance or if other circumstances dictate, the Form should be mailed promptly after the interview rather than with the next official communication.

The Form provides for recordation of the following information:

- Application Number (Series Code and Serial Number)
- Name of applicant
- Name of examiner
- Date of interview
- Type of interview (telephonic, video-conference, or personal)
- Name of participant(s) (applicant, attorney or agent, examiner, other PTO personnel, etc.)
- An indication whether or not an exhibit was shown or a demonstration conducted
- An identification of the specific prior art discussed
- An indication whether an agreement was reached and if so, a description of the general nature of the agreement (may be by attachment of a copy of amendments or claims agreed as being allowable). Note: Agreement as to allowability is tentative and does not restrict further action by the examiner to the contrary.
- The signature of the examiner who conducted the interview (if Form is not an attachment to a signed Office action)

It is desirable that the examiner orally remind the applicant of his or her obligation to record the substance of the interview of each case. It should be noted, however, that the Interview Summary Form will not normally be considered a complete and proper recordation of the interview unless it includes, or is supplemented by the applicant or the examiner to include, all of the applicable items required below concerning the substance of the interview.

A complete and proper recordation of the substance of any interview should include at least the following applicable items:

- 1) A brief description of the nature of any exhibit shown or any demonstration conducted,
- 2) an identification of the claims discussed,
- 3) an identification of the specific prior art discussed,
- 4) an identification of the principal proposed amendments of a substantive nature discussed, unless these are already described on the Interview Summary Form completed by the Examiner,
- 5) a brief identification of the general thrust of the principal arguments presented to the examiner,
(The identification of arguments need not be lengthy or elaborate. A verbatim or highly detailed description of the arguments is not required. The identification of the arguments is sufficient if the general nature or thrust of the principal arguments made to the examiner can be understood in the context of the application file. Of course, the applicant may desire to emphasize and fully describe those arguments which he or she feels were or might be persuasive to the examiner.)
- 6) a general indication of any other pertinent matters discussed, and
- 7) if appropriate, the general results or outcome of the interview unless already described in the Interview Summary Form completed by the examiner.

Examiners are expected to carefully review the applicant's record of the substance of an interview. If the record is not complete and accurate, the examiner will give the applicant an extendable one month time period to correct the record.

Examiner to Check for Accuracy

If the claims are allowable for other reasons of record, the examiner should send a letter setting forth the examiner's version of the statement attributed to him or her. If the record is complete and accurate, the examiner should place the indication, "Interview Record OK" on the paper recording the substance of the interview along with the date and the examiner's initials.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of: Inventors: Gregory I. Ostrow et al. Serial No.: 14/726,139 Filing Date: May 29, 2015 Title: OPHTHALMIC COMPOSITION	Group Art Unit: 1621 Confirmation No.: 7389 Examiner: Zohreh A. Fay Customer No.: 21971 <u>Certificate of Electronic Filing</u> I hereby certify that the attached Response and all marked attachments are being deposited by Electronic Filing on April 5, 2016 by using the EFS – Web patent filing system and addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450. By: /Linda Anders/

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

RESPONSE TO NON-FINAL OFFICE ACTION DATED MARCH 7, 2016

Madam:

This paper responds to the non-final Office Action dated March 7, 2016, setting forth an initial due date of June 7, 2016. Accordingly, this response is timely filed. In the event any additional fee is due, please charge it to Deposit Account No. 23-2415, referencing Docket No. 46682-701.201.

Listing of the Claims begins on page 2 of this paper.

Remarks begin on page 6 of this paper.

Conclusion begins on page 14 of this paper.

LISTING OF THE CLAIMS

This listing of claims will replace all prior versions and listings in the above-referenced patent application. The following amendments are without prejudice and do not constitute an admission regarding the patentability of the amended subject matter and should not so be construed.

1. (Currently Amended) An ophthalmic composition, comprising from about 0.001 wt% to about 0.05 wt% of a muscarinic antagonist and deuterated water, at a pD of from about 4.2 to about 7.9, wherein the muscarinic antagonist is atropine, or atropine sulfate.
2. (Cancelled).
3. (Cancelled).
4. (Original) The ophthalmic composition of claim 1, wherein the ophthalmic composition has a pD of one of: less than about 7.3, less than about 7.2, less than about 7.1, less than about 7, less than about 6.8, less than about 6.5, less than about 6.4, less than about 6.3, less than about 6.2, less than about 6.1, less than about 6, less than about 5.9, less than about 5.8, less than about 5.2, or less than about 4.8 after extended period of time under storage condition.
5. (Original) The ophthalmic composition of claim 1, wherein the ophthalmic composition comprises one of: at least about 80%, at least about 85%, at least about 90%, at least about 93%, at least about 95%, at least about 97%, at least about 98%, or at least about 99% of the muscarinic antagonist based on initial concentration after extended period of time under storage condition.
6. (Original) The ophthalmic composition of claim 1, wherein the ophthalmic composition further has a potency of one of: at least 80%, at least 85%, at least 90%, at least 93%, at least 95%, at least 97%, at least 98%, or at least 99% after extended period of time under storage condition.

7. (Original) The ophthalmic composition of claim 1, wherein the extended period of time is one of: about 1 week, about 2 weeks, about 3 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 8 months, about 10 months, about 12 months, about 18 months, about 24 months, about 36 months, about 4 years, or about 5 years.
8. (Original) The ophthalmic composition of claim 1, wherein the storage condition has a storage temperature of from about 2°C to about 10°C or from about 16°C to about 26°C.
9. (Original) The ophthalmic composition of claim 1, wherein the muscarinic antagonist is present in the composition at a concentration of one of: from about 0.001 wt% to about 0.04 wt%, from about 0.001 wt% to about 0.03 wt%, from about 0.001 wt% to about 0.025 wt%, from about 0.001 wt% to about 0.02 wt%, from about 0.001 wt% to about 0.01 wt%, from about 0.001 wt% to about 0.008 wt%, or from about 0.001 wt% to about 0.005 wt%.
10. (Original) The ophthalmic composition of claim 1, wherein the ophthalmic composition further comprises an osmolarity adjusting agent.
11. (Original) The ophthalmic composition of claim 10, wherein the osmolarity adjusting agent is sodium chloride.
12. (Original) The ophthalmic composition of claim 1, wherein the ophthalmic composition further comprises a preservative.
13. (Original) The ophthalmic composition of claim 12, wherein the preservative is selected from benzalkonium chloride, cetrimonium, sodium perborate, stabilized oxychloro complex, SofZia, polyquaternium-1, chlorobutanol, edetate disodium, polyhexamethylene biguanide, or combinations thereof.
14. (Original) The ophthalmic composition of claim 1, wherein the ophthalmic composition further comprises a buffer agent.

15. (Original) The ophthalmic composition of claim 14, wherein the buffer agent is selected from borates, borate-polyol complexes, phosphate buffering agents, citrate buffering agents, acetate buffering agents, carbonate buffering agents, organic buffering agents, amino acid buffering agents, or combinations thereof.
16. (Original) The ophthalmic composition of claim 1, wherein the ophthalmic composition is essentially free of procaine and benactyzine, or pharmaceutically acceptable salts thereof.
17. (Original) The ophthalmic composition of claim 1, wherein the ophthalmic composition has a dose-to-dose muscarinic antagonist concentration variation of one of: less than 50%, less than 40%, less than 30%, less than 20%, less than 10%, or less than 5%.
18. (Original) The ophthalmic composition of claim 17, wherein the dose-to-dose muscarinic antagonist concentration variation is based on one of: 10 consecutive doses, 8 consecutive doses, 5 consecutive doses, 3 consecutive doses, or 2 consecutive doses.
19. (Original) The ophthalmic composition of claim 1, wherein the ophthalmic composition further comprises a pD adjusting agent.
20. (Currently Amended) The ophthalmic composition of claim 19, wherein the pD adjusting agent comprises ~~DCl~~ deuterated hydrochloric acid, ~~NaOD~~ deuterated sodium hydroxide, ~~CD₃COOD~~ deuterated acetic acid, or ~~C₆D₈O₇~~ deuterated citric acid.
21. (Original) The ophthalmic composition of claim 1, wherein the ophthalmic composition comprises one of: less than 5% of H₂O, less than 4% of H₂O, less than 3% of H₂O, less than 2% of H₂O, less than 1% of H₂O, less than 0.5% of H₂O, less than 0.1% of H₂O, or 0% of H₂O.
22. (Original) The ophthalmic composition of claim 1, wherein the ophthalmic composition is not formulated as an injectable formulation.

23. (Original) The ophthalmic composition of claim 1, wherein the ophthalmic composition is formulated as an ophthalmic solution for the treatment of pre-myopia, myopia, or progression of myopia.
24. (Currently Amended) An ophthalmic solution, comprising from about 0.001 wt% to about 0.05 wt% of a muscarinic antagonist and deuterated water, at a pD of from about 4.2 to about 7.9, wherein the muscarinic antagonist is atropine, or atropine sulfate.
25. (Original) The ophthalmic solution of claim 24, wherein the ophthalmic solution has a pD of one of: less than about 7.3, less than about 7.2, less than about 7.1, less than about 7, less than about 6.8, less than about 6.5, less than about 6.4, less than about 6.3, less than about 6.2, less than about 6.1, less than about 6, less than about 5.9, less than about 5.8, less than about 5.2, or less than about 4.8 after extended period of time under storage condition.
26. (Cancelled).
27. (Original) The ophthalmic solution of claim 24, wherein the ophthalmic solution comprises one of: less than 5% of H₂O, less than 4% of H₂O, less than 3% of H₂O, less than 2% of H₂O, less than 1% of H₂O, less than 0.5% of H₂O, less than 0.1% of H₂O, or 0% of H₂O.
28. (Withdrawn) A method of arresting or preventing myopia development, comprising administering to an eye of an individual in need thereof an effective amount of an ophthalmic composition comprising from about 0.001 wt% to about 0.05 wt% of a muscarinic antagonist and deuterated water, at a pD of from about 4.2 to about 7.9.
29. (Withdrawn) The method of claim 28, wherein the ophthalmic composition is stored at between about 2 °C to about 10 °C prior to first use.
30. (Withdrawn) The method of claim 28, wherein the ophthalmic composition is stored at between about 16 °C to about 26 °C after first use.

REMARKS

This paper is filed in response to the non-final Office Action dated March 7, 2016, and in light of an Examiner Interview conducted on March 30, 2016. In the Office Action, claims 1-27 are rejected as allegedly failing to comply with the written description requirement; claim 20 is rejected as allegedly indefinite; and claims 1-27 are rejected as allegedly obvious over US5,716,952 (“WoldeMussie”) in view of US2012/0015035 (“Widsoet”) and US2012/0203161 (“Herekar”). In addition, all pending claims are rejected under double patenting over co-pending Application No. 14/859,042.

During the Examiner Interview conducted on March 30, 2016, Applicant’s attorney and Examiner Fay discussed potential claim amendments and remarks to overcome the rejections asserted in the Office Action. This response is prepared in furtherance of the discussion. Reconsideration and withdrawal of the rejections are respectfully requested.

Written Description

In the Office Action, claims 1-27 are rejected as allegedly failing to comply with the written description requirement because claim 1 recites “a muscarinic antagonist.” For the sole purpose of expediting prosecution of the present application, independent claims 1 and 24 are amended to recite that the muscarinic antagonist is atropine, or atropine sulfate. As such, the written description rejection should be withdrawn.

Indefiniteness

In the Office Action, claim 20 is rejected as allegedly indefinite for reciting “DCI, NaOD, CD₃COOD, or C₆D₈O₇.” As amended, claim 20 now recites “deuterated hydrochloric acid, deuterated sodium hydroxide, deuterated acetic acid, or deuterated citric acid.” Accordingly, this rejection should be withdrawn.

Obviousness

To support an obviousness rejection, MPEP §2143.03 requires “all words of a claim to be considered” and MPEP § 2141.02 requires consideration of the “[claimed] invention and prior art

as a whole.” Further, the Board of Patent Appeal and Interferences recently confirmed that a proper, post-KSR obviousness determination still requires the Office make “a searching comparison of the claimed invention – including all its limitations – with the teaching of the prior art.” See, *In re Wada and Murphy*, Appeal 2007-3733, citing *In re Ochiai*, 71 F.3d 1565, 1572 (Fed. Cir. 1995) (emphasis in original). In sum, it remains well-settled law that an obviousness rejection requires at least a suggestion of *all* of the claim elements. Applicant submits that the cited references, whether taken alone or in combination, do not teach or suggest D₂O-based atropine formulations, much less recognizing the technical effects thereof, as discussed in greater detail below.

WoldeMussie and Widsoet Are Irrelevant to D₂O-Based Atropine Formulations

In the Office Action, the Patent Office cites WoldeMussie for allegedly teaching ophthalmic formulations containing a muscarinic antagonist; and Widsoet for allegedly teaching atropine being used as a muscarinic antagonist in ophthalmic formulations (page 6 of the Office Action). Applicant notes that neither reference teaches or suggests D₂O-based atropine formulations, much less recognizing the technical effects thereof.

Herekar Cannot Be Properly Modified to Teach D₂O-Based Atropine Formulations

The Office Action cites Herekar for allegedly teaching: (1) use of deuterated water in combination with riboflavin in an ophthalmic formulation; (2) increased shelf life of such formulation; and (3) reduced amount of riboflavin if deuterated water is used instead of water (bottom of page 6 of the Office Action). According to the Office Action, one of ordinary in the art would use deuterated water to replace water in WoldeMussie’s ophthalmic formulation to “increase shelf life and to reduce the amount of active ingredient need for the pharmaceutical activity” (top of page 7 of the Office Action).

Applicant disagrees. Applicant first notes that Herekar entire disclosure includes only one sentence that mentions “shelf life.” Specifically, paragraph [0026] of Herekar states that:

Significantly, the riboflavin formulation can also be manufactured with a high concentration of dissolved oxygen. This oxygen enrichment enables the production of greater ROS concentration in a shorter period of time, and, in turn, this makes higher UVA irradiance practical. However, it should be noted that

there may be other means to diffuse oxygen gas into the stroma, which might include, among others, the use of a device that would deliver such oxygen gas to the corneal surface. This oxygen gas then diffuses (albeit slowly) into the stroma, thereby increasing dissolved oxygen. In summary, the ability to increase dissolved oxygen in the stroma enables the use of a higher UVA irradiance exposure to the collagen tissue. This concept of embedding dissolved oxygen in the stroma means that optimum cross-linking (i.e., adequate stiffness of the cornea with minimal side effects) can be achieved in a shorter period of time. **A means to manufacture the enriched oxygen deuterated Rf formulation, that will provide up to and over 1 year of extended shelf life,** is contemplated by this invention. The components of the formulation invention, which are set forth hereinabove, may be optimized for penetration rate, pH, hypotonicity, and lubricity by the proportions that each component is used within the formulation.

This general description of “extended shelf life” appear to be mere wishes as no detailed description or specific example of such “means to manufacture” or “shelf life extension” can be found in Herekar. More importantly, the “contemplated” shelf life extension as described in the cited paragraph is clearly tied to the “means to manufacture”, not to the components of the formulation, much less to deuterated water. Thus, one of ordinary skill in the art would not read Herekar as teaching the use of deuterated water for the purpose of extending shelf life of its riboflavin formulation, much less other ophthalmic formulations such as atropine formulations.

Turning to the Patent Office’s position that Herekar teaches D₂O-based riboflavin formulations in which the amount of riboflavin can be reduced if deuterated water is used instead of water, Applicant notes that such beneficial effect is specifically tied to the underlying biological mechanism disclosed in Herekar. For example, Herekar’s Abstract and Summary sections both start with the following characterization of Herekar’s invention:

a solution of deuterated water containing a riboflavin-based photosensitizer (Rf aka Vitamin B2) is provided in order to extend lifetimes of UVA/Rf photo-generated intra-stromal singlet oxygen, in combination with UVA delivery profiles of pulsing, fractionation, and optionally auxiliary stromal/Rf hyper-oxygenation in order to accelerate protein cross-linking density rates in ocular tissue.

Later, in the Summary section, Herekar makes it clear that it is the deuterated water in its riboflavin formulation that functions to provide the extended lifetimes of the singlet oxygen. See, e.g. paragraph [0011], stating that:

Lower concentrations of deuterated water with regular water, for example, yields shorter singlet oxygen lifetimes. These lifetimes have approximately a **linear relationship** to the concentration of deuterated water. These lower concentrations are considered acceptable in therapies known or being developed in the art of corneal cross-linking.

As such, one of ordinary skill in the art would understand that the “intended purpose” or “principle of operation” of using deuterated water in Herekar’s riboflavin formulation is such that lifetimes of the singlet oxygen can be extended, which in turn leads to the alleged benefit of requiring less riboflavin because of the singlet oxygen preservation.

With such understanding in mind, Applicant notes that there is insufficient rationale to combine Herekar and other references to produce a D₂O-based atropine formulation because atropine and its derivatives are recognized “singlet oxygen **scavengers**”. See, e.g. Abstract of Criado et al. “Scavenging of photogenerated oxidative species by antimuscarinic drugs: atropine and derivatives” Redox Rep 7(6):385-394 (2002), cited in Applicant’s IDS filed on March 29, 2016. In other words, any modification or combination of Herekar and other references to arrive at a D₂O-based atropine formulation as featured in the amended claims would inevitably frustrate the “intended purpose” or “principle of operation” of Herekar. See MPEP 2143.01 VI (“[i]f the proposed modification or combination of the prior art would change the principle of operation of the prior art invention being modified, then the teachings of the references are not sufficient to render the claims *prima facie* obvious”). See also, MPEP 2143.01 V (“[i]f proposed modification would render the prior art invention being modified unsatisfactory for its intended purpose, then there is no suggestion or motivation to make the proposed modification. *In re Gordon*, 733 F.2d 900, 221 USPQ 1125 (Fed. Cir. 1984).”

Unexpected Technical Effects of D2O-Based Atropine Formulation

Finally, Applicant draws the Patent Office’s attention to the surprising data presented in the application. In particular, the formulation with D₂O yield the following unexpected properties:

- Better stability
- Lower main degradant
- Longer shelf-life

Better stability

Tables 24A and 24B under paragraph [00329] (Example 11) and Table 15 under paragraph [00300] (Example 9) show atropine sulfate purity data of different formulations. Table 24A shows atropine sulfate formulations in water. Table 24B shows similar atropine sulfate formulations in deuterated water. The pair-wise formulations can be viewed as:

- Formulation 3 and Formulation 7
- Formulation 5 and Formulation 8
- Formulations 10/11 and Formulation 9
- Formulation 13 and Formulation 12

Formulation 8 shows a pD of 6.2 (with effective pH of 5.8; Table 24B). It is formulated similarly as Formulation 5 (Table 24A), with the exception of deuterated water. At week 0, both formulations (5 and 8) contain similar percentage of atropine sulfate (98.16% for Formulation 5 and 98.93% for Formulation 8). However, at week 2, a higher degradation is observed for Formulation 5 across all three temperature ranges while a much lower degradation is observed for Formulation 8. Similarly, at week 4, only 66.83% of atropine sulfate remains at 60 degree C in Formulation 5. In contrast, 92.72% of atropine sulfate remains at 60 degree C in Formulation 8.

Similarly, Formulation 9 shows a higher purity percentage at week 4 across all 3 temperatures than either Formulation 10 or 11. Formulation 12 (pD 7.2; effective pH of 6.8) shows about 46% atropine sulfate at week 4 and 60 degree C while Formulation 13 only retained about 28% atropine sulfate at week 4 and 60 degree C.

Table 24A. Atropine Sulfate Purity as Area-% for H₂O Formulations

Solvent	Condition	t=0	t=2 weeks	t=4 weeks
Formulation 3 ↓A H ₂ O pH 4.8	25/60	98.16	98.16	98.45
	40/75		97.98	97.35
	60 °C		95.94	94.65
Formulation 5 ↓C H ₂ O pH 5.8	25/60	98.16	97.92	97.54
	40/75		95.88	93.51
	60 °C		80.94	66.83
Formulation 10 ↓C H ₂ O pH 6.4	25/60	98.66	96.67	95.81
	40/75		91.07	85.27
	60 °C		59.77	42.87

Formulation 11 ↓C(2x) H ₂ O pH 6.4	25/60	99.47	97.87	96.69
	40/75		90.97	84.26
	60 °C		54.96	34.40
Formulation 13 ↓C H ₂ O pH 6.8	25/60	97.21	95.42	93.24
	40/75		83.05	73.00
	60 °C		43.99	27.50

Table 24B. Atropine Sulfate Purity as Area-% for D₂O Formulations

Solvent	Condition	t=0	t=2 weeks	t=4 weeks
Formulation 7 ↓A D ₂ O pD 5.2	25/60	98.93	99.07	98.39
	40/75		98.51	97.55
	60 °C		96.70	94.01
Formulation 8 ↓C D ₂ O pD 6.2	25/60	98.93	98.95	98.51
	40/75		98.53	97.44
	60 °C		95.97	92.72
Formulation 9 ↓C D ₂ O pD 6.8	25/60	99.29	98.42	98.07
	40/75		95.20	93.22
	60 °C		75.17	65.97
Formulation 12 ↓C D ₂ O pD 7.2	25/60	98.53	97.17	95.99
	40/75		90.75	84.64
	60 °C		56.78	46.05

Lower Main Degradant

With respect to the main degradant—tropic acid, Tables 25A and 25B (under paragraph [00330]) further show the percentage of tropic acid are present with each individual formulations. For example, only 4.03% of tropic acid was observed at 60 degree C in Formulation 8 while about 29% of tropic acid was observed at 60 degree C at week 4 in Formulation 5. Formulation 9 contains about 29% of tropic acid at 60 degree C at week 4 while Formulations 10 and 11 each contains about 54% or 62% tropic acid at 60 degree C, respectively. Similarly, Formulation 12 contains about 48% tropic acid at 60 degree C while Formulation 13 contains 69%. As such, the deuterated water stabilizes the atropine sulfate.

Table 25A. Tropic Acid as Area-% for H₂O Formulations

Solvent	Condition	t=0	t=2 weeks	t=4 weeks
Formulation 3 ↓A H ₂ O pH 4.8	25/60	<LOQ	0.12	0.05
	40/75		0.19	0.27
	60 °C		0.90	1.58
Formulation 5 ↓C H ₂ O pH 5.8	25/60	<LOQ	0.40	0.71
	40/75		2.22	4.35
	60 °C		16.62	29.13
Formulation 10	25/60	0.74	1.90	3.21

↓C H ₂ O pH 6.4	40/75		7.61	13.49
	60 °C		37.44	54.06
Formulation 11 ↓C(2x) H ₂ O pH 6.4	25/60	0.09	1.31	2.64
	40/75		7.61	14.68
	60 °C		42.43	62.23
Formulation 13 ↓C H ₂ O pH 6.8	25/60	2.21	3.66	6.11
	40/75		15.47	25.80
	60 °C		53.24	69.34

Table 25B. Tropic Acid as Area-% for D₂O Formulations

Solvent	Condition	t=0	t=2 weeks	t=4 weeks
Formulation 7 ↓A D ₂ O pD 5.2	25/60	<LOQ	0.07	0.08
	40/75		0.14	0.24
	60 °C		0.71	1.07
Formulation 8 ↓C D ₂ O pD 6.2	25/60	<LOQ	0.11	0.14
	40/75		0.33	0.65
	60 °C		2.32	4.03
Formulation 9 ↓C D ₂ O pD 6.8	25/60	0.06	0.55	1.06
	40/75		3.16	6.29
	60 °C		21.09	29.25
Formulation 12 ↓C D ₂ O pD 7.2	25/60	0.42	1.35	2.62
	40/75		7.27	13.53
	60 °C		38.58	48.15

Longer Shelf-life

Table 30 under Example 12 (paragraph [00334]) shows the predicted shelf life at temperatures of 40°C, 30°C, 25°C, and 2-8°C for Formulations 2-8 for total Related Substance and for the main degradant tropic acid, respectively. As shown from the table, Formulation 8 has longer shelf life across all 4 temperature ranges when compared with Formulation 5. In particular, at 2-8 degree C, Formulation 8 has a shelf life of about 158 months while Formulation 5 only has a shelf life of about 37 months.

Table 30

Stability Prediction		RS		Tropic Acid	
Formulation	Temperature (°C)	weeks	months	Temperature (°C)	weeks months
2	40	64.5	16.1	40	- -
	30	153.2	38.3	30	- -
	25	241.2	60.3	25	- -
	2-8	1747.9	437.0	2-8	- -
3	40	31.1	7.8	40	99.5 24.9
	30	73.9	18.5	30	268.3 67.1
	25	116.3	29.1	25	451.8 113.0

	2-8	842.9	210.7	2-8	4382.0	1095.5
4	40	30.7	7.7	40	42.1	10.5
	30	73.0	18.2	30	113.7	28.4
	25	114.9	28.7	25	191.5	47.9
	2-8	832.6	208.1	2-8	1857.0	464.2
5	40	5.5	1.4	40	4.9	1.2
	30	13.1	3.3	30	13.2	3.3
	25	20.6	5.2	25	22.2	5.5
	2-8	149.3	37.3	2-8	215.0	53.8
6	40	10.7	2.7	40	8.8	2.2
	30	25.5	6.4	30	23.7	5.9
	25	40.1	10.0	25	39.8	10.0
	2-8	290.5	72.6	2-8	386.5	96.6
7	40	27.9	7.0	40	129.6	32.4
	30	66.4	16.6	30	349.6	87.4
	25	104.5	26.1	25	588.7	147.2
	2-8	757.3	189.3	2-8	5709.4	1427.4
8	40	23.3	5.8	40	33.6	8.4
	30	55.3	13.8	30	90.6	22.6
	25	87.2	21.8	25	152.5	38.1
	2-8	631.6	157.9	2-8	1479.2	369.8

For reasons stated above, the cited references do not teach or suggest the use of deuterated water in an atropine ophthalmic formulation, much less recognizing the technical effect thereof, as summarized above. Accordingly, the obviousness rejections should be withdrawn.

Double Patenting

Turning to the double patenting rejections, all pending claims are over the claims of co-pending Application No. 14/859,042. Applicant requests the Office to hold this rejection in abeyance until an allowable set of claims has been indicated for the present application.

CONCLUSION

Applicants submit that this response fully addresses the Office Action dated March 7, 2016 and respectfully request consideration and allowance of the claims.

Should the Examiner have any questions, the Examiner is encouraged to contact the undersigned attorney at (858) 350-2339. If additional fees are believed to be required, the Commissioner is authorized to charge any additional fees to Deposit Account No. 23-2415 (Attorney Docket No. 46682-701.201).

Respectfully submitted,

WILSON SONSINI GOODRICH & ROSATI
Professional Corporation

Date: April 5, 2016

By: /Xiaofan Yang/
Xiaofan Yang, Ph.D., Esq.
Reg. No. 61,459

650 Page Mill Road
Palo Alto, CA 94304
Direct Dial: (858) 350-2306
Customer No. 021971

Electronic Acknowledgement Receipt

EFS ID:	25406997
Application Number:	14726139
International Application Number:	
Confirmation Number:	7389
Title of Invention:	OPHTHALMIC COMPOSITION
First Named Inventor/Applicant Name:	Gregory I. Ostrow
Customer Number:	21971
Filer:	Xiaofan Yang/Linda Anders
Filer Authorized By:	Xiaofan Yang
Attorney Docket Number:	46682-701.201
Receipt Date:	05-APR-2016
Filing Date:	29-MAY-2015
Time Stamp:	18:07:26
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		46682-701-201-responseOA.pdf	277851 1563fcb10b6353c2afa70f20e6897b3ce5af303b	yes	14

Multipart Description/PDF files in .zip description			
Document Description	Start	End	
Amendment/Req. Reconsideration-After Non-Final Reject	1	1	
Claims	2	5	
Applicant Arguments/Remarks Made in an Amendment	6	14	

Warnings:

Information:

Total Files Size (in bytes):	277851

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New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 14/726,139	Filing Date 05/29/2015	☐ To be Mailed

ENTITY: ☐ LARGE ☒ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

(Column 1)

(Column 2)

FOR	NUMBER FILED	NUMBER EXTRA	RATE (\$)	FEE (\$)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (l), or (m))	N/A	N/A	N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X \$ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X \$ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is \$310 (\$155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	

APPLICATION AS AMENDED – PART II

(Column 1)

(Column 2)

(Column 3)

AMENDMENT	04/05/2016	CLAIMS REMAINING AFTER AMENDMENT	MINUS	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE (\$)	ADDITIONAL FEE (\$)
	Total (37 CFR 1.16(i))	* 27	Minus	** 30	= 0	X \$40 =	0
	Independent (37 CFR 1.16(h))	* 3	Minus	***3	= 0	X \$210 =	0
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	0

(Column 1)

(Column 2)

(Column 3)

AMENDMENT	CLAIMS REMAINING AFTER AMENDMENT	MINUS	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE (\$)	ADDITIONAL FEE (\$)
	Total (37 CFR 1.16(i))	*	Minus	**	=	X \$ =
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X \$ =
	☐ Application Size Fee (37 CFR 1.16(s))					
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))					
						TOTAL ADD'L FEE

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".

The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

LIE
/PATRICIA F. LEWIS/

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Substitute for form 1449/PTO INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use as many sheets as necessary)				Complete if Known	
				Application Number	14726139
				Filing Date	05-29-2015
				First Named Inventor	OSTROW; Gregory I.
				Art Unit	1621
				Examiner Name	Zohreh A. Fay
				Attorney Docket Number	46682-701.201
Sheet	1	of	1		

U. S. PATENT DOCUMENTS					
Examiner Initials*	Cite No.	Document Number	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		Number-Kind Code ² (if known)			
		None			

FOREIGN PATENT DOCUMENTS						
Examiner Initials*	Cite No.	Foreign Patent Document	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T ⁶
		Country Code ³ -Number ⁴ -Kind Code ⁵ (if known)				
		None				

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No. ¹	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T ²
	001	CRIADO et al. Scavenging of photogenerated oxidative species by antimuscarinic drugs: atropine and derivatives. Redox Rep 7(6):385-394 (2002).	☐

Examiner Signature		Date Considered	

EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. ¹ Applicant's unique citation designation number (optional). ² See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. ³ Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). ⁴ For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. ⁵ Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. ⁶ Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND**
TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

Electronic Patent Application Fee Transmittal

Application Number:	14726139			
Filing Date:	29-May-2015			
Title of Invention:	OPHTHALMIC COMPOSITION			
First Named Inventor/Applicant Name:	Gregory I. Ostrow			
Filer:	Michael J. Hostetler/FY/Jennifer Vail			
Attorney Docket Number:	46682-701.201			
Filed as Small Entity				
Filing Fees for Utility under 35 USC 111(a)				
Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Miscellaneous:				
Submission- Information Disclosure Stmt	2806	1	90	90
Total in USD (\$)				90

Electronic Acknowledgement Receipt

EFS ID:	25338772
Application Number:	14726139
International Application Number:	
Confirmation Number:	7389
Title of Invention:	OPHTHALMIC COMPOSITION
First Named Inventor/Applicant Name:	Gregory I. Ostrow
Customer Number:	21971
Filer:	Michael J. Hostetler/FY/Jennifer Vail
Filer Authorized By:	Michael J. Hostetler
Attorney Docket Number:	46682-701.201
Receipt Date:	29-MAR-2016
Filing Date:	29-MAY-2015
Time Stamp:	19:43:11
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	\$90
RAM confirmation Number	20407
Deposit Account	232415
Authorized User	YANG, XIAOFAN

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

File Listing:					
Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		46682-701-201_IDS.pdf	228722	yes	5
			36a27c43d4fae9e4b3670a01cd3ca07655fc7f58		
	Multipart Description/PDF files in .zip description				
	Document Description		Start	End	
	Transmittal Letter		1	4	
	Information Disclosure Statement (IDS) Form (SB08)		5	5	
Warnings:					
Information:					
2	Non Patent Literature	CRIADO_Scavengingofphotogenerated_385-394.pdf	675623	no	11
			88d1b10681fbf27729688e7e165dbe91e029863c		
Warnings:					
Information:					
3	Fee Worksheet (SB06)	fee-info.pdf	30609	no	2
			b35c38fa4eb512aa4d66a0f771b6c8ba8b782ff7		
Warnings:					
Information:					
Total Files Size (in bytes):			934954		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor:	OSTROW; Gregory I. et al.	Group Art Unit:	1621
Serial Number:	14/726,139	Examiner:	Zohreh A. Fay
Filing or 371 (c) Date:	2015-05-29	CONFIRMATION NO:	7389
Title:	OPHTHALMIC COMPOSITION		

FILED ELECTRONICALLY ON: March 29, 2016

Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

INFORMATION DISCLOSURE STATEMENT
UNDER 37 CFR § 1.97

Commissioner for Patents:

An Information Disclosure Statement along with attached PTO/SB/08 is hereby submitted. A copy of each listed publication is submitted, if required, pursuant to 37 CFR §§1.97-1.98, as indicated below.

The Examiner is requested to review the information provided and to make the information of record in the above-identified application. The Examiner is further requested to initial and return the attached PTO/SB/08 in accordance with MPEP § 609.

The right to establish the patentability of the claimed invention over any of the information provided herewith, and/or to prove that this information may not be prior art, and/or to prove that this information may not be enabling for the teachings purportedly offered, is hereby reserved.

This statement is not intended to represent that a search has been made or that the information cited in the statement is, or is considered to be, prior art or material to patentability as defined in § 1.56.

- A. ☐ 37 CFR § 1.97 (b). This Information Disclosure Statement should be considered by the Office because:
- ☐ (1) It is being filed within 3 months of the filing date of a national application and is other than a continued prosecution application under § 1.53 (d);
-- OR --
 - ☐ (2) It is being filed within 3 months of entry of the national stage as set forth in § 1.491 in an international application;
-- OR --
 - ☐ (3) It is being filed before the mailing of a first Office action on the merits;
-- OR --
 - ☐ (4) It is being filed before the mailing of a first Office action after the filing of a request for continued examination under § 1.114.
- B. ☒ 37 CFR § 1.97(c). Although this Information Disclosure Statement is being filed after the period specified in 37 CFR § 1.97(b), above, it is filed before the mailing date of the earlier of (1) a final office action under § 1.113, (2) a notice of allowance under § 1.311, or (3) an action that otherwise closes prosecution on the merits, this Information Disclosure Statement should be considered because it is accompanied by one of:
- ☐ a statement as specified in §1.97 (e) provided concurrently herewith;
-- OR --
 - ☒ a fee of \$90.00 as set forth in § 1.17 (p) authorized below, enclosed, or included with the payment of other papers filed together with this statement.
- C. ☐ 37 CFR § 1.97 (d). Although this Information Disclosure Statement is being filed after the mailing date of the earlier of (1) a final office action under § 1.113 or (2) a notice of allowance under § 1.311, it is being filed before payment of the issue fee and should be considered because it is accompanied by:
- i. a statement as specified in § 1.97 (e);
-- AND --
 - ii. a fee of \$180.00 as set forth in §1.17(p) is authorized below, enclosed, or included with the payment of other papers filed together with this Statement.
- D. ☐ 37 CFR §1.97 (e). Statement.
- ☐ A statement is provided herewith to satisfy the requirement under 37 CFR §§ 1.97 (c);
-- AND/OR --
 - ☐ A statement is provided herewith to satisfy the requirement under 37 CFR §§ 1.97 (d);
-- AND/OR --
 - ☐ A copy of a dated communication from a foreign patent office clearly showing that the information disclosure statement is being submitted within 3 months of the filing date on the communication is provided in lieu of a statement under 37 C.F.R. § 1.97(e) (1) as provided for under MPEP 609.04(b) V.

- E. ☐ *Statement Under 37 C.F.R. §1.704(d).* Each item of information contained in the information disclosure statement was first cited in any communication from a patent office in a counterpart foreign or international application or from the Office or is a communication that was issued by a patent office in a counterpart foreign or international application or by the Office that was received by an individual designated in § 1.56(c) not more than thirty (30) days prior to the filing of this information disclosure statement. This statement is made pursuant to the requirements of 37 C.F.R. §1.704(d) to avoid reduction of the period of adjustment of the patent term for Applicant(s) delay.
- F. ☒ *37 CFR §1.98 (a) (2).* The content of the Information Disclosure Statement is as follows:
- ☐ Copies of each of the references listed on the attached Form PTO/SB/08 are enclosed herewith.
- OR --
- ☐ Copies of U.S. Patent Documents (issued patents and patent publications) listed on the attached Form PTO/SB/08 is NOT enclosed.
- AND/OR --
- ☒ Copies of Foreign Patent Documents and/or Non Patent Literature Documents listed on the attached Form PTO/SB/08 are enclosed in accordance with 37 CFR §1.98 (a)(2).
- AND/OR --
- ☐ Copies of pending unpublished U.S. patent applications are enclosed in accordance with 37 CFR §1.98 (a) (2) (iii).
- G. ☐ *37 CFR §1.98(a)(3).* The Information Disclosure Statement includes non-English patents and/or references.
- ☐ Pursuant to 37 CFR §1.98(a)(3)(i), a concise explanation of the relevance of each patent, publication or other information provided that is not in English is provided herewith.
- ☐ Pursuant to MPEP 609(B), an English language copy of a foreign search report is submitted herewith to satisfy the requirement for a concise explanation where non-English language information is cited in the search report.
- OR --
- ☐ A concise explanation of the relevance of each patent, publication or other information provided that is not in English is as follows: _____
- ☐ Pursuant to 37 CFR §1.98(a) (3) (ii), a copy of a translation, or a portion thereof, of the non-English language reference(s) is provided herewith.
- H. ☐ *37 CFR §1.98(d).* Copies of patents, publications and pending U.S. patent applications, or other information specified in 37 C.F.R. § 1.98(a) are not provided herewith because:
- ☐ Pursuant to 37 CFR §1.98(d)(1) the information was previously submitted in an Information Disclosure Statement, or cited by examiner for another application under which this application claims priority for an earlier effective filing date under 35 U.S.C. 120.
- Application in which the information was submitted: _____
- Information Disclosure Statement(s) filed on: _____
- AND
- ☐ The information disclosure statement submitted in the earlier application complied with paragraphs (a) through (c) of 37 CFR §1.98.

- I. ☒ *Fee Authorization.* The Commissioner is hereby authorized to charge the above-referenced fees of \$90.00 and charge any additional fees or credit any overpayment associated with this communication to Deposit Account No. 23-2415 (Docket No. 46682-701.201).

Respectfully submitted,

WILSON SONSINI GOODRICH & ROSATI

Dated: March 29, 2016

By: /Xiaofan Yang/
Xiaofan Yang, Reg. No. 61459

650 Page Mill Road
Palo Alto, CA 94304-1050
(650) 493-9300
Customer No. 21971



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/726,139	05/29/2015	Gregory I. Ostrow	46682-701.201	7389

21971 7590 03/07/2016
WILSON, SONSINI, GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304-1050

EXAMINER

FAY, ZOHREH A

ART UNIT	PAPER NUMBER

1621

NOTIFICATION DATE	DELIVERY MODE

03/07/2016

ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

patentdocket@wsgr.com

Office Action Summary	Application No. 14/726,139	Applicant(s) OSTROW ET AL.	
	Examiner ZOHREH FAY	Art Unit 1621	AIA (First Inventor to File) Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 12/2/2015.
☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on ____.
- 2a) ☐ This action is **FINAL**. 2b) ☒ This action is non-final.
- 3) ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on ____; the restriction requirement and election have been incorporated into this action.
- 4) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

- 5) ☒ Claim(s) 1-30 is/are pending in the application.
5a) Of the above claim(s) 28-30 is/are withdrawn from consideration.
- 6) ☐ Claim(s) ____ is/are allowed.
- 7) ☒ Claim(s) 1-27 is/are rejected.
- 8) ☐ Claim(s) ____ is/are objected to.
- 9) ☐ Claim(s) ____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

- 10) ☐ The specification is objected to by the Examiner.
- 11) ☐ The drawing(s) filed on ____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

- a) ☐ All b) ☐ Some** c) ☐ None of the:
1. ☐ Certified copies of the priority documents have been received.
 2. ☐ Certified copies of the priority documents have been received in Application No. ____.
 3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- 1) ☒ Notice of References Cited (PTO-892)
- 2) ☒ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b)
Paper No(s)/Mail Date 1/7/2016
- 3) ☐ Interview Summary (PTO-413)
Paper No(s)/Mail Date. ____.
- 4) ☐ Other: ____.

DETAILED ACTION

Claims 1-30 are pending in the instant application.

Claims 1-27 are presented for examination.

Claims 28-30 are withdrawn from consideration.

The response to the restriction requirement of December 2, 2015 has been received and entered.

Applicant elected Group I, claims 1-27 and the species of atropine without traverse for examination purpose.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112(a):

(a) IN GENERAL.—The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor or joint inventor of carrying out the invention.

The following is a quotation of the first paragraph of pre-AIA 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-27 are rejected under 35 U.S.C. 112(a) or 35 U.S.C. 112 (pre-AIA), first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor or a joint inventor, or for pre-AIA the inventor(s), at the time the application was filed, had possession of

the claimed invention. The claims are drawn to “a muscarinic antagonist”. The instant specification discloses a few compounds within the scope of what is claimed. However, in the absence of the compounds being used, the artisan, would not have accepted that applicant was in possession of the claimed method of use. The description requirement of the patent statute requires a description of an invention, not an indication of a result that one might achieve if one made that invention. See, e.g., In re Wilder, 22 USPQ 369, 372-3 (Fed. Cir. 1984). (Holding that a claim was not adequately described because the specification did ‘little more than outline goals appellants hope the claimed invention achieves and the problems the invention will hopefully ameliorate.’) Mere indistinct terms (such as known classes of compounds which are now claimed based on a limited functional capability, such as “a muscarinic antagonist” however, may not suffice to meet the written description requirement. This is particularly true when known compound classes, which otherwise meet the written description requirement, are limited to a smaller subgroup claimed in purely functional terms, such as the case in the instant claims. See Univ. of Rochester v. G.D. Searle, 69USPQ2d 1886 (CAFC 2004) at 1892, stating: The appearance of mere indistinct words in a specification or a claim, even an original claim, does not necessarily satisfy that requirement. A description of an anti-inflammatory steroid, i.e., a steroid (a generic structural term) described even in terms of its functioning of lessening inflammation of tissues fails to distinguish any steroid from others having the same activity or function. A description of what a material does, rather than of what it is, usually does not suffice. The disclosure must allow one skilled in the art to visualize or recognize the identity of the subject matter purportedly described. (Emphasis added). Conversely, a description of a chemical genus will usually comprise a recitation of structural features common to the members of the genus, which features constitute a substantial portion of the genus. See Univ. of Calif. V. Eli Lilly, 43 USPQ 2d 1398, 1406 (Fed. Cir. 1997). This is

analogous to enablement of a genus under Section 112, I[1, by showing the enablement of a representative number of species within the genus. A chemical genus can be adequately described if the disclosure presents a sufficient number of representative species that encompass the genus. If the genus has substantial variance, the disclosure must describe a sufficient number of species to reflect the variation within that genus. See MPEP 2163. The MPEP lists factors that can be used to determine if sufficient evidence of possession has been furnished in the disclosure of the Application. These include the level of skill and knowledge in the art, partial structure, physical and/or chemical properties, functional characteristics alone or coupled with a known or disclosed correlation between structure and function, and the method of making the claimed invention. Disclosure of any combination of such identifying characteristics that distinguish the claimed invention from other materials and would lead one of skill in the art to the conclusion that the applicant was in possession of the claimed species is sufficient. MPEP 2163. Here, the specification does not provide a reasonably representative disclosure of useful compounds which are “muscarinic antagonists”.

Claim Rejections - 35 USC § 112

The following is a quotation of 35 U.S.C. 112(b):

(b) CONCLUSION.—The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the inventor or a joint inventor regards as the invention.

The following is a quotation of 35 U.S.C. 112 (pre-AIA), second paragraph:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim 20 is rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the inventor or a joint inventor, or for pre-AIA the applicant regards as the invention.

Claim 20 is indefinite as to the expression "DCI, NaOD, CD₃COOD, or C₆D₈O₇". Such expression fails to set forth the intended meaning and the chemical structure of the intended compound.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103 which forms the basis for all obviousness rejections set forth in this Office action:

A patent for a claimed invention may not be obtained, notwithstanding that the claimed invention is not identically disclosed as set forth in section 102 of this title, if the differences between the claimed invention and the prior art are such that the claimed invention as a whole would have been obvious before the effective filing date of the claimed invention to a person having ordinary skill in the art to which the claimed invention pertains. Patentability shall not be negated by the manner in which the invention was made.

The factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103 are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

This application currently names joint inventors. In considering patentability of the claims the examiner presumes that the subject matter of the various claims was commonly owned as of the effective filing date of the claimed invention(s) absent any

evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and effective filing dates of each claim that was not commonly owned as of the effective filing date of the later invention in order for the examiner to consider the applicability of 35 U.S.C. 102(b)(2)(C) for any potential 35 U.S.C. 102(a)(2) prior art against the later invention.

Claims 1-27 are rejected under 35 U.S.C. 103 as being unpatentable over WoldeMussie et al. (US 5,716,952) as applied to claim 1-27 above, and further in view of Wildsoet et al. (US 20120015035) and Herekar (20120203161).

WoldeMussie et al. Teach the use of the claimed muscarinic antagonists in an ophthalmic formulation in combination with purified water. See the abstract, column 2, lines 46-67 and column 3, lines 1-20 and column 4, lines 23-32. The use of sodium chloride is taught in column 5, lines 18-22. The use of the buffering agents, such as phosphate, borate and citrate is taught in column 2, lines 23-29. The use of preservatives is taught in column 5, lines 9-14. WoldeMussie differs from the claimed invention in using deuterated water and the use of atropine. Wildsoet et al. teach the use of atropine as a muscarinic antagonist, being used in ophthalmic formulations. See Para [0031]. Herekar teaches the use of deuterated water in combination with riboflavin in an ophthalmic formulation. Herekar teaches the increased shelf life and the need for the use of lower concentration of riboflavin if deuterated water is used instead of water. See para [0026] and [0029]. The combination of water and deuterated water is taught in claim 2. It would have been obvious to use deuterated water instead of water or in combination with water in the composition of WoldeMussie et al., motivated

by the teachings of Herekar, which teaches the use of deuterated water instead of water or in combination with water increases the shelf life and reduces the amount of active ingredient needed for the pharmaceutical activity. Herekar makes clear that the use of deuterated water increases the shelf life of a pharmaceutical formulation. Therefore, it would have been obvious to a person skilled in the art to add deuterated water to a pharmaceutical formulation with the expectation of getting an increased shelf life and stability.

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the “right to exclude” granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the claims at issue are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory

double patenting ground provided the reference application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The USPTO internet Web site contains terminal disclaimer forms which may be used. Please visit <http://www.uspto.gov/forms/>. The filing date of the application will determine what form should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to <http://www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp>.

Claims 1-27 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-3, 25-39 of copending Application No. 14/859,042. Although the claims at issue are not identical, they are not patentably distinct from each other because they overlap. The claims of the instant application are drawn to an ophthalmic formulation of a muscarinic antagonist and deuterated water at a pD of 4.2 to about 7.9. The claims of the co-pending application are drawn to the use of a muscarinic antagonist and deuterated water. The pD of 4.2 to about 7.9 is the expected property of the claims of the copending application since it uses the same components at the same concentrations as claimed herein.

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ZOHREH FAY whose telephone number is (571)272-0573. The examiner can normally be reached on Monday to Friday 9:30-6:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Melenie McCormick can be reached on (571) 272-8037. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

ZF
/ZOHREH FAY/
Primary Examiner, Art Unit 1621

Notice of References Cited	Application/Control No. 14/726,139	Applicant(s)/Patent Under Reexamination OSTROW ET AL.	
	Examiner ZOHREH FAY	Art Unit 1621	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	CPC Classification	US Classification
*	A	US-2012/0203161 A1	08-2012	Herekar; Satish V.	A61K9/0048	604/20
	B	US-				
	C	US-				
	D	US-				
	E	US-				
	F	US-				
	G	US-				
	H	US-				
	I	US-				
	J	US-				
	K	US-				
	L	US-				
	M	US-				

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	CPC Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Search Notes 	Application/Control No. 14726139	Applicant(s)/Patent Under Reexamination OSTROW ET AL.
	Examiner ZOHREH FAY	Art Unit 1621

CPC- SEARCHED		
Symbol	Date	Examiner

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
West	2/29/2016	ZF
Inventor search	2/29/2016	ZF

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner

Substitute for form 1449/PTO INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use as many sheets as necessary)				Complete if Known	
				Application Number	14726139
				Filing Date	05-29-2015
				First Named Inventor	OSTROW; Gregory I.
				Art Unit	1621
				Examiner Name	Fay, Zohreh A
				Attorney Docket Number	46682-701.201
Sheet	1	of	7		

U. S. PATENT DOCUMENTS					
Examiner Initials*	Cite No.	Document Number	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		Number-Kind Code ² (if known)			
	001	US-3863633	02-04-1975	RYDE; Emma Marta et al.	
	002	US-4014335	03-29-1977	ARNOLD; Randall K.	
	003	US-4474751	10-02-1984	HASLAM; John L. et al.	
	004	US-5259998	11-09-1993	REICH; Cary et al.	
	005	US-5716952	02-10-1998	WOLDEMUSSIE; Elizabeth et al.	
	006	US-5858375	01-12-1999	FURMINGER; Ian George Stephen et al.	
	007	US-5900360	05-04-1999	WELCH; William J. et al.	
	008	US-6218428	04-17-2001	CHYNN; Emil	
	009	US-6270954	08-07-2001	WELCH; William J. et al.	
	010	US-6410048	06-25-2002	FOTINOS; Spiros	
	011	US-6720001	04-13-2004	CHEN; Feng-Jing et al.	
	012	US-7691099	04-06-2010	BERRY; Michael J.	
	013	US-7858582	12-28-2010	JIN; Lei et al.	
	014	US-8333985	12-18-2012	KNAACK; David et al.	
	015	US-20030157187	08-21-2003	HUNTER; William L.	
	016	US-20050249770	11-10-2005	HUNTER; William L.	
	017	US-20060159771	07-20-2006	KADRMAS; Eddie F. et al.	
	018	US-20070207192	09-06-2007	HOLL; Richard et al.	

Examiner Signature	/ZOHREH A FAY/	Date Considered	02/29/2016

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				First Named Inventor	OSTROW; Gregory I.
				Art Unit	1621
				Examiner Name	Fay, Zohreh A
				Attorney Docket Number	46682-701.201
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		Number-Kind Code ² (if known)			
	019	US-20070280992	12-06-2007	MARGARON; Philippe M.C. et al.	
	020	US-20080113035	05-15-2008	HUNTER; William L.	
	021	US-20080153900	06-26-2008	HUNTER; William L.	
	022	US-20100022495	01-28-2010	HOTAMISLIGIL; Gokhan S. et al.	
	023	US-20100196285	08-05-2010	BAYERL; Thomas	
	024	US-20120015035	01-19-2012	WILDSOET; Christine F. et al.	
	025	US-20120135084	05-31-2012	BAYERL; Thomas	
	026	US-20120203161	08-09-2012	HEREKAR; Satish V.	
	027	US-20130302855	11-14-2013	SELBER; Klaus et al.	
	028	US-20140011761	01-09-2014	HOTAMISLIGIL; Gokhan S. et al.	
	029	US-20140088199	03-27-2014	SHARMA; Anant	
	030	US-20140350049	11-27-2014	HOVNANIAN; Alain et al.	
	031	US-20150290125	10-15-2015	HORN; Gerald et al.	

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Examiner Initials*	Cite No ¹	Foreign Patent Document	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T ⁶
		Country Code ³ -Number ⁴ -Kind Code ⁵ (if known)				

Examiner Signature	/ZOHREH A FAY/	Date Considered	02/29/2016

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Sheet	3	of	7		

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		Country Code ³ -Number ⁴ -Kind Code ⁵ (if known)				
	001	CN-101327216-A	12-24-2008	ZHENGLIAN XIAO [CN]	Abstract:	☒
	002	CN-1456161-A	11-19-2003	LIU JIDONG [CN]	Abstract:	☒
	003	EP-0332826-A1	09-20-1989	TEVA PHARMA [IL]		☐
	004	WO-0049990-A2	08-31-2000	KHAMAR BAKULESH MAFATLAL [IN]		☐
	005	WO-02096418-A1	12-05-2002	VALLEY FORGE PHARMACEUTICALS [US], et al.		☐
	006	WO-2008005053-A1	01-10-2008	CYDEX INC [US], et al.		☐
	007	WO-2011098578-A2	08-18-2011	BIONEER AS [DK], et al.		☐
	008	WO-2012161655-A1	11-29-2012	SINGAPORE HEALTH SERVICE PTE LTD [SG], et al.		☐
	009	WO-2014140105-A1	09-18-2014	D2 BIOSCIENCE GROUP LTD., et al.		☐

Examiner Signature	/ZOHREH A FAY/	Date Considered	02/29/2016

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		Country Code ³ -Number ⁴ -Kind Code ⁵ (if known)				
	010	WO-9421298-A1	09-29-1994	PASTEUR INSTITUT [FR], et al.	Abstract:	☒
	011	WO-9624331-A1	08-15-1996	USTAV MAKROMOLEKUL ARNI CHEMIE [CZ], et al.		☐
	012	WO-9716192-A1	05-09-1997	MERCK & CO INC [US], et al.		☐

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	001	ABRAHAM et al. Draize rabbit eye test compatibility with eye irritation thresholds in humans: a quantitative structure-activity relationship analysis. Toxicol Sci 76:384-391 (2003).	☐
	002	BADARO et al. Retinal biocompatibility of brilliant Blue G with deuterated water for chromovitrectomy. J. Ophthalmic and Vision Research 9(2): 204-209 (2014).	☐
	003	CHENG et al. Water movement in the rabbit eye. Exp. Eye Res. 52:337-339 (1991).	☐

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				Application Number	14726139
				Filing Date	05-29-2015
				First Named Inventor	OSTROW; Gregory I.
				Art Unit	1621
				Examiner Name	Fay, Zohreh A
				Attorney Docket Number	46682-701.201
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	004	CHENG. Fate of water in the soft contact lens immediately after lens placement onto the cornea. Optometry and Vision Science 68(6):414-417 (1991).	☐
	005	CHIRIERI et al. Investigations concerning the changes induced by deuterium for hydrogen substitution in bioelectric activity of the frog retina. Physiologie 14(2):119-123 (1977).	☐
	006	Co-pending US patent application No. US14/859042, filed on 09-18-2015.	☐
	007	GANEA. Heavy water effect on certain energetic processes in retina. Physiologie 6(1):59-62 (1979).	☐
	008	GETTINGS et al. A comparison of low volume, Draize and in vitro eye irritation test data. III. Surfactant-based formulations. Food Chem Toxicol 36(3):209-231 (1998).	☐
	009	GLASOE et al. Use of glass electrodes to measure acidities in deuterium oxide. Journal of Physical Chemistry. 64:188-190 (1960).	☐
	010	JANUSCHOWSKI et al. Evaluating retinal toxicity of a new heavy intraocular dye, using a model of perfused and isolated retinal cultures of bovine and human origins. Graefes Arch Clin Exp Ophthalmol. 250:1013-1022 (2012).	☐
	011	JEONG et al. Biodegradable block copolymers as injectable drug-delivery systems. Nature 388(6645):860-862 (1997).	☐

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	012	JEONG et al. Drug release from biodegradable injectable thermosensitive hydrogel of PEG-PLGA-PEG triblock copolymers. J Control Release 63(1-2):155-163 (2000).	☐
	013	JEONG et al. Thermosensitive sol-gel reversible hydrogels. Advanced Drug Delivery Reviews 54:37-51 (2002).	☐
	014	KREZEL et al. A formula for correlating pKa values determined in D2O and H2O. Journal of Inorganic Chemistry 98:161-166 (2008).	☐
	015	LAI et al. Rapid transport of large polymeric nanoparticles in fresh undiluted human mucus. PNAS USA 104(5):1482-1487 (2007).	☐
	016	LUND et al. The Kinetics of atropine and apoatropine in aqueous solutions. ACTA Chemica Scandinavica 22:3085-3097 (1968).	☐
	017	MCCALL et al. Mechanisms of corneal tissue cross-linking in response to treatment with topical riboflavin and long-wavelength ultraviolet radiation (UVA). Investigative Ophthalmology & Visual Science 51(1):129-138 (2010).	☐
	018	OBATA et al. Deuterium magnetic resonance imaging of rabbit eye in Vivo. Magnetic Resonance in Medicine. 33(4):569-572 (1995).	☐
	019	OBATA et al. Deuterium MR in vivo imaging of the rat eye using 2H2O. Acta Radiologica, 36:552-555 (1995).	☐

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	020	PCT/US2015/037249 International Search Report and Written Opinion dated September 30, 2015.	☐
	021	RICHARD et al. Effects of sterilizing-grade filters on the physico-chemical properties of onion-like vesicles. International Journal of Pharmaceutics 312(1-2):144-150 (2006).	☐
	022	SIEGEL et al. Stability of procaine in deuterium oxide. Journal of Pharmaceutical Sciences 53:978-979 (1964).	☐
	023	TAKTAK et al. Assay of Pyrogens by Interleukin-6 Release from Monocytic Cell Lines. J. Pharm. Pharmacol. 43:578-582 (1991).	☐
	024	The U. S. Food and Drug Administration has provided regulatory guidance in the publication: Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing. available at: http://www.fda.gov/cder/guidance/5882fn1.htm (August 2003).	☐
	025	VIEGAS et al. Osmotic behavior of poloxamer 407 and other non-ionic surfactants in aqueous solutions. Int J Pharm 160:157-162 (1998).	☐

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				Examiner Name	Fay, Zohreh A
				Attorney Docket Number	46682-701.201
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U. S. PATENT DOCUMENTS					
Examiner Initials*	Cite No.	Document Number	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		Number-Kind Code ² (if known)			
	001	US-3863633	02-04-1975	RYDE; Emma Marta et al.	
	002	US-4014335	03-29-1977	ARNOLD; Randall K.	
	003	US-4474751	10-02-1984	HASLAM; John L. et al.	
	004	US-5259998	11-09-1993	REICH; Cary et al.	
	005	US-5716952	02-10-1998	WOLDEMUSSIE; Elizabeth et al.	
	006	US-5858375	01-12-1999	FURMINGER; Ian George Stephen et al.	
	007	US-5900360	05-04-1999	WELCH; William J. et al.	
	008	US-6218428	04-17-2001	CHYNN; Emil	
	009	US-6270954	08-07-2001	WELCH; William J. et al.	
	010	US-6410048	06-25-2002	FOTINOS; Spiros	
	011	US-6720001	04-13-2004	CHEN; Feng-Jing et al.	
	012	US-7691099	04-06-2010	BERRY; Michael J.	
	013	US-7858582	12-28-2010	JIN; Lei et al.	
	014	US-8333985	12-18-2012	KNAACK; David et al.	
	015	US-20030157187	08-21-2003	HUNTER; William L.	
	016	US-20050249770	11-10-2005	HUNTER; William L.	
	017	US-20060159771	07-20-2006	KADRMAS; Eddie F. et al.	
	018	US-20070207192	09-06-2007	HOLL; Richard et al.	

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	019	US-20070280992	12-06-2007	MARGARON; Philippe M.C. et al.	
	020	US-20080113035	05-15-2008	HUNTER; William L.	
	021	US-20080153900	06-26-2008	HUNTER; William L.	
	022	US-20100022495	01-28-2010	HOTAMISLIGIL; Gokhan S. et al.	
	023	US-20100196285	08-05-2010	BAYERL; Thomas	
	024	US-20120015035	01-19-2012	WILDSOET; Christine F. et al.	
	025	US-20120135084	05-31-2012	BAYERL; Thomas	
	026	US-20120203161	08-09-2012	HEREKAR; Satish V.	
	027	US-20130302855	11-14-2013	SELBER; Klaus et al.	
	028	US-20140011761	01-09-2014	HOTAMISLIGIL; Gokhan S. et al.	
	029	US-20140088199	03-27-2014	SHARMA; Anant	
	030	US-20140350049	11-27-2014	HOVNANIAN; Alain et al.	
	031	US-20150290125	10-15-2015	HORN; Gerald et al.	

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	001	CN-101327216-A	12-24-2008	ZHENGLIAN XIAO [CN]	Abstract:	☒
	002	CN-1456161-A	11-19-2003	LIU JIDONG [CN]	Abstract:	☒
	003	EP-0332826-A1	09-20-1989	TEVA PHARMA [IL]		☐
	004	WO-0049990-A2	08-31-2000	KHAMAR BAKULESH MAFATLAL [IN]		☐
	005	WO-02096418-A1	12-05-2002	VALLEY FORGE PHARMACEUTICALS [US], et al.		☐
	006	WO-2008005053-A1	01-10-2008	CYDEX INC [US], et al.		☐
	007	WO-2011098578-A2	08-18-2011	BIONEER AS [DK], et al.		☐
	008	WO-2012161655-A1	11-29-2012	SINGAPORE HEALTH SERVICE PTE LTD [SG], et al.		☐
	009	WO-2014140105-A1	09-18-2014	D2 BIOSCIENCE GROUP LTD., et al.		☐

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	003	CHENG et al. Water movement in the rabbit eye. Exp. Eye Res. 52:337-339 (1991).	☐

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Substitute for form 1449/PTO INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use as many sheets as necessary)				Complete if Known	
				Application Number	14726139
				Filing Date	05-29-2015
				First Named Inventor	OSTROW; Gregory I.
				Art Unit	1621
				Examiner Name	Fay, Zohreh A
				Attorney Docket Number	46682-701.201
Sheet	5	of	7		

NON-PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No. ¹	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T ²
	004	CHENG. Fate of water in the soft contact lens immediately after lens placement onto the cornea. Optometry and Vision Science 68(6):414-417 (1991).	☐
	005	CHIRIERI et al. Investigations concerning the changes induced by deuterium for hydrogen substitution in bioelectric activity of the frog retina. Physiologie 14(2):119-123 (1977).	☐
	006	Co-pending US patent application No. US14/859042, filed on 09-18-2015.	☐
	007	GANEA. Heavy water effect on certain energetic processes in retina. Physiologie 6(1):59-62 (1979).	☐
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				Filing Date	05-29-2015
				First Named Inventor	OSTROW; Gregory I.
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				Examiner Name	Fay, Zohreh A
				Attorney Docket Number	46682-701.201
Sheet	6	of	7		

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	012	JEONG et al. Drug release from biodegradable injectable thermosensitive hydrogel of PEG-PLGA-PEG triblock copolymers. J Control Release 63(1-2):155-163 (2000).	☐
	013	JEONG et al. Thermosensitive sol-gel reversible hydrogels. Advanced Drug Delivery Reviews 54:37-51 (2002).	☐
	014	KREZEL et al. A formula for correlating pKa values determined in D2O and H2O. Journal of Inorganic Chemistry 98:161-166 (2008).	☐
	015	LAI et al. Rapid transport of large polymeric nanoparticles in fresh undiluted human mucus. PNAS USA 104(5):1482-1487 (2007).	☐
	016	LUND et al. The Kinetics of atropine and apotatropine in aqueous solutions. ACTA Chemica Scandinavica 22:3085-3097 (1968).	☐
	017	MCCALL et al. Mechanisms of corneal tissue cross-linking in response to treatment with topical riboflavin and long-wavelength ultraviolet radiation (UVA). Investigative Ophthalmology & Visual Science 51(1):129-138 (2010).	☐
	018	OBATA et al. Deuterium magnetic resonance imaging of rabbit eye in Vivo. Magnetic Resonance in Medicine. 33(4):569-572 (1995).	☐
	019	OBATA et al. Deuterium MR in vivo imaging of the rat eye using 2H2O. Acta Radiologica, 36:552-555 (1995).	☐

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				Attorney Docket Number	46682-701.201
Sheet	7	of	7		

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	020	PCT/US2015/037249 International Search Report and Written Opinion dated September 30, 2015.	☐
	021	RICHARD et al. Effects of sterilizing-grade filters on the physico-chemical properties of onion-like vesicles. International Journal of Pharmaceutics 312(1-2):144-150 (2006).	☐
	022	SIEGEL et al. Stability of procaine in deuterium oxide. Journal of Pharmaceutical Sciences 53:978-979 (1964).	☐
	023	TAKTAK et al. Assay of Pyrogens by Interleukin-6 Release from Monocytic Cell Lines. J. Pharm. Pharmacol. 43:578-582 (1991).	☐
	024	The U. S. Food and Drug Administration has provided regulatory guidance in the publication: Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing. available at: http://www.fda.gov/cder/guidance/5882fn1.htm (August 2003).	☐
	025	VIEGAS et al. Osmotic behavior of poloxamer 407 and other non-ionic surfactants in aqueous solutions. Int J Pharm 160:157-162 (1998).	☐

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Bibliographic data

Title: Atropine sulfate in situ forming eye gel
Pub/Pat no: CN101327216A
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Inventor(s): XIAO ZHENGLIAN
Applicant(s): ZHENGLIAN XIAO[CN]
Classification: A61K31/46AI; A61K9/00AI; A61K47/34AI; A61P27/02AI
Application number: CN20071111597 2007-06-22
Priority number: CN20071111597 2007-06-22;

Abstract of CN101327216A

The invention discloses an instant atropine sulphate eye gel and a preparation method thereof. According to the invention, 1000ml of the instant atropine sulphate eye gel contains 1g to 20g of atropine sulphate, 0.05g to 1g of preservative, 1.0g to 7.5g of osmotic pressure adjusting agent, and 30g to 150g of gel stroma, and the rest includes acid-alkali buffer and water for injection. The invention, through the optimization of accessories and the improvement of the technique, enriches the pharmaceutical dosage form of the atropine sulphate, greatly prolongs the time for which the medicine stays in the eyes, and not only improves the curative effect and shortens the time for reaching the function peak, but also reduces the amount of the medicine flowing into and absorbed by dacryocysts and relieves the side effect and toxic reaction of the atropine sulphate.

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权利要求书 1 页 说明书 14 页

[54] 发明名称

一种硫酸阿托品眼用即型凝胶

[57] 摘要

本发明公开了一种硫酸阿托品眼用即型凝胶及其制备方法, 本发明的 1000 毫升硫酸阿托品眼用即型凝胶中含有: 硫酸阿托品 1-20 克, 防腐剂 0.05-1 克, 渗透压调节剂 1.0-7.5 克, 凝胶基质 30-150 克, 余量为酸碱缓冲剂和注射用水。 本发明通过辅料的优化和工艺的改进, 丰富了硫酸阿托品的药物剂型, 大大延长了药物在眼部滞留时间, 不仅改善了疗效, 缩短达到作用峰值的时间, 并且可以减少药物流入泪囊吸收, 减轻阿托品的副作用和中毒反应。

1. 一种硫酸阿托品眼用即型凝胶，其特征在于 1000 毫升所述凝胶中含有：硫酸阿托品 1—20 克，防腐剂 0.05—1 克，渗透压调节剂 1.0—7.5 克，凝胶基质 30—150 克，余量为酸碱缓冲剂和注射用水。
2. 根据权利要求 1 所述硫酸阿托品眼用即型凝胶，其特征在于 1000 毫升所述凝胶中含有：硫酸阿托品 1—5 克，防腐剂 0.05—1 克，渗透压调节剂 1.0—5.5 克，凝胶基质 30—100 克，余量为酸碱缓冲剂和注射用水。
3. 根据权利要求 2 所述硫酸阿托品眼用即型凝胶，其特征在于，所述酸碱缓冲剂将所述硫酸阿托品眼用即型凝胶的 pH 值控制在 4.35—8.89 之间。
4. 根据权利要求 1—3 任一所述硫酸阿托品眼用即型凝胶，其特征在于，所述防腐剂选自苯乙醇、苯甲醇、苯甲酸钠、羟苯甲酯和羟苯乙酯中的一种或多种；所述渗透压调节剂为氯化钠；所述酸碱缓冲剂选自盐酸、硫酸、硼酸、氢氧化钠、氢氧化钾、柠檬酸钠、柠檬酸、三乙醇胺、二乙醇胺、乙醇胺中的 2 种或多种；所述凝胶基质选自聚乙二醇、甲基纤维素、乙基纤维素、聚丙烯酸和聚丙烯酸钠中的一种或多种。
5. 根据权利要求 4 所述硫酸阿托品眼用即型凝胶，其特征在于所述酸碱缓冲剂选自盐酸和三乙醇胺，二者的摩尔比为 1:0.5—2。
6. 根据权利要求 4 所述硫酸阿托品眼用即型凝胶，其特征在于所述酸碱缓冲剂选自盐酸和柠檬酸钠，二者的摩尔比为 1:0.2—2。
7. 根据权利要求 4 所述硫酸阿托品眼用即型凝胶，其特征在于所述凝胶基质选自聚乙二醇和甲基纤维素，二者的重量比为 1:50—5000。
8. 根据权利要求 7 所述硫酸阿托品眼用即型凝胶，其特征在于所述聚乙二醇选自 PEG-400、PEG-600、PEG-1000、PEG-1500、PEG-4000、PEG-6000、PEG-10000 和 PEG-20000 中的一种或多种。
9. 根据权利要求 8 所述硫酸阿托品眼用即型凝胶，其特征在于所述聚乙二醇选自 PEG-1500、PEG-4000 和 PEG-6000 中的一种或多种。

一种硫酸阿托品眼用即型凝胶

【技术领域】

本发明属于化学药物技术领域，具体涉及一种硫酸阿托品眼用即型凝胶。

【背景技术】

眼用即型凝胶也叫眼用在位凝胶或眼用原位凝胶，其制剂在常温放置时为液态，当滴入眼内后，由于温度、pH 值以及离子强度的变化转化为凝胶状，能够在较长时间内滞留在眼内，从而达到延长治疗时间，增强治疗效果减少药物损失的作用。

眼用即型凝胶目前而言还不是一种非常成熟的药物制剂，依赖温度、pH 值以及离子强度等原理的眼用即型凝胶在商业化上还尚未成熟，这是因为该剂型除了需要精细的工艺，适当的辅料，还需要眼用即型凝胶中药物与工艺和辅料的恰当匹配，否则就不能达到常温放置为液态，滴入眼内后转化为凝胶状，这个领域工作仍然需要大量的基础研究，目前只有美国食品药品监督管理局批准了一种马来酸噻吗洛尔凝胶 (Timolol Malaeate Ophthalmic Gel forming Solution) 上市，属于离子强度敏感型眼用即型凝胶，而其他眼科用药尚无眼内在位凝胶面世。

专利文献 CN1377706A 公开了一种具有适宜相转变温度的眼用原位凝胶制剂，它融合利用不同型号泊洛沙姆的组合，制备了含有药物和水溶性高分子辅料的眼用原位凝胶制剂，制剂中含有泊洛沙姆 407 和泊洛沙姆 188，还可加入浓度小于 3% 的水溶性高分子辅料，该原位凝胶具有适宜的相转变温度，能够在室温条件下以液体状态给药并在活体角膜表面形成凝胶。但是该专利使用泊洛沙姆 407 和 188 是非常昂贵的药用辅料，该发明的实施过程也比较复杂，因而还需要进一步研究开发新的眼用即型凝胶的工艺方法。

硫酸阿托品滴眼液可阻断乙酰胆碱的作用，使瞳孔括约肌和睫状肌麻痹而引起散瞳及调节麻痹，临床用于角膜炎、虹膜睫状体炎、白内障手术前后及验光等。

硫酸阿托品滴眼液是一种常用的眼用制剂，为典型的乙酰胆碱受体阻滞剂，能阻断副交感神经对睫状肌和瞳孔括约肌的兴奋作用，使睫状肌麻痹和瞳孔扩大，它是最强的睫状肌麻痹剂，能完全消除调节紧张，所以，阿托品散瞳验光会得到准确的结果。但是临床上存在滴入眼内的眼药水损失大，滞留时间短，达到作用峰值的时间过长等问题。而且 1% 的硫酸阿托品滴眼液局部点眼，易流入泪囊吸收，可造成

全身皮肤潮红、皮疹、红斑、口干、心悸等副作用，严重者可出现中毒症状，特别是儿童对此药易中毒。将眼药水改为即型凝胶，因为药物的流动性降低，大大延长了药物在眼部滞留时间，不仅改善了疗效，缩短达到作用峰值的时间，并且可以减少药物流入泪囊吸收，减轻阿托品的副作用和中毒反应。

【发明内容】

本发明的目的在于提供一种硫酸阿托品眼用即型凝胶。

本发明的另一目的在于提供硫酸阿托品眼用即型凝胶的制备方法。

具体地说，本发明所述 1000 毫升硫酸阿托品眼用即型凝胶含有：硫酸阿托品 1—20 克，防腐剂 0.05—1 克，渗透压调节剂 1.0—7.5 克，凝胶基质 30—150 克，余量为酸碱缓冲剂和注射用水。

上述 1000 毫升凝胶中含有：硫酸阿托品 1—5 克，防腐剂 0.05—1 克，渗透压调节剂 1.0—5.5 克，凝胶基质 30—100 克，余量为酸碱缓冲剂和注射用水。

上述酸碱缓冲剂将所述硫酸阿托品眼用即型凝胶的 pH 值控制在 4.35—8.89 之间。

上述防腐剂选自苯乙醇、苯甲醇、苯甲酸钠、羟苯甲酯和羟苯乙酯中的一种或多种；所述渗透压调节剂为氯化钠；所述酸碱缓冲剂选自盐酸、硫酸、硼酸、氢氧化钠、氢氧化钾、柠檬酸钠、柠檬酸、三乙醇胺、二乙醇胺、乙醇胺中的 2 种或多种；所述凝胶基质选自聚乙二醇、甲基纤维素、乙基纤维素、聚丙烯酸和聚丙烯酸钠中的一种或多种。

上述酸碱缓冲剂选自盐酸和三乙醇胺，二者的摩尔比为 1:0.5—2。

上述酸碱缓冲剂选自盐酸和柠檬酸钠，二者的摩尔比为 1:0.2—2。

上述凝胶基质选自聚乙二醇和甲基纤维素，二者的重量比为 1:50—5000。

上述聚乙二醇选自 PEG-400、PEG-600、PEG-1000、PEG-1500、PEG-4000、PEG-6000、PEG-10000 和 PEG-20000 中的一种或多种。

上述聚乙二醇选自 PEG-1500、PEG-4000 和 PEG-6000 中的一种或多种。

本发明的硫酸阿托品眼用即型凝胶的制备方法为：将注射用水加热煮沸，冷却至 80℃，加入硫酸阿托品，防腐剂溶解于乙醇中，边搅拌边滴加入以上溶液内，并加入酸碱缓冲剂后搅拌，过微孔滤膜滤过，滤液再加热至 40℃，趁热加入渗透压调节剂和凝胶基质，搅拌至室温，再加注射用水补足重量至 1000 毫升，搅拌使其形

成均匀的凝胶，无菌分装即得。

眼用即型凝胶分为三种：温度敏感型、pH 值敏感型以及离子强度敏感型，其中温度敏感型眼用即型凝胶相对易于工业化，本发明也是通过温度敏感型眼用即型凝胶实现的，但是经过发明人的实验摸索发现，温度敏感型凝胶的稳定性以及能否在人眼球的局部温度下即刻转化为凝胶状与即型凝胶的 pH 值是否稳定关系密切。发明人采用较大容量的酸碱缓冲剂确保眼用即型凝胶的 pH 值的稳定，从而保证了凝胶具有敏感和适宜的相转化温度。

本发明通过辅料的优化和工艺的改进，丰富了硫酸阿托品的药物剂型，大大延长了药物在眼部滞留时间，不仅改善了疗效，缩短达到作用峰值的时间，并且可以减少药物流入泪囊吸收，减轻阿托品的副作用和中毒反应。

【具体实施方式】

下面实施例和实验例进一步描述本发明，但所述实施例和实验例仅用于说明本发明而不是限制本发明。发明中甲基纤维素中甲氧基含量为 27-32%。

实施例 1

处方：硫酸阿托品 5 克 盐酸（0.05mol/L）400 毫升
柠檬酸钠 8.5 克 甲基纤维素 5.0 克 苯乙醇 0.5 克
乙醇 5 毫升 氯化钠 1.0 克 PEG-1000 20 克
PEG-400 5 克 注射用水 适量

制法：将注射用水加热煮沸，冷却至 80℃，加入硫酸阿托品，苯乙醇溶解于乙醇中，边搅拌边滴加入以上溶液内，并加入柠檬酸钠，搅拌 30 分钟以上，再加入盐酸后搅拌，过微孔滤膜（0.22 μm）滤过，滤液再加热至 40℃，趁热加入氯化钠、甲基纤维素、PEG-1000 和 PEG-400，搅拌至室温（18-25℃），再加注射用水补足重量至 1000 毫升，搅拌使其形成均匀的凝胶，无菌分装即得。

实施例 2

处方：硫酸阿托品 10 克 硫酸（0.05mol/L）200 毫升
柠檬酸钠 8.6 克 甲基纤维素 5.0 克 苯甲醇 0.5 克
乙醇 5 毫升 氯化钠 5.0 克 PEG-1000 70 克
注射用水 适量

制法：将注射用水加热煮沸，冷却至 80℃，加入硫酸阿托品，苯甲醇溶解于乙醇中，边搅拌边滴加入以上溶液内，并加入柠檬酸钠，搅拌 30 分钟以上，再加入硫酸后

搅拌，过微孔滤膜（0.22 μm）滤过，滤液再加热至 40℃，趁热加入氯化钠、甲基纤维素和 PEG-1000，搅拌至室温（18-25℃），再加注射用水补足重量至 1000 毫升，搅拌使其形成均匀的凝胶，无菌分装即得。

实施例 3

处方：硫酸阿托品 1 克 氢氧化钠（0.1mol/L）200 毫升

硼酸 5.6 克 甲基纤维素 5.0 克 苯甲酸钠 0.5 克

乙醇 5 毫升 氯化钠 25.0 克 PEG-4000 100 克

注射用水 适量

制法：将注射用水加热煮沸，冷却至 80℃，加入硫酸阿托品，并加入柠檬酸钠、氢氧化钠和苯甲酸钠，搅拌 30 分钟以上，再加入硼酸后搅拌，过微孔滤膜（0.22 μm）滤过，滤液再加热至 40℃，趁热加入 PEG-4000 和甲基纤维素，搅拌至室温（18-25℃），再加注射用水补足重量至 1000 毫升，搅拌使其形成均匀的凝胶，无菌分装即得。

实施例 4

处方：硫酸阿托品 5 克 氢氧化钾（0.1mol/L）200 毫升

硼酸 5.6 克 甲基纤维素 5.0 克 羟苯甲酯 0.5 克

乙醇 5 毫升 氯化钠 5.0 克 PEG-6000 100 克

注射用水 适量

制法：将注射用水加热煮沸，冷却至 80℃，加入硫酸阿托品，羟苯甲酯溶解于乙醇中，边搅拌边滴加入以上溶液内，并加入硼酸和氢氧化钾，搅拌 30 分钟以上，过微孔滤膜（0.22 μm）滤过，滤液再加热至 40℃，趁热加入 PEG-6000 和甲基纤维素，搅拌至室温（18-25℃），再加注射用水补足重量至 1000 毫升，搅拌使其形成均匀的凝胶，无菌分装即得。

实施例 5

处方：硫酸阿托品 2 克 氢氧化钾（0.1mol/L）200 毫升

柠檬酸 14.0 克 甲基纤维素 5.0 克 羟苯乙酯 0.5 克

乙醇 5 毫升 氯化钠 5.0 克 PEG-6000 50 克

PEG-20000 5 克 注射用水 适量

制法：将注射用水加热煮沸，冷却至 80℃，加入硫酸阿托品，羟苯乙酯溶解于乙醇中，边搅拌边滴加入以上溶液内，并加入柠檬酸和氢氧化钾，搅拌 30 分钟以上，

过微孔滤膜(0.22 μm)滤过,滤液再加热至 40℃,趁热加入 PEG-20000、PEG-6000 和甲基纤维素,搅拌至室温(18-25℃),再加注射用水补足重量至 1000 毫升,搅拌使其形成均匀的凝胶,无菌分装即得。

实施例 6

处方: 硫酸阿托品 1 克 盐酸(0.05mol/L) 250 毫升
 三乙醇胺 30 克 甲基纤维素 5.0 克 苯乙醇 0.5 克
 乙醇 5 毫升 氯化钠 1.0 克 PEG-1500 35 克
 PEG-400 5 克 注射用水 适量

制法: 将注射用水加热煮沸,冷却至 80℃,加入硫酸阿托品,苯乙醇溶解于乙醇中,边搅拌边滴加入以上溶液内,并加入三乙醇胺,搅拌 30 分钟以上,再加入盐酸后搅拌,过微孔滤膜(0.22 μm)滤过,滤液再加热至 40℃,趁热加入 PEG-400、PEG-1500 和甲基纤维素,搅拌至室温(18-25℃),再加注射用水补足重量至 1000 毫升,搅拌使其形成均匀的凝胶,无菌分装即得。

实施例 7

处方: 硫酸阿托品 3 克 盐酸(0.05mol/L) 400 毫升
 二乙醇胺 38 克 甲基纤维素 5.0 克 苯乙醇 0.5 克
 乙醇 5 毫升 氯化钠 1.0 克 PEG-1000 20 克
 PEG-400 5 克 注射用水 适量

制法: 将注射用水加热煮沸,冷却至 80℃,加入硫酸阿托品,苯乙醇溶解于乙醇中,边搅拌边滴加入以上溶液内,并加入二乙醇胺,搅拌 30 分钟以上,再加入盐酸后搅拌,过微孔滤膜(0.22 μm)滤过,滤液再加热至 40℃,趁热加入 PEG-400、PEG-1500 和甲基纤维素,搅拌至室温(18-25℃),再加注射用水补足重量至 1000 毫升,搅拌使其形成均匀的凝胶,无菌分装即得。

实施例 8

处方: 硫酸阿托品 6 克 盐酸(0.05mol/L) 400 毫升
 乙醇胺 41 克 甲基纤维素 5.0 克 苯乙醇 0.5 克
 乙醇 5 毫升 氯化钠 1.0 克 PEG-1000 20 克
 PEG-400 5 克 注射用水 适量

制法: 将注射用水加热煮沸,冷却至 80℃,加入硫酸阿托品,苯乙醇溶解于乙醇中,边搅拌边滴加入以上溶液内,并加入乙醇胺,搅拌 30 分钟以上,再加入盐酸后搅

拌, 过微孔滤膜 ($0.22\ \mu\text{m}$) 滤过, 滤液再加热至 40°C , 趁热加入 PEG-400、PEG-1000 和甲基纤维素, 搅拌至室温 ($18-25^{\circ}\text{C}$), 再加注射用水补足重量至 1000 毫升, 搅拌使其形成均匀的凝胶, 无菌分装即得。

实施例 9

处方: 硫酸阿托品 10 克 盐酸 (0.05mol/L) 400 毫升

柠檬酸钠	8.5 克	甲基纤维素	5.0 克	苯乙醇	0.5 克
乙醇	5 毫升	氯化钠	1.0 克	PEG-1000	20 克
PEG-400	5 克	注射用水	适量		

制法: 将注射用水加热煮沸, 冷却至 80°C , 加入硫酸阿托品, 苯乙醇溶解于乙醇中, 边搅拌边滴加入以上溶液内, 并加入柠檬酸钠, 搅拌 30 分钟以上, 再加入盐酸后搅拌, 过微孔滤膜 ($0.22\ \mu\text{m}$) 滤过, 滤液再加热至 40°C , 趁热加入氯化钠、甲基纤维素、PEG-1000 和 PEG-400, 搅拌至室温 ($18-25^{\circ}\text{C}$), 再加注射用水补足重量至 1000 毫升, 搅拌使其形成均匀的凝胶, 无菌分装即得。

实施例 10

处方: 硫酸阿托品 10 克 硫酸 (0.05mol/L) 200 毫升

柠檬酸钠	8.5 克	甲基纤维素	5.0 克	苯甲醇	0.5 克
乙醇	5 毫升	氯化钠	5.0 克	PEG-1000	70 克
注射用水	适量				

制法: 将注射用水加热煮沸, 冷却至 80°C , 加入硫酸阿托品, 苯甲醇溶解于乙醇中, 边搅拌边滴加入以上溶液内, 并加入柠檬酸钠, 搅拌 30 分钟以上, 再加入硫酸后搅拌, 过微孔滤膜 ($0.22\ \mu\text{m}$) 滤过, 滤液再加热至 40°C , 趁热加入氯化钠、甲基纤维素和 PEG-1000, 搅拌至室温 ($18-25^{\circ}\text{C}$), 再加注射用水补足重量至 1000 毫升, 搅拌使其形成均匀的凝胶, 无菌分装即得。

实施例 11

处方: 硫酸阿托品 2 克 氢氧化钠 (0.1mol/L) 200 毫升

硼酸	5.6 克	甲基纤维素	5.0 克	苯甲酸钠	0.5 克
乙醇	5 毫升	氯化钠	5.0 克	PEG-4000	100 克
注射用水	适量				

制法: 将注射用水加热煮沸, 冷却至 80°C , 加入硫酸阿托品, 苯甲酸钠溶解于乙醇中, 边搅拌边滴加入以上溶液内, 并加入柠檬酸钠、氢氧化钠和苯甲酸钠, 搅拌 30

分钟以上，再加入硼酸后搅拌，过微孔滤膜（0.22 μm）滤过，滤液再加热至 40℃，趁热加入 PEG-4000 和甲基纤维素，搅拌至室温（18-25℃），再加注射用水补足重量至 1000 毫升，搅拌使其形成均匀的凝胶，无菌分装即得。

实施例 12

处方：硫酸阿托品 2 克 氢氧化钾（0.1mol/L）200 毫升

硼酸 5.6 克 甲基纤维素 5.0 克 羟苯甲酯 0.5 克

乙醇 5 毫升 氯化钠 5.0 克 PEG-6000 100 克

注射用水 适量

制法：将注射用水加热煮沸，冷却至 80℃，加入硫酸阿托品，羟苯甲酯溶解于乙醇中，边搅拌边滴加入以上溶液内，并加入硼酸和氢氧化钾，搅拌 30 分钟以上，过微孔滤膜（0.22 μm）滤过，滤液再加热至 40℃，趁热加入 PEG-6000 和甲基纤维素，搅拌至室温（18-25℃），再加注射用水补足重量至 1000 毫升，搅拌使其形成均匀的凝胶，无菌分装即得。

实施例 13

处方：硫酸阿托品 1 克 氢氧化钾（0.1mol/L）200 毫升

柠檬酸 14.0 克 甲基纤维素 5.0 克 羟苯乙酯 0.5 克

乙醇 5 毫升 氯化钠 5.0 克 PEG-6000 50 克

PEG-20000 5 克 注射用水 适量

制法：将注射用水加热煮沸，冷却至 80℃，加入硫酸阿托品，羟苯乙酯溶解于乙醇中，边搅拌边滴加入以上溶液内，并加入柠檬酸和氢氧化钾，搅拌 30 分钟以上，过微孔滤膜（0.22 μm）滤过，滤液再加热至 40℃，趁热加入 PEG-20000、PEG-6000 和甲基纤维素，搅拌至室温（18-25℃），再加注射用水补足重量至 1000 毫升，搅拌使其形成均匀的凝胶，无菌分装即得。

实施例 14

处方：硫酸阿托品 1 克 盐酸（0.05mol/L）250 毫升

三乙醇胺 30 克 甲基纤维素 5.0 克 苯乙醇 0.5 克

乙醇 5 毫升 氯化钠 1.0 克 PEG-1500 35 克

PEG-400 5 克 注射用水 适量

制法：将注射用水加热煮沸，冷却至 80℃，加入硫酸阿托品，苯乙醇溶解于乙醇中，边搅拌边滴加入以上溶液内，并加入三乙醇胺，搅拌 30 分钟以上，再加入盐酸后

搅拌,过微孔滤膜(0.22 μ m)滤过,滤液再加热至 40℃,趁热加入 PEG-400、PEG-1500 和甲基纤维素,搅拌至室温(18-25℃),再加注射用水补足重量至 1000 毫升,搅拌使其形成均匀的凝胶,无菌分装即得。

实施例 15

处方: 硫酸阿托品 1 克 盐酸(0.05mol/L) 400 毫升

二乙醇胺	38 克	甲基纤维素	5.0 克	苯乙醇	0.5 克
乙醇	5 毫升	氯化钠	1.0 克	PEG-1000	20 克
PEG-400	5 克	注射用水	适量		

制法: 将注射用水加热煮沸,冷却至 80℃,加入硫酸阿托品,苯乙醇溶解于乙醇中,边搅拌边滴加入以上溶液内,并加入二乙醇胺,搅拌 30 分钟以上,再加入盐酸后搅拌,过微孔滤膜(0.22 μ m)滤过,滤液再加热至 40℃,趁热加入 PEG-400、PEG-1500 和甲基纤维素,搅拌至室温(18-25℃),再加注射用水补足重量至 1000 毫升,搅拌使其形成均匀的凝胶,无菌分装即得。

实施例 16

处方: 硫酸阿托品 2 克 盐酸(0.05mol/L) 400 毫升

乙醇胺	41 克	甲基纤维素	5.0 克	苯乙醇	0.5 克
乙醇	5 毫升	氯化钠	1.0 克	PEG-1000	20 克
PEG-400	5 克	注射用水	适量		

制法: 将注射用水加热煮沸,冷却至 80℃,加入硫酸阿托品,苯乙醇溶解于乙醇中,边搅拌边滴加入以上溶液内,并加入乙醇胺,搅拌 30 分钟以上,再加入盐酸后搅拌,过微孔滤膜(0.22 μ m)滤过,滤液再加热至 40℃,趁热加入 PEG-400、PEG-1000 和甲基纤维素,搅拌至室温(18-25℃),再加注射用水补足重量至 1000 毫升,搅拌使其形成均匀的凝胶,无菌分装即得。

实施例 17

处方: 硫酸阿托品 3 克 盐酸(0.05mol/L) 400 毫升

柠檬酸钠	8.5 克	甲基纤维素	5.0 克	苯乙醇	0.5 克
乙醇	5 毫升	氯化钠	1.0 克	PEG-1000	20 克
PEG-400	5 克	注射用水	适量		

制法: 将注射用水加热煮沸,冷却至 80℃,加入硫酸阿托品,苯乙醇溶解于乙醇中,边搅拌边滴加入以上溶液内,并加入柠檬酸钠,搅拌 30 分钟以上,再加入盐酸后

搅拌，过微孔滤膜（0.22 μm）滤过，滤液再加热至 40℃，趁热加入氯化钠、甲基纤维素、PEG-1000 和 PEG-400，搅拌至室温（18-25℃），再加注射用水补足重量至 1000 毫升，搅拌使其形成均匀的凝胶，无菌分装即得。

实施例 18

处方：硫酸阿托品 10 克 硫酸（0.05mol/L）200 毫升

柠檬酸钠 8.5 克 甲基纤维素 5.0 克 苯甲醇 0.5 克

乙醇 5 毫升 氯化钠 5.0 克 PEG-1000 70 克

注射用水 适量

制法：将注射用水加热煮沸，冷却至 80℃，加入硫酸阿托品，苯甲醇溶解于乙醇中，边搅拌边滴加入以上溶液内，并加入柠檬酸钠，搅拌 30 分钟以上，再加入硫酸后搅拌，过微孔滤膜（0.22 μm）滤过，滤液再加热至 40℃，趁热加入氯化钠、甲基纤维素和 PEG-1000，搅拌至室温（18-25℃），再加注射用水补足重量至 1000 毫升，搅拌使其形成均匀的凝胶，无菌分装即得。

实施例 19

处方：硫酸阿托品 10 克 氢氧化钠（0.1mol/L）200 毫升

硼酸 5.6 克 甲基纤维素 5.0 克 苯甲酸钠 0.5 克

乙醇 5 毫升 氯化钠 5.0 克 PEG-4000 100 克

注射用水 适量

制法：将注射用水加热煮沸，冷却至 80℃，加入硫酸阿托品，并加入柠檬酸钠、氢氧化钠和苯甲酸钠，搅拌 30 分钟以上，再加入硼酸后搅拌，过微孔滤膜（0.22 μm）滤过，滤液再加热至 40℃，趁热加入 PEG-4000 和甲基纤维素，搅拌至室温（18-25℃），再加注射用水补足重量至 1000 毫升，搅拌使其形成均匀的凝胶，无菌分装即得。

实施例 20

处方：硫酸阿托品 10 克 氢氧化钾（0.1mol/L）200 毫升

硼酸 5.6 克 甲基纤维素 5.0 克 羟苯甲酯 0.5 克

乙醇 5 毫升 氯化钠 5.0 克 PEG-6000 100 克

注射用水 适量

制法：将注射用水加热煮沸，冷却至 80℃，加入硫酸阿托品，羟苯甲酯溶解于乙醇中，边搅拌边滴加入以上溶液内，并加入硼酸和氢氧化钾，搅拌 30 分钟以上，过

微孔滤膜 (0.22 μm) 滤过, 滤液再加热至 40℃, 趁热加入 PEG-6000 和甲基纤维素, 搅拌至室温 (18-25℃), 再加注射用水补足重量至 1000 毫升, 搅拌使其形成均匀的凝胶, 无菌分装即得。

实施例 21

处方: 硫酸阿托品 10 克 氢氧化钾 (0.1mol/L) 200 毫升
 柠檬酸 14.0 克 甲基纤维素 5.0 克 羟苯乙酯 0.5 克
 乙醇 5 毫升 氯化钠 5.0 克 PEG-6000 50 克
 PEG-20000 5 克 注射用水 适量

制法: 将注射用水加热煮沸, 冷却至 80℃, 加入硫酸阿托品, 羟苯乙酯溶解于乙醇中, 边搅拌边滴加入以上溶液内, 并加入柠檬酸和氢氧化钾, 搅拌 30 分钟以上, 过微孔滤膜 (0.22 μm) 滤过, 滤液再加热至 40℃, 趁热加入 PEG-20000、PEG-6000 和甲基纤维素, 搅拌至室温 (18-25℃), 再加注射用水补足重量至 1000 毫升, 搅拌使其形成均匀的凝胶, 无菌分装即得。

实施例 22

处方: 硫酸阿托品 10 克 盐酸 (0.05mol/L) 250 毫升
 三乙醇胺 30 克 甲基纤维素 5.0 克 苯乙醇 0.5 克
 乙醇 5 毫升 氯化钠 1.0 克 PEG-1500 35 克
 PEG-400 5 克 注射用水 适量

制法: 将注射用水加热煮沸, 冷却至 80℃, 加入硫酸阿托品, 苯乙醇溶解于乙醇中, 边搅拌边滴加入以上溶液内, 并加入三乙醇胺, 搅拌 30 分钟以上, 再加入盐酸后搅拌, 过微孔滤膜 (0.22 μm) 滤过, 滤液再加热至 40℃, 趁热加入 PEG-400、PEG-1500 和甲基纤维素, 搅拌至室温 (18-25℃), 再加注射用水补足重量至 1000 毫升, 搅拌使其形成均匀的凝胶, 无菌分装即得。

实施例 23

处方: 硫酸阿托品 10 克 盐酸 (0.05mol/L) 400 毫升
 二乙醇胺 38 克 甲基纤维素 5.0 克 苯乙醇 0.5 克
 乙醇 5 毫升 氯化钠 1.0 克 PEG-1000 20 克
 PEG-400 5 克 注射用水 适量

制法: 将注射用水加热煮沸, 冷却至 80℃, 加入硫酸阿托品, 苯乙醇溶解于乙醇中, 边搅拌边滴加入以上溶液内, 并加入二乙醇胺, 搅拌 30 分钟以上, 再加入盐酸后

搅拌,过微孔滤膜(0.22 μm)滤过,滤液再加热至 40℃,趁热加入 PEG-400、PEG-1500 和甲基纤维素,搅拌至室温 (18-25℃),再加注射用水补足重量至 1000 毫升,搅拌使其形成均匀的凝胶,无菌分装即得。

实施例 24

处方: 硫酸阿托品 10 克 盐酸 (0.05mol/L) 400 毫升

乙醇胺	41 克	甲基纤维素	5.0 克	苯乙醇	0.5 克
乙醇	5 毫升	氯化钠	1.0 克	PEG-10000	10 克
PEG-400	5 克	注射用水	适量		

制法: 将注射用水加热煮沸,冷却至 80℃,加入硫酸阿托品,苯乙醇溶解于乙醇中,边搅拌边滴加入以上溶液内,并加入乙醇胺,搅拌 30 分钟以上,再加入盐酸后搅拌,过微孔滤膜(0.22 μm)滤过,滤液再加热至 40℃,趁热加入 PEG-400、PEG-10000 和甲基纤维素,搅拌至室温 (18-25℃),再加注射用水补足重量至 1000 毫升,搅拌使其形成均匀的凝胶,无菌分装即得。

实验例 1-本发明凝胶的 pH 值测定

1 方法提要

本方法以玻璃电极作指示电极,以饱和甘汞电极作参比电极,以 pH4、7 或 9 标准缓冲液定位,测定凝胶的 pH 值。

2 仪器

2.1 酸度计: 测量范围 0~14pH; 读数精度 $\leq 0.02\text{pH}$ 。

2.2 pH 玻璃电极, 等电位点在 pH7 左右。

2.3 饱和甘汞电极。

2.4 温度计: 测量范围 0~100℃。

2.5 塑料杯: 50 毫升。

2.6 带线性回归方程的科学计算器。

3 试剂

3.1 pH4 标准缓冲液

准确称取 10.21 克邻苯二甲酸氢钾(KHC₈H₂O₄),溶于试剂水并定容至 1L。由于此溶液稀释效应小,称量前不必干燥。此溶液放置几周后会发霉,加入少许微溶性酚或其化合物(如百里酚)作防霉剂即可防止此现象发生。

3.2 pH7 标准缓冲液

分别准确称取 3.5 克经 $120 \pm 10^\circ\text{C}$ 干燥 2h 并冷却至室温的优级纯无水磷酸氢二钠 (Na_2HPO_4), 及 3.40 克优级纯磷酸二氢钾 (KH_2PO_4), 一起溶于试剂水并定容至 1L。配好的溶液应避免被大气中的二氧化碳沾污。6 周后应重新制备。

3.3 pH9 标准缓冲液

准确称取 3.81 克优级纯硼砂 ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$), 溶于无二氧化碳的试剂水并定容至 1L。配好的溶液应尽可能避免与大气中的二氧化碳接触。四周后应重新制备。

上述标准缓冲液在不同温度条件下的 pH 值如表 1 所示。

表 1 标准缓冲液在不同温度下的 pH 值

4 分析步骤

4.1 电极的准备

4.1.1 新玻璃电极或久置不用的玻璃电极, 应预先置于 pH4 标准缓冲液中浸泡一昼夜。使用完毕, 亦应放在上述缓冲液中浸泡, 不要放在试剂水中长期浸泡。使用中若发现有油渍污染, 最好放在 0.1mol/L 盐酸, 0.1mol/L 氢氧化钠, 0.1mol/L 盐酸中循环浸泡各 5 分钟。用试剂水洗净后, 再在 pH4 缓冲液中浸泡。

4.1.2 饱和氯化钾电极使用前最好浸泡在饱和氯化钾溶液稀释 10 倍的稀溶液中。贮存时把上端的注入口塞紧, 使用时则启开。应经常注意从注入口注入氯化钾饱和溶液至一定液位。

4.2 仪器校正

仪器开启半小时后, 按仪器说明书的规定, 进行调零、温度补偿和满刻度校正等操作步骤。

4.3 pH 定位

根据具体情况, 选择下列一种方法定位。

4.3.1 单点定位

选用一种 pH 值与被测凝胶相接近的标准缓冲液。定位前先用试剂水冲洗电极及塑料杯 2 次以上。然后用干净滤纸将电极底部水滴轻轻地吸干 (勿用滤纸去擦拭, 以免电极底部带静电导致读数不稳定)。将定位缓冲液倒入塑料杯内, 浸入电极, 稍摇动塑料杯数秒钟。测量凝胶温度 (要求与定位缓冲液温度一致), 查出该温度下定位缓冲液的 pH 值, 将仪器定位至该 pH 值。重复调零、校正及定位 1~2 次, 直

至稳定为止。

4.3.2 两次定位

先取 pH7 标准缓冲液依上法定位。电极洗干净后，将另一定位标准缓冲液(若 被测凝胶为酸性，选 pH4 缓冲液；若为碱性，选 pH9 缓冲液)倒入塑料杯内，电极 底部水滴用滤纸轻轻吸干后，把电极浸入杯内，稍摇动数秒钟，按下读数开关。调 整斜率旋钮使读数指示或显示该测试温度下第二定位缓冲液的 pH 值。重复 1~2 次两 点定位操作至稳定为止。

4.3.3 三点回归定位

洗干净三个塑料杯，分别置入 pH4、7、9 标准缓冲液。取其中一个先按 4.3.1 定 位后，再测定另两个标准缓冲液的 pH 值。把三个标准缓冲液在测试温度下的标准 值与相应的 pH 值读数在计算器上进行回归储存。若由三个读数求出的回归值 与标准值相差都不大于 0.02pH 单位，可认为仪器及电极正常，可进行凝胶的 pH 测 定。

4.4 凝胶的测定

将塑料杯及电极用试剂水洗净后，再用被测凝胶冲洗 2 次或以上。然后，浸入 电极并进行 pH 值测定。记下读数。

5. 本发明的凝胶的 pH 值数据见表 1

表 1 本发明的凝胶的 pH 值

实施例	pH 值数据	实施例	pH 值数据	实施例	pH 值数据
实施例 1	5.63	实施例 9	4.35	实施例 17	4.63
实施例 2	5.83	实施例 10	5.30	实施例 18	5.81
实施例 3	5.03	实施例 11	5.24	实施例 19	5.21
实施例 4	8.13	实施例 12	8.44	实施例 20	8.08
实施例 5	4.68	实施例 13	4.85	实施例 21	4.06
实施例 6	6.77	实施例 14	6.54	实施例 22	6.72
实施例 7	8.89	实施例 15	8.83	实施例 23	8.70
实施例 8	5.67	实施例 16	5.43	实施例 24	5.61

由此实验说明，本发明的凝胶 pH 值处于 4.35-8.89 之间。

实验例 2-本发明凝胶的相转化温度测定

相转化温度的测定方法参照专利文献 CN1377706A 说明书第 3 页实验一的测试

方法，所得数据为温度区间，见下表 2

表 2 本发明凝胶的相转化温度

实施例	相转化温度	实施例	相转化温度	实施例	相转化温度
实施例 1	31-35	实施例 9	31-32	实施例 17	25-28
实施例 2	29-31	实施例 10	28-32	实施例 18	28-29
实施例 3	30-34	实施例 11	28-31	实施例 19	27-29
实施例 4	35-36	实施例 12	30-34	实施例 20	27-29
实施例 5	29-34	实施例 13	31-32	实施例 21	27-30
实施例 6	29-32	实施例 14	29-32	实施例 22	28-31
实施例 7	30-31	实施例 15	30-33	实施例 23	28-30
实施例 8	31-32	实施例 16	31-32	实施例 24	32-33

由此实验说明，本发明的凝胶的相转变温度介于 27-36℃，能够满足在本发明的即型凝胶常温处于液态，滴入眼内后转化为固体状凝胶，达到延长药物滞留作用的效果。

眼用即型凝胶分为三种：温度敏感型、pH 值敏感型以及离子强度敏感型，其中温度敏感型眼用即型凝胶相对易于工业化，本发明也是通过温度敏感型眼用即型凝胶实现的，但是经过发明人的实验摸索发现，温度敏感型凝胶的稳定性以及能否在人眼球的局部温度下即刻转化为凝胶状与即型凝胶的 pH 值是否稳定关系密切。发明人采用较大容量的酸碱缓冲剂确保眼用即型凝胶的 pH 值的稳定，从而保证了凝胶具有敏感和适宜的相转化温度。

实验例 3-本发明眼用即型凝胶的眼部刺激的测试

将 40 只新西兰白兔分为 10 组，分别在各组兔眼结膜囊内滴入本发明的眼用即型凝胶（实施例 1、4、7、9、12、16、18、20、22 和 24），刺激实验以内高频点左眼，每 45 分钟一次，每次 1 滴，并同时以生理盐水点右眼为对照，连续 4 次。于点眼后定时检查各组动物结膜、虹膜及角膜组织，均未见充血、水肿、分泌物粘连等反应。这表明本发明眼用即型凝胶是安全的，对眼组织无刺激。

Bibliographic data

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Abstract of CN1456161A

An atropine sulfate gel for examining eyeground, enlarging pupil, and treating keratitis and iridocyclitis is prepared from atropine sulfate, hydroxypropyl methylcellulose, boric acid, borax and phenylmethylhydroxy amine chloride. Its advantages are long stay time in eye, and high curative effect and biological utilization rate.



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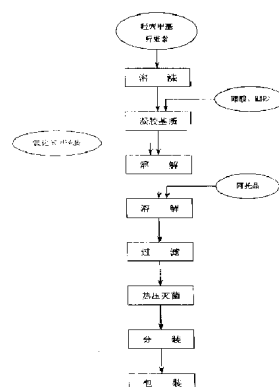
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[54] 发明名称 硫酸阿托品眼用凝胶

[57] 摘要

本发明涉及一种药物有效成分—硫酸阿托品—首次制成眼用凝胶剂，硫酸阿托品能选择性的阻断 M—胆碱能受体，能对抗乙酰胆碱及其拟胆碱药的 M—受体激动作用，能接触平滑肌痉挛，能拟制腺体分泌，散大瞳孔。本发明首次将硫酸阿托品制成眼用凝胶，不仅解决了滴眼液易被泪液稀释、眼内停留时间短的问题；而且，提高药物有效成分的生物利用度，增加疗效和用药安全性。主要内容是：硫酸阿托品，羟丙甲基纤维素，硼酸，硼砂，氯化苯甲烃胺制成稳定的水溶性透明乳膏体，使用后在眼表形成一层透气的网状膜，作用持久，主要用于眼底检查及验光前的散瞳，眼科手术术前散瞳，术后防止粘连，也可以用于角膜炎、虹膜睫状体炎等的治疗。

工艺流程图



1. 一种用于眼底检查及验光前的散瞳，眼科手术术前散瞳，术后防止粘连，用于角膜炎、虹膜睫状体炎等治疗的硫酸阿托品眼用凝胶单方制剂；其通用名为硫酸阿托品眼用凝胶；化学名为 α - (羟甲基) 苯乙酸 - 8 - 甲基 - 氮杂双环[3、2、1] - 3 - 辛酯硫酸盐一水合物；英文名为 Atropine sulfate ophthalmic gel；汉语拼音为 Liusuan Atropin Yanyongningjiao；硫酸阿托品眼用凝胶为硫酸阿托品的单方制剂，根据制剂名称与原料相一致的原则，本品命名为硫酸阿托品眼用凝胶。硫酸阿托品药物与主要辅料成分及其按下列比例组成的应用于眼科疾患的制剂：其中含硫酸阿托品为 1.0% (w/w)；含羟丙甲基纤维素为 4.0% (w/w)；含硼酸为 0.5% (w/w)；含硼砂为 1.0% (w/w)；含氯化苯甲烃胺为 0.01% (w/w)。取处方量的羟丙甲基纤维素用适量注射用水溶胀，备用。取处方量的硼砂、硼酸加入其中，搅拌均匀，制成透明凝胶基质；加入氯化苯甲烃胺，搅拌使溶解；加入硫酸阿托品药物有效成分，补水至全量，充分搅拌均匀；过滤，热压灭菌，分装，即得。另外，硫酸阿托品药物有效成分与羟丙甲基纤维素系列、甲基纤维素系列、聚乙烯醇系列等基质均可形成凝胶，卡泊姆系列基质效果最好。上述制备工艺中，其核心技术是：(1)羟丙甲基纤维素酸性条件下 (pH=5.0-6.0) 以注射用水溶胀，(2)与硼砂、硼酸等辅料成分混合加热溶解 (温度范围 60-80℃) 是保证膏体透明的关键，(3)加入硫酸阿托品药物有效成分适当温度下 (45-55℃) 搅拌溶解，(4)热压灭菌 (115℃，30 分钟)。

2. 按照权利要求 1 所述的硫酸阿托品眼用凝胶，其特征在于该药物有效成分硫酸阿托品首次制成眼用凝胶剂，药物与主要辅料成分及其按下列比例组成的应用于眼科眼底检查及验光前的散瞳，眼科手术术前散瞳，术后防止粘连，用于角膜炎、虹膜睫状体炎等治疗的制剂：其中硫酸阿托品为其药效成分，浓度为 0.5-5.0% (w/w)；主要辅料含羟丙甲基纤维素为 0-20.0% (w/w)；含硼酸为 0-5.0% (w/w)；含硼砂为 0-10.0% (w/w)；含氯化苯甲烃胺为 0-0.05% (w/w)。

硫酸阿托品眼用凝胶

所属技术领域

本发明涉及一种药物有效成分——硫酸阿托品——首次制成眼用凝胶，应用于眼科手术术前散瞳，术后防止粘连。也可以用于角膜炎、虹膜睫状体炎等的治疗。

背景技术

国内临床上除使用片剂、注射液外，眼科制剂常使用 1.0%眼膏（以凡士林为基质）；国外眼科用药中除使用 1.0%眼膏外，还使用 1.0%硫酸阿托品滴眼液。硫酸阿托品眼用凝胶的开发和使用未见报道，在国内医疗机构的医院制剂中有使用凝胶剂者（非眼用），据反映，效果很好。硫酸阿托品眼用凝胶剂目前还未见有研究和生产的报道。

发明内容

为了克服给药量大，口服药物难以通过血眼屏障的不足，发明一种眼用凝胶，眼科局部给药，不仅解决了滴眼液易被泪液稀释，眼内停留时间短的问题，且使用量少，提高药物有效成分的生物利用度，增加疗效和用药安全性。

本发明的技术方案是：硫酸阿托品药物成分与羟丙甲基纤维素膏体基质形成稳定的透明膏体，施用后在眼表形成一层透气的网状膜，作用持久。其具体特征如下：

硫酸阿托品眼用凝胶单方制剂，通用名：硫酸阿托品眼用凝胶；化学名： α -（羟甲基）苯乙酸 - 8 - 甲基 - 氮杂双环[3、2、1] - 3 - 辛酯硫酸盐一水合物；英文名：Atropine sulfate ophthalmic gel；汉语拼音：Liusuan Atuopin yanyong ningjiao；硫酸阿托品眼用凝胶为硫酸阿托品的单方制剂，根据制剂名称与原料相一致的原则，本品命名为硫酸阿托品眼用凝胶。硫酸阿托品药物与主要辅料成分及其按下列比例组成的应用于眼科疾患的制剂：其中含硫酸阿托品为 0.1-5.0%

(w/w); 含羟丙甲基纤维素为 0-20.0% (w/w); 含硼酸为 0-5.0% (w/w); 含硼砂为 0-10.0% (w/w); 含氯化苯甲烃胺为 0-0.05% (w/w)。取处方量的羟丙甲基纤维素用 400ml 注射用水溶胀, 过夜。取处方量的硼砂、硼酸加入其中, 搅拌均匀, 制成透明凝胶基质; 加入氯化苯甲烃胺, 搅拌使溶解; 加入硫酸阿托品药物有效成分, 补水至全量, 充分搅拌均匀; 用 220 目筛布过滤, 热压灭菌, 分装, 即得。另外, 硫酸阿托品与卡泊姆系列、甲基纤维素系列、聚乙烯醇系列等基质均可形成凝胶, 羟丙甲基纤维素系列基质效果最好。上述制备工艺中, 其核心技术是: (1)羟丙甲基纤维素酸性条件下 (pH=5.0-6.0) 以注射用水溶胀, (2)与硼砂、硼酸等辅料成分混合加热溶解 (温度范围 60-80℃) 是保证膏体透明的关键, (3)加入硫酸阿托品药物有效成分适当温度下 (45-55℃) 搅拌溶解, (4)热压灭菌 (115℃, 30 分钟)。

本发明的积极效果: 硫酸阿托品眼用凝胶的研制与开发有以下优点: (1)、硫酸阿托品眼用凝胶直接作用于患处, 能充分有效地发挥作用, 可避免口服药物治疗所带来的胃肠道刺激和其他副作用。(2)、相对于其它眼用制剂, 凝胶剂具有无可比拟的优点。和滴眼液相比硫酸阿托品眼用凝胶不易被泪液稀释, 在眼内停留时间长, 有利于主要的充分吸收和利用; 和凡士林基质的眼膏相比, 硫酸阿托品眼用凝胶水溶性好, 容易清洗, 不污染衣物, 使用方便。另一方面, 硫酸阿托品眼用凝胶不造成视野模糊, 昼夜都可使用。本产品的开发成功, 不仅可填补国内眼科用药的一项空白, 还可带来很好的社会效益和经济效益。(3)、我们企业是国内专业的眼科用药生产厂家, 30 多年来一直致力于眼药的生产、研制、开发, 积极参加社会公益事业, 眼科用药占总产值的 90%以上。尤其是, 我们有环丙沙星滴眼液、氧氟沙星滴眼液 (眼膏) 的生产、开发经验。硫酸阿托品眼用凝胶一旦开发成功, 必然会受到广大医生和患者的欢迎。

实施例

处方 1:

硫酸阿托品	1.0 克
羟丙甲基纤维素	4.0 克
硼砂	1.0 克

硼酸	0.5 克
氯化苯甲烃胺	0.01 克
注射用水	制成 100 克

操作过程:

- ①. 取处方量的羟丙甲基纤维素用适量注射用水溶胀, 过夜。
- ②. 取处方量的硼砂、硼酸加入其中, 搅拌均匀, 加热制成透明凝胶基质;
- ③. 加入氯化苯甲烃胺, 搅拌使溶解;
- ④. 加入硫酸阿托品, 补水至全量, 充分搅拌均匀;
- ⑤. 用 220 目筛布过滤, 热压灭菌, 分装, 即得。

处方 2:

硫酸阿托品	0.1-5.0 克
羟丙甲基纤维素	0.1-20.0 克
硼砂	0.1-5.0 克
硼酸	0.1-2.5 克
氯化苯甲烃胺	0.01-0.5 克
注射用水	制成 100 克

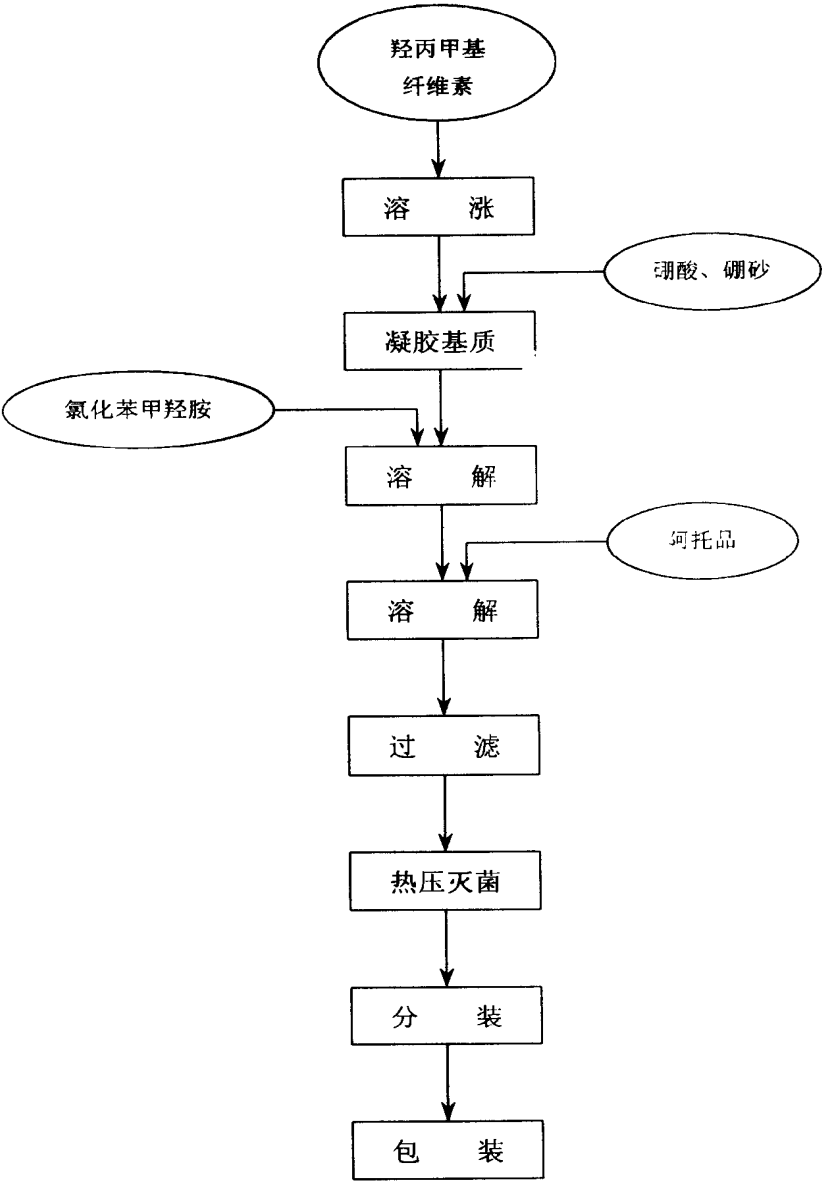
操作过程:

- ①. 取处方量的羟丙甲基纤维素用适量注射用水溶胀, 过夜。
- ②. 取处方量的硼砂、硼酸加入其中, 搅拌均匀, 加热制成透明凝胶基质;
- ③. 加入氯化苯甲烃胺, 搅拌使溶解;
- ④. 加入硫酸阿托品, 补水至全量, 充分搅拌均匀;
- ⑤. 用 220 目筛布过滤, 热压灭菌, 分装, 即得。

本发明的创造性, 通过临床、毒理药理数据来实现, 实质审查时一并提供。

图 1 为硫酸阿托品眼用凝胶制备工艺流程图。

工艺流程图



Bibliographic data

Title: Injectable pharmaceutical compositions having improved stability, and methods of producing the same.

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Inventor(s): TASHMA, ZEV

Applicant(s): TEVA PHARMA[IL]

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Abstract of EP0332826A1

Improved pharmaceutical compositions having a long term shelf life, which comprises certain pharmacologically active water-hydrolyzable derivatives of alpha -amino- and alpha - 5 hydroxy-carboxylic acids and a solvent medium containing an aqueous component, characterized in that at least a major proportion by volume of the ordinary water normally used in formulating such compositions is replaced by deuterium oxide.

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54 **Injectable pharmaceutical compositions having improved stability, and methods of producing the same.**

57 Improved pharmaceutical compositions having a long term shelf life, which comprises certain pharmacologically active water-hydrolyzable derivatives of α -amino- and α - 5 hydroxy-carboxylic acids and a solvent medium containing an aqueous component, characterized in that at least a major proportion by volume of the ordinary water normally used in formulating such compositions is replaced by deuterium oxide.

EP 0 332 826 A1

BACKGROUND OF THE INVENTION

Injectable pharmaceutical compositions usually require the presence of water. In certain cases, however, water has a destabilizing influence on the pharmaceutically active ingredients of such compositions, e.g. it may cause such ingredients to hydrolyze and in consequence lose their pharmacological potency. In these cases, where the compositions are to be used in clinics or hospitals, one solution of the problem is to store the active ingredients in a form in which injectable compositions can be readily formulated therefrom by addition of water, and to prepare such compositions only on demand. There exist, however, situations in which such a solution to the problem of destabilization in presence of water cannot be applied. For example, for reasons of public health, in order to combat or prevent a disease, it may be desirable to maintain a stock of the order of hundred of thousands, or even millions of units, of injectable pharmaceutical compositions. Similarly, in order to provide an antidote in case of chemical warfare it may be desirable to be able to distribute very large number of injectable compositions which are ready for use to the civilian population or to military personnel.

Moreover, for reasons of convenience or economy, it may be desirable to store these injectable pharmaceutical compositions for long terms. The expression "long term", when used herein in this context, means a period of at least one year. That is to say, it is contemplated that such injectable compositions may be stored under ambient conditions with a tolerable degree of deterioration of their pharmacological effectiveness, for periods of at least one year, possibly even for several years.

It has previously been proposed to replace some of the water in injectable compositions by propylene glycol. Thus, in U.S. 4,212,886 (Homan), the contents of which are to be regarded as incorporated herein by reference, there is described and claimed a pharmaceutical composition comprising (as active ingredient) benactyzine hydrochloride dissolved in a solvent mixture of 30-50% propylene glycol and 70-50% water, by volume, subject to the qualifications that the amount of propylene glycol is sufficient to increase the stability of the active ingredient over that in water alone and that the amount of water is sufficient to prevent dehydration of the active ingredient. Additionally, ethanol may be present in order to reduce the viscosity so as to render the composition more easily injectable; thus, the solvent mixture may comprise by volume, 50% propylene glycol, 40% water and 10% ethanol.

However, Homan's compositions suffer from a number of disadvantages. Firstly, the degree of stabilization appears to be insufficient for practical

application. Secondly, over the long term, propylene glycol may give rise to undesirable degradation products *per se* and also to undesirable reaction products with the active ingredients, thereby detracting from the pharmaceutical purity of the compositions. Thirdly, the viscosity characteristics of aqueous solutions of propylene glycol are inconvenient, both from a handling and an administration point of view.

Siegel et al J. Pharm. Sci. 53: 978 (1964) and 51: 1166 (1962) proposed to stabilize solutions of procaine, which may be suitable for ophthalmic use, by substituting the ordinary water in these solutions by deuterium oxide. Siegel's experiments relate to the stabilization of these solutions in the alkaline pH range at 40° C; in no case does such stabilization lead to a half-life greater than 115 hours. Thus, there is no suggestion that it could be practicable to use deuterium oxide for long term stabilization of pharmaceutical compositions of any kind.

In accordance with the invention it has now been surprisingly discovered that the stability of certain aqueous pharmaceutical compositions intended for long term storage can be enhanced by replacing at least a major part of the ordinary water in such compositions by deuterium oxide.

SUMMARY OF THE INVENTION

It is, therefore an object of the present invention to stabilize and thus prolong the long term shelf-life of certain injectable pharmaceutical compositions by replacing the greater part of the ordinary water normally used therein by deuterium oxide.

Another object of the invention is to significantly reduce the amount of ordinary water present in certain injectable pharmaceutical compositions, by replacing at least a major part thereof by deuterium oxide, whereby the deteriorative reaction with water of the active ingredient(s) in the long term will be significantly retarded.

Other objects of the invention will become apparent from the following description.

Throughout the present specification and claims, "%" in relation to ingredients of solvent media is intended to signify % by volume.

The present invention accordingly provides, in one aspect, an injectable pharmaceutical composition having a long term shelf life, which composition comprises:

at least one pharmacologically active ingredient which is susceptible to deterioration due to the presence of water, selected from the group consist-

ing of water-hydrolyzable derivatives of α -amino- and α -hydroxy- carboxylic acids, pharmaceutically acceptable acid addition salts and alkali metal, alkaline earth metal and ammonium salts of such derivatives; and

a solvent medium containing an aqueous component, said aqueous component comprising at least a major proportion by volume of deuterium oxide.

In another aspect, the invention provides a method of producing an injectable pharmaceutical composition having a long term shelf-life, which comprises dissolving at least one pharmacologically active ingredient which is susceptible to deterioration due to the presence of water, selected from the group consisting of water-hydrolyzable derivatives of α -amino and α -hydroxy- carboxylic acids, pharmaceutically acceptable acid addition salts and alkali metal, alkaline earth metal and ammonium salts of such derivatives; in a solvent medium containing an aqueous component, said aqueous component comprising at least a major proportion by volume of deuterium oxide.

The term "ordinary water", as used herein, is intended to mean water suitable for preparing injectable pharmaceutical formulations, but which in any event contains no more than the normally occurring amount of deuterium oxide. As previously mentioned, "long term" (storage or shelf life) in the present context signifies a period of at least one year. Thus, in accordance with the present invention, the injectable pharmaceutical compositions will have a storage or shelf-life of at least one year, and the initial storage or shelf-life, whatever it may be, is prolonged by the replacement of at least the major part by volume of the ordinary water present, by deuterium oxide.

DETAILED DESCRIPTION OF THE INVENTION

Contrary to the prior art teaching, it has been found in accordance with the present invention that there is no minimum amount of ordinary water required "sufficient to prevent dehydration of the active ingredient". In accordance with the invention, 99.9 or 100% of the ordinary water present in the injectable composition could be replaced by deuterium oxide, but for practical reasons there will usually be present a small residual amount of ordinary water. Thus from a practical and economic point of view, the aqueous component of the solvent medium in the injectable pharmaceutical compositions may contain, say, about 98% or 99% deuterium oxide, the balance being ordinary water.

However, since in accordance with the invention a major proportion of the ordinary water present in the aqueous component of the solvent medium is replaced by deuterium oxide, it will be

evident that other percentages than those just mentioned are viable. Thus, for example, the aqueous component of the injectable composition according to the invention may comprise from about 75 to about 100%, preferably from about 90 to about 100% deuterium oxide and from about 25 to about 0%, preferably from about 10 to about 0% ordinary water. Most preferably, the aqueous component comprises from about 95 to about 100% deuterium oxide and from about 5 to about 0% ordinary water. It is especially preferred that in the compositions according to the invention, the aqueous component comprises from about 98 to about 100% deuterium oxide and from about 2 to about 0% ordinary water, and more particularly from about 99 to about 99.75% deuterium oxide and from about 1 to about 0.25% ordinary water.

As will be appreciated by those skilled in the pharmaceutical art, the solvent medium may be wholly comprised of an aqueous component or, alternatively, the solvent medium may be a mixture of an aqueous component with a water-miscible non-aqueous liquid.

As is well known, deuterium oxide is itself both stable and effectively non-toxic (in the amounts presently contemplated for use herein, e.g. up to about 10 ml). It is therefore an ideal solvent medium for the present purposes.

Deuterium oxide is present in small amounts in ordinary water, including the water content of the human body; an average adult male contains about 6 ml deuterium oxide. It is close in chemical and physical properties to ordinary water. In connection with its physiological properties, it is to be noted that deuterium oxide has been routinely used over a period of years for measuring total body water content. For this purpose, quantities up to about 100 ml are used. In a published experiment (Taylor et al, Arch. Path., 81: 213 (1966)), considerable amounts of deuterium oxide were administered continuously to humans over a period of about 6 to 7 weeks, with little or no notable ill effects.

The deuterium oxide according to the invention should preferably pass the tests for ordinary water for injection as required by a recent edition of a recognized pharmacopoeia, adapted, if and as necessary, for the specific characteristics of deuterium oxide.

The use of deuterium oxide in accordance with the present invention is believed to have the following advantages over the possible use of other solvents (except ordinary water) for the same purpose:

1. it is far less toxic than other known solvents, except for ordinary water;

2. no new decomposition products due to drug-solvent interactions are to be expected, besides those which are known from ordinary water (or analogues of such known products);

3. no contamination due to dehydration, re-sinification, oxidation or other chemical changes of the solvent per se, is to be expected, as compared with the possible use of non-aqueous solvents; and

4. unlike most other non-aqueous solvents, deuterium oxide can be brought to a high and uniform standard of chemical and microbiological purity, by adapting the pharmacopoeia standards and tests for ordinary water for injection.

It is preferred that the compositions of the present invention have a pH within the range of about 1.5 to about 5.5. The manner of adjustment of pH values is of course well known to those skilled in the art.

The compositions of the present invention may contain additionally at least one further pharmacologically active ingredient, besides the at least one carboxylic acid derivative susceptible to hydrolysis as defined herein.

As previously noted, the at least one active ingredient is a water-hydrolyzable derivative of an α -amino- or α -hydroxy-carboxylic acid (including salts thereof). Such derivatives include, particularly, esters and amides, including N-substituted amides. The carboxylic acid derivative is also characterized by the fact that it contains an α -amino or α -hydroxy group. A presently preferred sub-group comprises the α -hydroxy-carboxylic acid derivatives. An exemplary member of this sub-group is benactyzine and its pharmaceutically acceptable acid addition salts, e.g. the HCl salt. In the case of benactyzine and its pharmaceutically acceptable acid addition salts, the pH of the composition of the invention is preferably within the range of about 2.0 to about 4.0, more preferably within the range of about 2.7 to about 2.8.

As already indicated generally, such compositions which contain benactyzine (or its salts) may contain additionally at least one further pharmacologically active ingredient; the latter may, for example, be selected from cholinolytic drugs, pharmaceutically accepted oximes and pharmaceutically acceptable salts thereof. It is preferably selected from atropine, pharmaceutically acceptable salts thereof and trimedoxime (which is also known as "TMB-4"). Atropine may be used as the sulfate salt, for example. Trimedoxime is a diquaternary salt in which the anion may be, e.g., bromide.

The medical treatment of an acute exposure to certain organophosphorus insecticides or nerve gases, which are anticholinesterase agents, demands immediate administration of cholinolytics, to control the excessive cholinergic stimulation. For

this purpose, it is well known to use atropine, and a combination of atropine with benactyzine. Addition of oximes to cholinolytic drugs has the effect of reactivating and restoring the cholinesterase activity. Zvirbliis and Ellin, in *J. Pharm. Sci.* 71:321 (1982), described a 3-component mixture containing 2 ml of injection solution: 40 mg of trimedoxime bromide, 1 mg of atropine sulfate and 4.1 mg of benactyzine hydrochloride. It will be evident to those skilled in the art that the proportions and quantities of the ingredients in such a three-component mixture can be varied. Other related drug mixtures are described in Schenk et al., *Arch. Toxicol.*, 36:71 (1976). The contents of both the Zvirbliis and Ellin article and the Schenk et al. article are to be regarded as incorporated herein by reference.

The susceptibility of benactyzine to hydrolysis (which under optimal conditions of pH 2.7-2.8 may amount to about 20% per year at 25°C and about 2% per year at 5°C according to Zvirbliis and Ellin), detracts from its usefulness as a component of a large scale antidote to organophosphorus poisoning, because the evident necessity for refrigeration requires considerable expense and logistics to store the quantity of units required. In accordance with the present invention, making use of deuterium oxide, the storage characteristics of benactyzine, whether kept alone or in combination with other ingredients, are considerably enhanced.

The invention moreover extends to an automatic injector characterized by the presence therein of an injectable composition according to the invention. Normally, the automatic injector will be a single stage injector, i.e. comprising a single compartment containing the injectable composition. However, in cases where an active ingredient which is beneficially stabilized in the long term is to be used together with one or more other active ingredients which are sufficiently stable (without substitution of deuterium oxide for ordinary water), then it will be apparent that economy in the consumption of deuterium oxide may be achieved by use of a multistage automatic injector comprising one compartment containing the relatively less stable active ingredient dissolved in deuterium oxide, and one or more other compartments containing the relatively more stable active ingredients dissolved in ordinary water.

The invention will be illustrated by the following non-limitative examples, in which 99.75% deuterium oxide was used initially. Owing to the hygroscopic nature of this substance, and to the presence of small amounts of ordinary water in the composition ingredients and in the apparatus, the solvent medium in the composition end-products contained about 99% deuterium oxide, the balance being ordinary water. The deuterium oxide should

pass the tests for Water for Injection of U.S.P. vol. XXI, adapted as appropriate for deuterium oxide.

EXAMPLE I

Benactyzine hydrochloride (80 mg) is dissolved in 18 ml of deuterium oxide, the pH is adjusted by known methods (see e.g. A.K. Covington, Analytical Chemistry, 40: 700 (1968)) to 2.7 with strong acid, and the volume is brought to 20 ml with more deuterium oxide. The resulting solution is sterilized by passing through a pharmaceutically acceptable and antimicrobial filtering device, and then divided into 1 ml portions. The resultant composition is suitable for use in a single stage automatic injector, where benactyzine is the only active ingredient to be administered, or in a multistage automatic injector together with relatively more stable active ingredients (such as atropine and trimedoxime) dissolved in ordinary water in one or more further separate compartment(s).

EXAMPLE II

Benactyzine hydrochloride (40 mg), atropine sulfate (10 mg) and trimedoxime bromide (400 mg) are dissolved in 18 ml of deuterium oxide. The pH is adjusted by known methods to 2.7 with strong acid, and the volume is brought to 20 ml with more deuterium oxide. The resulting solution is sterilized by passing through a pharmaceutically acceptable and antimicrobial filtering device, and then divided into 2 ml portions. The resultant composition is suitable for use in a single stage automatic injector.

It was found that the shelf life at room temperature of the compositions of Examples I and II was considerably enhanced as compared to similar compositions prepared with ordinary water only.

Since it will be apparent to those skilled in the art that many modifications and variations could be made in the particular description of the invention hereinabove, the invention is not to be construed as limited thereby, but rather the invention will be defined only by the claims which follow.

The features disclosed in the foregoing description, in the claims and/or in the accompanying drawings may, both, separately and in any combination thereof, be material for realising the invention in diverse forms thereof.

Claims

1. An injectable pharmaceutical composition having a long term shelf life, which composition comprises:
 - at least one pharmacologically active ingredient which is susceptible to deterioration due to the presence of water, selected from the group consisting of water-hydrolyzable derivatives of α -amino- and α -hydroxy- carboxylic acids, pharmaceutically acceptable acid addition salts and alkali metal, alkaline earth metal and ammonium salts of such derivatives; and
 - a solvent medium containing an aqueous component, said aqueous component comprising at least a major proportion by volume of deuterium oxide.
2. A composition according to claim 1, wherein said aqueous component comprises from about 75 to about 100% by volume of deuterium oxide and from about 25 to about 0% by volume of ordinary water.
3. A composition according to claim 2, wherein said aqueous component comprises from about 90 to about 100% by volume of deuterium oxide and from about 10 to about 0% by volume of ordinary water.
4. A composition according to claim 3, wherein said aqueous component comprises from about 95 to about 100% by volume of deuterium oxide and from about 5 to about 0% by volume of ordinary water.
5. A composition according to claim 4, wherein said aqueous component comprises from about 98 to about 100% by volume of deuterium oxide and from about 2 to about 0% by volume of ordinary water.
6. A composition according to claim 5, wherein said aqueous component comprises from about 99 to about 99.75% by volume of deuterium oxide and from about 1 to about 0.25% by volume of ordinary water.
7. A composition according to claim 5, wherein said aqueous component consists of substantially 100% by volume of deuterium oxide.
8. A composition according to any one of claims 1 to 7, having a pH within the range of about 1.5 to about 5.5.
9. A composition according to any one of claims 1 to 8, wherein said solvent medium comprises a mixture of an aqueous component with a water-miscible solvent.
10. A composition according to any one of claims 1 to 9, wherein said at least one pharmacologically active ingredient is an ester of an α -hydroxy-carboxylic acid.
11. A composition according to claim 10, wherein said at least one active ingredient comprises at least one member selected from the

group consisting of benactyzine and its pharmaceutically acceptable acid addition salts, and the pH of the composition is within the range of about 2.0 to about 4.0.

12. A composition according to claim 11, having a pH within the range of about 2.7. to about 2.8. 5

13. A composition according to any one of claims 1 to 12, which contains additionally at least one further pharmacologically active ingredient.

14. A composition according to claim 13, wherein said at least one further pharmacologically active ingredient is selected from the group consisting of cholinolytic drugs, pharmaceutically acceptable oximes and pharmaceutically acceptable salts thereof. 10 15

15. A composition according to claim 14, wherein said at least one further pharmacologically active ingredient is selected from the group consisting of atropine, pharmaceutically acceptable salts thereof and trimedoxime. 20

16. A single stage automatic injector characterised by the presence therein of a composition according to any of claims 1 to 15.

17. A multistage automatic injector characterised by the presence therein of a composition according to any of claims 1 to 15. 25

18. A method of producing an injectable pharmaceutical composition having a long term shelf-life, which comprises dissolving at least one pharmacologically active ingredient which is susceptible to deterioration due to the presence of water, selected from the group consisting of water-hydrolyzable derivatives of α -amino and α -hydroxycarboxylic acids, pharmaceutically acceptable acid addition salts and alkali metal, alkaline earth metal and ammonium salts of such derivatives; in a solvent medium containing an aqueous component, said aqueous component comprising at least a major proportion by volume of deuterium oxide. 30 35 40

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DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
D,A	JOURNAL OF PHARMACEUTICAL SCIENCES, vol. 71, no. 3, March 1982, pages 321-325, American Pharmaceutical Association, Washington, DC, US; P. ZVIRBLIS et al.: "Kinetics and stability of a multicomponent organophosphate antidote formulation in glass and plastic" * Whole document *	1-18	A 61 K 9/08 A 61 K 47/00
D,A	JOURNAL OF PHARMACEUTICAL SCIENCES, vol. 53, no. 8, August 1964, pages 978-979, American Pharmaceutical Association, Washington, DC, US; F.P. SIEGEL et al.: "Stability of procaine in deuterium oxide" * Whole document *	1-18	
D,A	US-A-4 212 886 (G. HOMAN) * Whole document *	1-18	
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The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 14-07-1989	Examiner MUELLNERS W.
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document			



DOCUMENTS CONSIDERED TO BE RELEVANT					
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A	JOURNAL OF PHARMACEUTICAL SCIENCES, vol. 68, no. 7, July 1979, pages 856-859, American Pharmaceutical Association, Washington, DC, US; L.A. HULL et al.: "3-Quinuclidinyl benzilate hydrolysis in dilute aqueous solution" * Page 856, left-hand column; page 858, right-hand column - page 859, left-hand column *	1-18			
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The present search report has been drawn up for all claims			TECHNICAL FIELDS SEARCHED (Int. Cl.4)		
Place of search THE HAGUE		Date of completion of the search 14-07-1989	Examiner MUELLNERS W.		
<table border="0"><tr><td>CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</td><td>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ----- & : member of the same patent family, corresponding document</td></tr></table>				CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document	T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ----- & : member of the same patent family, corresponding document
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Bibliographic data

Title: THE PROCESS FOR MANUFACTURING TOPICAL OPHTHALMIC PREPARATIONS WITHOUT SYSTEMIC EFFECTS

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Inventor(s): KHAMAR, BAKULESH, MAFATLAL

Applicant(s): KHAMAR BAKULESH MAFATLAL[IN]

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Abstract of WO0049990A2

For treating eye diseases, various kinds of medications are used. They are used in form of topical preparations or in form of systemic preparations to be taken orally or parenterally. Of topical preparations some are for topical use only e.g. framycetin, neomycin, loteprednol ebanoate, etc. However, large majority of topical ophthalmic drugs are for systemic use also, e.g., Ciprofloxacin, Gentamicin, Timolol, Clonidine, Dexamethasone, Betamethasone, Carbachol, etc. Some of these drugs when used topically are also found to have systemic effects and if they are of serious nature limits the use of that drug, e.g. cardiopulmonary effects of B-blockers like timolol. Dryness of mouth, flush, fever, tachy cardia, urinary retention, convulsion irritability with atropine. Hypertension with phenylephrine. Increased salivation, nausea, vomiting, diarrhea, stomach cramps, bronchial secretions, bronchial constriction, asthma, bradycardia, paresthesia with miotics, Hypotension with clonidine. Dry mouth, fatigue and drowsiness with apraclonidine and brimonidine. The invention relates to topical ophthalmic preparations resp. a process for manufacturing the same using polymers in such a way that they have desired topical effects but no systemic effects. The polymers (e.g. polyacrylic acids, hydroxyethyl cellulose, etc.) used for this purpose have pseudo-plastic, mucoadhesive and viscosity enhancing properties.



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(21) International Application Number: PCT/IN00/00008 (22) International Filing Date: 2 February 2000 (02.02.00) (30) Priority Data: 90/BOM/99 3 February 1999 (03.02.99) IN (71)(72) Applicant and Inventor: KHAMAR, Bakulesh, Mafatlal [IN/IN]; 201 "Ashadha", Vasundhara Colony, Gulbai Tekra, Ellisbridge, Ahmedabad 380 006, Gujarat (IN).		(81) Designated States: AE, AL, AU, BA, BB, BG, BR, CA, CN, CR, CU, CZ, EE, GE, HU, ID, IL, IS, KP, KR, LC, LK, LR, LS, LT, LV, MG, MK, MN, MX, NO, PL, RO, SG, SI, SL, TR, TT, UA, US, UZ, VN, YU, ZA, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>Without international search report and to be republished upon receipt of that report.</i>
(54) Title: THE PROCESS FOR MANUFACTURING TOPICAL OPHTHALMIC PREPARATIONS WITHOUT SYSTEMIC EFFECTS		
(57) Abstract For treating eye diseases, various kinds of medications are used. They are used in form of topical preparations or in form of systemic preparations to be taken orally or parenterally. Of topical preparations some are for topical use only e.g. framycetin, neomycin, loteprednol ebanoate, etc. However, large majority of topical ophthalmic drugs are for systemic use also, e.g.. Ciprofloxacin, Gentamicin, Timolol, Clonidine, Dexamethasone, Betamethasone, Carbachol, etc. Some of these drugs when used topically are also found to have systemic effects and if they are of serious nature limits the use of that drug, e.g. cardiopulmonary effects of B-blockers like timolol. Dryness of mouth, flush, fever, tachy cardia, urinary retention, convulsion irritability with atropine. Hypertension with phenylephrine. Increased salivation, nausea, vomiting, diarrhea, stomach cramps, bronchial secretions, bronchial constriction, asthma, bradycardia, paresthesia with miotics, Hypotension with clonidine. Dry mouth, fatigue and drowsiness with apraclonidine and brimonidine.		

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THE PROCESS FOR MANUFACTURING TOPICAL OPHTHALMIC PREPARATIONS WITHOUT SYSTEMIC EFFECTS.

The present invention relates to the process for manufacturing topical ophthalmic preparations without systemic effects. Many topical ophthalmic preparations have systemic effects. These systemic effects are responsible for contraindications, side effects, toxicity of some of the topical ophthalmic preparations. Similarly, due to systemic effects certain topical ophthalmic preparations have not been commercialized.

The present invention is directed to manufacturing topical ophthalmic preparations in such a way that systemic effects of that topical ophthalmic preparation do not manifest.

Topical ophthalmic preparations can be divided into two groups. One of the group includes preparations in which active ingredients are for topical use only and have no systemic effects. These group of drugs include antibiotics like Framycetin, Neomycin, Fucidin, steroids like Loteprednol Ebanoate, Triamcinolone, alpha agonist like Apraclonidine, Brimonidine, etc. The other group of topical ophthalmic preparations have active ingredients which are generally used for their effects. These group of preparations include antibiotics like Ciprofloxacin, Norfloxacin, Ofloxacin, Gentamicin, Tobramycin, steroids like Dexamethasone, Betamethasone, β -blockers like Timolol, Betaxolol, etc. Some of these drugs when used topically are also found to have systemic effects. When systemic effects are serious in nature, it results in limiting the use of a drug in the form of contraindication or amount of drug to be used.

Examples of well known systemic effects of topical ophthalmic preparations include cardiopulmonary effects of β -blockers like Timolol, Levobunolol, Metipranolol, Carteolol, etc. Dryness of mouth, flush, fever, tachycardia, urinary retention, convulsion irritability are found with Atropine eye drops. Systemic hypertension is associated with topical mydriatic phenylephrine. Increased salivation, nausea, vomiting, diarrhoea, stomach cramp, bronchial secretions, bronchial constriction, asthma, bradycardia, parasthesia is seen with miotics. Systemic hypotension is main limiting factor for use of clonidine in management of glaucoma. Dry mouth, fatigue and drowsiness seen with Brimonidine and Apraclonidine are some of their systemic effects.

The systemic side effects, manifesting with the use of topical ophthalmic preparations results in discontinuation of therapy or not initiating a therapy or reducing the amount of drug or drug not having wide spread acceptance.

Because of this reason, attempts are made to reduce systemic effects of topically applied drugs.

Systemic effects are due to plasma concentration of a drug. It depends on absorption of the drug from conjunctiva or nasal mucosa into systemic circulation (serum levels of drug).

The mechanisms to reduce plasma concentration of a drug includes reduction in drop size. It reduces the amount of drug available through conjunctiva as well as nasal mucosa.

The blockage of nasolacrimal duct temporarily or permanently also reduces drug reaching to nasal mucosa through nasolacrimal passages and thus reduces the amount of drug available systemically. Increasing the viscosity of a formulation also reduces the plasma concentration of a drug. Slow release of a drug through sustained release mechanism/device are known to reduce plasma concentration of topical ophthalmic preparations. Including vasoconstrictive agents into a topical ophthalmic preparation also reduces the plasma level of topically applied drugs.

The other mechanism used to reduce systemic effects include use of a prodrug as topical ophthalmic preparation which gets converted to active compound only at the site of action, e.g. Dipivetrin for epinephrine and Phenylephrine Oxazoline for Phenylephrine.

The other mechanism known includes formulating a preparation as an ointment. The tear film formed with the use of ointment is thick, with poor light transmission and irregular anterior surface. This results in blurring of vision and so have not been popular. It also causes stickiness of lashes and lid margin. This limits its use to a great extent and whenever used, its use is restricted for bed time application.

None of the above described methods in isolation or in combination with each other have been successful in eliminating systemic effects of topical ophthalmic preparations. Majority of efforts are centered around topical β -blockers to reduce their systemic effect, e.g. reduction in pulse rate. All known methods have been able to decrease the reduction in pulse rate, but none of them have been able to eliminate it completely.

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The objective of present invention is to provide topical ophthalmic preparations without systemic effects without reducing the concentration of active ingredient.

The further objective of present invention is to provide topical ophthalmic preparations which does not cause significant visual disturbances to limit its use during waking hours.

The further objective of present invention is to provide topical ophthalmic preparations which are equally effective after longer period of storage.

The further objective of present invention is to provide topical ophthalmic preparations which do not require special storage conditions.

Accordingly there is provided a process of manufacturing topical ophthalmic preparations without systemic side effects which comprises of the following steps.

1. Liquid formulation of a selected drug is prepared which contains excipients buffers and preservatives in distilled water. The pH of this solution is adjusted to provide stable formulation for topical ophthalmic use.
2. In a separate vessel polymer is dissolved into a solvent preferably water and stirred well till gel is formed.
3. Solution containing selected drug as formulated in step 1 is gradually added to the gel as formed in step 2.

4. Volume is made up by adding distilled water/solvent as required.
5. pH is checked and adjusted as necessary to provide stable formulation for topical instillation into eye.

The drug described above can be any of the existing ophthalmic preparations or any other drug which cannot be used as a topical preparation in a desired concentration for instillation into eye. The drugs which are most frequently used and are known to have systemic effects include β -blockers like Timolol, Levobunolol, Metipranalol, etc.

Similarly, mydriatics like phenylephrine, atropine, cyclopentolate, tropicamide have systemic effects and their use is restricted by it.

Clonidine is an example of a drug which lowers I.O.P. significantly but cannot be used as 0.1% or 0.2% concentration due to its systemic hypotensive effect.

The polymer to be used for preparing topical formulation as per present invention should form a gel when solubilized. For the purpose of present invention it is desirable to select a polymer with mucoadhesive properties. To avoid discomfort and visual disturbances associated with use of viscous solutions, it is desirable that polymer selected demonstrates pseudoplastic behaviour in a formulation.

Polymer to be used for the purpose of present invention having above properties are known and includes polyacrylic acid, polyacrylic esters, polycarbophile, polyvinyl Acetate, Acrypol, Xanthan gum, Guar gum, hydroxy ethyl cellulose, polyvinyl alcohol, PVP, carbomers, hydrogels prepared by combination of various polymers etc. Names of above polymer exemplifies the process and are not restricted to for the purpose of invention.

For the purpose of avoiding systemic effects of a formulation prepared as per present invention, it is necessary to have viscosity above 100,000 cps (one hundred thousand cps), preferably above 400,000 (four hundred thousand cps).

The final volume adjustment and amount of polymer to be used has to be designed considering these requirements. The amount of polymer in a final formulation to get desired viscosity is variable but is well known. It varies with polymer to polymer and also with molecular weight of some polymer.

Pharmaceutical preparations so manufactured can be sterilized by known methods of sterilizing, including autoclaving. If sterilization process is likely to result in unstabilization of drug, it can be prepared as a sterile product throughout the process.

Examples of formulations**I. Timolol****A.. Timolol 0.5%**

Timolol Maleate	0.72 gm. equivalent to 0.5 gm of Timolol
Benzylconium chloride	0.0107 gm
Polyacrylic Acid (Carbopol 940)	1.5 gm to 2.5 gm
Sodium hydroxide to adjust pH	6.5 to 7.5
Water for injection	QS to make 100 ml.

B. Timolol 0.25%

Timolol Maleate	0.36 gm equivalent to 0.5 gm of Timolol
Benzylconium chloride	0.0107 gm
Polyacrylic Acid (Carbopol 940)	1.5 gm to 2.5 gm
Sodium hydroxide to adjust pH	6.5 to 7.5
Water for injection	QS to make 100 ml

II. Timolol**A.. Timolol 0.5%**

Timolol Maleate	0.72 gm. equivalent to 0.5 gm of Timolol
Benzylconium chloride	0.0107 gm
Carbopol ETD 2001	1.5 gm to 2.5 gm
Sodium hydroxide to adjust pH	6.5 to 7.5
Water for injection	Q.S. to make 100 ml.

B. Timolol 0.25%

Timolol Maleate	0.36 gm equivalent to 0.5 gm of Timolol
Benzylconium chloride	0.0107 gm
Carbopol ETD 2001	1.5 gm to 2.5 gm
Sodium hydroxide to adjust pH	6.5 to 7.5
Water for injection	Q.S. to make 100 ml

III. Timolol**A.. Timolol 0.5%**

Timolol Maleate	0.72 gm. equivalent to 0.5 gm of Timolol
Benzylconium chloride	0.0107 gm
Carbopol 981	1.5 gm to 2.5 gm
Sodium hydroxide to adjust pH	6.5 to 7.5
Water for injection	Q.S. to make 100 ml.

- B. Timolol 0.25%
Timolol Maleate 0.36 gm equivalent to 0.5 gm of Timolol
Benzylconium chloride 0.0107 gm
Carbopol 981 1.5 gm to 2.5 gm
Sodium hydroxide to adjust pH 6.5 to 7.5
Water for injection Q.S. to make 100 ml

IV. **Timolol**

- A.. Timolol 0.5%
Timolol Maleate 0.72 gm. equivalent to 0.5 gm of Timolol
Benzylconium chloride 0.0107 gm
Polycarbophil 1.5 gm to 2.5 gm
Sodium hydroxide to adjust pH 6.5 to 7.5
Water for injection Q.S. to make 100 ml.

- B. Timolol 0.25%
Timolol Maleate 0.36 gm equivalent to 0.5 gm of Timolol
Benzylconium chloride 0.0107 gm
Polycarbophil 1.5 gm to 2.5 gm
Sodium hydroxide to adjust pH 6.5 to 7.5
Water for injection Q.S. to make 100 ml

V. **Clonidine**

- A. Clonidine 0.1%
Clonidine hydrochloride 0.1 gm
Benzylconium chloride 0.0107 gm
Polyacrylic Acid 1.5 gm to 2.5 gm
(Carbopol 940)
Sodium hydroxide to adjust pH 6.5 to 7.5
Water for injection QS to make 100 ml

- B. Clonidine 0.2%
Clonidine hydrochloride 0.2 gm
Benzylconium chloride 0.0107 gm
Polyacrylic Acid 1.5 gm to 2.5 gm
(Carbopol 940)
Sodium hydroxide to adjust pH 6.5 to 7.5
Water for injection QS to make 100 ml

VI. Clonidine**A. Clonidine 0.1%**

Clonidine hydrochloride . 0.1 gm
Benzylconium chloride 0.0107 gm
Carbopol ETD 2001 1.5 gm to 2.5 gm
Sodium hydroxide to adjust pH 6.5 to 7.5
Water for injection Q.S. to make 100 ml

B. Clonidine 0.2%

Clonidine hydrochloride 0.2 gm
Benzylconium chloride 0.0107 gm
Carbopol ETD 2001 1.5 gm to 2.5 gm
Sodium hydroxide to adjust pH 6.5 to 7.5
Water for injection Q.S. to make 100 ml

VII. Clonidine**A. Clonidine 0.1%**

Clonidine hydrochloride 0.1 gm
Benzylconium chloride 0.0107 gm
Carbopol 981 1.5 gm to 2.5 gm
Sodium hydroxide to adjust pH 6.5 to 7.5
Water for injection Q.S. to make 100 ml

B. Clonidine 0.2%

Clonidine hydrochloride 0.2 gm
Benzylconium chloride 0.0107 gm
Carbopol 981 1.5 gm to 2.5 gm
Sodium hydroxide to adjust pH 6.5 to 7.5
Water for injection Q.S. to make 100 ml

VIII. Clonidine**A. Clonidine 0.1%**

Clonidine hydrochloride	0.1 gm
Benzylconium chloride	0.0107 gm
Polycarbophil	1.5 gm to 2.5 gm
Sodium hydroxide to adjust pH	6.5 to 7.5
Water for injection	Q.S. to make 100 ml

B. Clonidine 0.2%

Clonidine hydrochloride	0.2 gm
Benzylconium chloride	0.0107 gm
Polycarbophil	1.5 gm to 2.5 gm
Sodium hydroxide to adjust pH	6.5 to 7.5
Water for injection	Q.S. to make 100 ml

IX. Betaxolol**Betaxolol 0.5%**

Betaxolol hydrochloride	0.56 gm. equivalent to 0.5 gm of Betaxolol
Benzylconium chloride	0.01 gm
Dibasic sodium phosphate	0.05 gm
Sodium phosphate monobasic	0.025 gm
Disodium EDTA	0.05 gm
Sodium chloride	0.3 gm
Propylene glycol	2.5 gm
Carbopol ETD 2001	2.0 gm
Water for injection	Q.S. to make 100 ml.

X. Betaxolol

Betaxolol 0.5%

Betaxolol hydrochloride 0.56 gm. equivalent to 0.5 gm of Betaxolol

Benzylconium chloride 0.01 gm

Dibasic sodium phosphate 0.05 gm

Sodium phosphate monobasic 0.025 gm

Disodium EDTA 0.05 gm

Sodium chloride 0.3 gm

Propylene glycol 2.5 gm

Polyacrylic acid 2.0 gm

(Carbopol 940)

Water for injection Q.S. to make 100 ml

X.I Betaxolol

Betaxolol 0.5%

Betaxolol hydrochloride 0.56 gm. equivalent to 0.5 gm of Betaxolol

Benzylconium chloride 0.01 gm

Dibasic sodium phosphate 0.05 gm

Sodium phosphate monobasic 0.025 gm

Disodium EDTA 0.05 gm

Sodium chloride 0.3 gm

Propylene glycol 2.5 gm

Carbopol 981 2.0 gm

Water for injection Q.S. to make 100 ml.

XI.I. Betaxolol

Betaxolol 0.5%

Betaxolol hydrochloride 0.56 gm. equivalent to 0.5 gm of Betaxolol

Benzylconium chloride 0.01 gm

Dibasic sodium phosphate 0.05 gm

Sodium phosphate monobasic 0.025 gm

Disodium EDTA 0.05 gm

Sodium chloride 0.3 gm

Propylene glycol 2.5 gm

Polycarbophil 2.0 gm

Water for injection Q.S. to make 100 ml.

Pharmaceutical composition so manufactured is evaluated for stability and efficacy.

The Pharmaceutical composition so manufactured is evaluated at different test conditions of temperature and humidity (as per ICH guidelines, 40°C/75% RH, 25°C/60% RH) for time interval extending upto 12 months.

The samples of formulations so prepared were used for study.

The topical ophthalmic preparation of Clonidine 0.1% and 0.2% so prepared were evaluated in vivo studies.

Healthy normal volunteers (10) had instillation of drug in their eyes. I.O.P. and B.P. were measured every 2 hours. Control group (10) received placebo.

Drop in I.O.P. was seen only in eyes receiving Clonidine. It was found to be in the range of 30% of initial I.O.P. The effect on I.O.P. was found to last 8 – 10 hours.

Effect on blood pressure in both groups i.e. Clonidine and placebo was identical for systolic as well as diastolic blood pressure and it was insignificant. Peak reduction in systolic B.P. was 4.4 mm of Hg was for placebo, 4.11 mm of Hg for 0.1% Clonidine and 3.93 mm. of Hg for 0.2% Clonidine. Peak reduction in diastolic B.P. was 4.23 mm of Hg for placebo, 4.49 mm of Hg. for Clonidine 0.1% and 2.89 mm of Hg for Clonidine 0.2%.

Clonidine eye drops even when used at 0.05 % to 0.06 % concentration are associated with reduction in systemic B.P.

The topical ophthalmic preparation of Timolol Maleate 0.5% made according to present invention was evaluated for systemic effects and compared with Timolol eye drops and Timoptic XE.

In healthy normal volunteers (10 in each group) various formulations of Timolol and placebo drug were instilled in both eyes. I.O.P. and resting pulse rate were measured every 2 hours. The peak reduction in mean I.O.P. was 25% with Timolol drops, 27% with Timoptic XE and 40% with Timolol made as per present invention. The change in resting pulse rate was identical in placebo and Timolol as per present invention. It was 13.2% with Timolol drops and 13.33% with Timoptic XE.

Thus, both the preparations of Clonidine as well as Timolol made according to present invention were found to have no systemic effect. The efforts were made to find out plasma concentration of drugs but none of the drugs achieved detectable plasma concentrations.

Thus, present invention provides process for manufacturing of topical ophthalmic preparations without systemic effects.

The above examples of formulations are provided as a proof of working of this invention and should not be restricted to this only. Any drug useful after instillation into eye can be formulated according to present invention without systemic effect.

I claim :

1. The process of manufacturing of formulation of topical ophthalmic preparations without systemic effect comprising the following steps.
 - i. Making a gel using polymers with or without physiologically acceptable excipients buffers and preservatives
 - ii. Adding liquid formulation of a drug into a prepared gel of step (i) while stirring slowly.
 - iii. Adjusting the pH and volume before final packing
2. A process as claimed in claim 1 wherein Polymer can be any polymer having pseudoplastic behaviour.
3. A process as claimed in claim 1 & 2 wherein polymer should have mucoadhesive property.
4. A process as claimed in claim 1 to 3 wherein polymer can be but not restricting to Carbopol 940 (Polyacrylic acid), Carbopol ETD 2001, Carbopol 981, Polycarbophil, Polyvinyl Alcohol, Hydroxyethyl Cellulose, Polyacrylic esters, Acrypol, Xanthan gum, Guar gum, polyvinyl ester, Carbomer etc.
5. Polymer as claimed in claim 1 to 4 can be used alone or in combination with other polymers.

6. The process as claimed in claim 1 to 5 wherein viscosity of final formulation is more than 100,000 cps (One hundred thousand cps).
7. The process as claimed in claim 1 to 6 physiologically acceptable buffers excipients and preservatives are used.
8. The process as claimed in claim 1 to 7 wherein liquid formulation of a drug can be in the form of aqueous solution, suspension or emulsion.
9. The process as claimed in claim 1 to 8 wherein volume of formulation is adjusted to get desired concentration of a drug into final formulation.
10. A process as claimed in claim 1 to 9 and substantially described in example in accompanying specification.

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It is a primary object of the present invention to provide an aqueous ophthalmic formulation, for treating myopia, comprising pirenzepine in combination with a pharmaceutically acceptable gel carrier.

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(54) Title: PIRENZEPINE OPHTHALMIC GEL

(57) Abstract: It is a primary object of the present invention to provide an aqueous ophthalmic formulation, for treating myopia, comprising pirenzepine in combination with a pharmaceutically acceptable gel carrier.

PIRENZEPINE OPHTHALMIC GEL

Background of the Invention

5 Field of the Invention

The present invention is in the field of aqueous ophthalmic pharmaceutical formulations.

Background of the Invention

- 10 Myopia, axial elongation of the eye, affects a large proportion of the population. Commonly, the onset of myopia is during the grade school years and progresses until growth of the eye is completed. A pharmacologic therapy which prevents or retards the developmental abnormality of myopia would represent a major advance in the treatment of myopia.
- 15 The potential use for a pharmacologic therapy has been stimulated by evidence that atropine prevents the development of myopia in humans (DA Goss. 1982. Attempts to reduce the rate of increase of myopia in young people--a critical literature review. *Am. J. Optom. Physiol. Opt.* 59: 828-841.), tree shrews (McKanna JA, and VA Casagrande. 1978. Reduced lens development in lid-suture myopia. *Exp. Eye Res.* 26: 715-723.),
- 20 stump-tailed monkeys and chicks (McBrien NA, Moghaddam HO, Reeder AP, and S. Moules. 1991a. Structural and biochemical changes in the sclera of experimentally myopic eyes. *Biochem. Soc. Trans.* 19: 861-865; McBrien NA, Moghaddam HO, and AP Reeder. 1991b. Atropine reduces axial elongation and myopia in visually impaired chick eyes. *Invest. Ophthalmol. Vis. Sci.* 32: 1203; Tigges M, Sugrue MF, Mallorga P, Stone
- 25 RA, Laties AM, Fernandes A, and PM Iuvone. 1996. Effects of atropine, ATR, and pirenzepine, PIR, on ocular growth and muscarinic cholinergic receptors in young rhesus monkeys. *Invest. Ophthalmol. Vis. Sci.* 37: S326.). The clinical use of atropine as a therapy has been limited due to its ocular side effects including glare from pupillary dilation and blurred vision due to loss of accommodation. Mild cycloplegic agents like
- 30 tropicamide have been effective in a number of studies but failed in other studies (Curtin BJ and DB Karlin. 1971. Axial length measurements and fundus changes of the myopic eye. *Am. J. Ophthalmol.* 71: 42-53.).

Stone and Laties found that subconjunctival injections of atropine, a nonselective muscarinic antagonist, and pirenzepine, a relatively selective M1-antagonist marketed for systemic use in Europe for its anti-dyspepsia properties, attenuated axial eye growth in a chick model of myopia. M2 and M3 antagonists did not prevent axial elongation (Stone RA, Lin T, and AM Laties. 1991. Muscarinic antagonist effects on experimental chick myopia. *Exp. Eye Res.* 52: 755-758; U.S. Patent No. 5,112,522, Filed May 11, 1990.). Unlike atropine, a selected concentration of pirenzepine may prevent myopia without inducing unwanted side effects such as disabling mydriasis and cycloplegia.

Pirenzepine is a relatively selective M1-muscarinic antagonist. It is being investigated for its topical ocular use to moderate and halt the progression of pediatric myopia. Administered as a solution in up to 2% strength, it was found comfortable and without systemic effects in adult volunteers (Shedden AH, Sciberras D, Hutzelmann J, and C van Nispen. 1998. Tolerability of pirenzepine ophthalmic solution in adult male volunteers. *Invest. Ophthalmol. Vis. Sci.* 39: S279.).

However, work on a solution dosage form of pirenzepine indicated a physical appearance problem. Pirenzepine is stable in solution especially at pH 5, but its degradation product is insoluble in water. Thus, the accumulation of even a small amount of degradation product over the shelf-life of the solution results in an unacceptable product due to the unattractive appearance of the precipitate in the solution.

There are no "standard" formulation solutions for such a problem. One approach is to use a refrigerated solution. Another approach is to use a lyophilized product for reconstitution prior to dispensing to the patient. However, neither of these approaches is optimal. Lyophilization adds considerably to the cost of the product and requires cumbersome reconstitution processes. Refrigeration is not always convenient. Thus, there is a need for pirenzepine in a dosage form that solves the physical appearance problem using a formulation approach that is deemed desirable.

Summary of the Invention

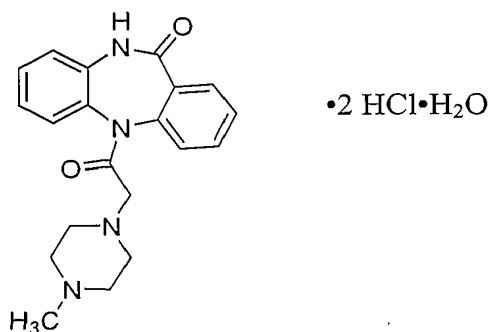
Accordingly, it is a primary object of the present invention to provide an aqueous ophthalmic formulation, for treating myopia, comprising pirenzepine in combination with a pharmaceutically acceptable gel carrier.

Detailed Description of the Preferred Embodiment

The ophthalmic aqueous gel formulation of the present invention for treating myopia comprises a pharmaceutically effective amount of pirenzepine in combination with a water soluble cellulose derivative.

5 The concentration of the pirenzepine in the present formulation may range from about 0.001 to 3% (w/v), preferably about 0.005 to 2% (w/v). Pirenzepine and its dihydrochloride salt are known in the art.

Below is the structure of pirenzepine dihydrochloride:



10 Molecular formula: C₁₉H₂₁N₅O₂•2 HCl•H₂O
 Molecular weight: 442.3; 351.4 (anhydrous free base)
 Chemical Names: 5,11-dihydro-11-[(4-methyl-1-piperazinyl)acetyl]-6H
 pyrido[2,3-b][1,4]-benzodiazepin-6-one dihydrochloride
 monohydrate
 15 11-[(4-methyl-1-piperazinyl)acetyl]-pyrido[2,3-b][1,4]-
 benzodiazepin-6(5H)-one dihydrochloride monohydrate

Cellulose derivatives are used as gelling agents in the formulation of this invention. Most preferred is hydroxypropyl methylcellulose. Any cellulose derived gelling agent,
 20 however, that forms an aqueous gel at the desired viscosity, i.e., is soluble in water and forms a gel, can be used. Such derivatives are well known, as are their properties, and are described, e.g., in the U.S. Pharmacopeia (2000) (UNITED STATES PHARMACOPEIAL
 CONVENTION, INC., THE UNITED STATES PHARMACOPEIA/THE NATIONAL
 FORMULARY (2000)). Such gelling agents include, but are not limited to, methyl
 25 cellulose, hydroxypropyl cellulose, carboxymethyl cellulose, hydroxyethyl cellulose, and cellulose gum. Combinations of various derivatives may also be used. Cellulose based

gelling agents are advantageous over, for example, cross-linked acrylic polymers. For example, Carbopol™, a cross-linked acrylic polymer, has been used to form an aqueous gel containing pilocarpine hydrochloride for ophthalmic use. Cellulose based gelling agents, however, are less likely to cause adverse reactions.

5 The formulations of the invention are substantially viscous enough to form a viscous gel. The viscosity preferably is in the range of 10,000 to 300,000 centipoise (cps), most preferably 15,000-200,000 cps, at about 20 °C and shear rate of 1s^{-1} based on Brookfield RVDV analysis.

10 In the aqueous gel for ophthalmic use, the amount of cellulose based gelling agent is preferably from about 0.5 wt. % to 5 wt. %, most preferably from about 1 wt. % to 5 wt. %.

15 Suitable cellulose based preparations for use in the invention are commonly commercially available. For example, sources of hydroxypropyl methylcellulose that are suitable for making a cellulose based ophthalmic gel according to the invention include Ashland Distribution Co., Asiaamerica International Inc., Biddle Sawyer Corp., Carbomer Inc., Colorcon Inc., Dow Chemical Co., FOB Chemicals, Hercules Inc., Mutchler Inc., Penta Mfg Co., Spectrum Laboratory Products Inc., Van Waters & Rogers Inc., and Warner Jenkinson.

20 The formulation may contain additional pharmaceutically inactive substances. For example, it may contain one or more solubilizing agents, such as polysorbate 20, polysorbate 40, polysorbate 60, or polysorbate 80. The formulation may also contain a dispersant, such as lecithin or glycerin. Collagen can also be added. Other additives include cyclodextrins, in particular alpha, beta, and gamma cyclodextrins. Also, vitamin E, particularly in solubilized form, or other antioxidants, including butylate hydroxyanisole (BHA) and butylate hydroxytoluene (BHT), may be added. Some additional examples of
25 inactives follow: sodium chloride, cetrimide, thimerosal, benzalkonium chloride, boric acid, sodium carbonate, potassium chloride, propylene glycol, polyoxyethylene, polyoxypropylene, polyoxyl 40 stearate, polyvinyl alcohol, poloxamer 188, sodium citrate, sodium thiosulfate, sodium bisulfite, dextran 70, acetic acid, polyethylene glycol, povidone,
30 dextrose, magnesium chloride, alginic acid, sodium acetate, sodium borate, edetate disodium, sodium hydroxide, and hydrochloric acid. The optimal amount of inactive ingredient employed in the formulation can be conventionally determined based on the particular active pharmaceutical, and the intended use.

The formulation of the invention can be placed in any desired dispensing device suitable for an ophthalmic formulation. The device can be an ophthalmic delivery system such as a sterile ophthalmic tube, for example, a conventional 3.5-5 g tube having an ophthalmic tip and containing the ophthalmic formulation of the invention, or a sterile,
5 single or daily use container containing 0.1-0.5 g of the formulation.

The pharmaceutical formulations can be administered via various routes including ocular instillation, subconjunctival administration, and intravitreal administration. A typical daily dose of pirenzepine may range 6 mg or less/whole body weight, preferably 4 mg or less/whole body weight, and can be administered in a single dose or in divided doses.
10 However, it should be understood that the amount of pirenzepine actually administered ought to be determined in light of various relevant factors including the myopia to be treated, the chosen route of administration and the severity of the patient's symptom; and, therefore, the above dose should not be intended to limit the scope of the invention in any way.

15 The stability data generated on the gel and solution dosage forms show the superiority of the gel over the solution in maintaining an acceptable physical appearance in the presence of the small amounts of the water-insoluble degradation product referred to in the background of the invention.

The following Examples are intended to further illustrate the scope of the invention
20 without limiting its scope.

Example 1

An aqueous ophthalmic gel of 2.0% pirenzepine for the treatment of myopia according to the present invention was prepared as follows:
25

Table 1. Pirenzepine ophthalmic gel formulations.

<u>Ingredient</u>	0.5% (in mg/g)	1.0% (in mg/g)	2.0% (in mg/g)
Pirenzepine dihydrochloride	6.3	12.6	25.2
<i>(base equivalent)</i>	<i>(5.0)</i>	<i>(10.0)</i>	<i>(20.0)</i>
Hydroxypropyl Methyl-Cellulose (K100M, Dow Chemical Co.)	20	20	20
Sodium acetate	0.40	0.40	0.40
Benzalkonium chloride	0.05	0.05	0.05
Edetate disodium	0.15	0.15	0.15
Sodium chloride	5.0	3.5	0.0
Sodium Hydroxide (q.s. to pH)	5.0	5.0	5.0
Purified Water, q.s. to	1.00g	1.00g	1.00g

Part 1: Purified water was heated to 80-90 °C. Hydroxypropyl methylcellulose (HPMC) was added and mixed until it was uniformly dispersed. The pH was adjusted to 5.0 ± 1.0 with sodium hydroxide, but this was not a critical step and can be eliminated. After being placed in a pressure vessel, the mixture was sterilized at 121 °C for 30-45 minutes. In another embodiment, autoclaving is conducted under nitrogen when oxygen plays a role in viscosity loss upon autoclaving. The mixture was cooled to 25° to 30 °C and mixed for several hours to yield a homogenous viscous gel. Batches manufactured in the appropriate jacketed pressure vessel showed that chilling Part 1 (the hydroxypropyl methylcellulose phase) to about 10 °C rather than to 25° to 30 °C after autoclaving greatly enhanced the hydration and consequently the viscosity of the gel. The gel was stored at 25° to 30 °C for several hours to aid in dissolution and then maintained at 25° to 30 °C for storage.

Part 2: The rest of the ingredients were mixed and dissolved in water until a clear solution was obtained. The pH was adjusted to 5.0 ± 1.0 with sodium hydroxide. The solution was sterilized by membrane filtration (0.2 microns).

WHAT IS CLAIMED IS:

1. An aqueous ophthalmic gel formulation for the treatment of myopia comprising pirenzepine and an amount of gelling agent effective to form an aqueous gel, said gel having a Brookfield RVDV viscosity of from about 10,000 to about 300,000 cps at about 20°C and sheer rate of 1s^{-1} , wherein said gelling agent is a water soluble cellulose derivative.
2. A formulation according to claim 1 wherein the concentration of said pirenzepine is from about 0.001 to 3 % (w/v).
3. A formulation according to claim 1 wherein the concentration of said pirenzepine is from about 0.005 to 2 % (w/v).
4. A formulation according to claim 1 wherein said water soluble cellulose derivative is soluble in said aqueous formulation at a viscosity of about 15,000 to about 200,000 cps at about 20°C and sheer rate of 1s^{-1} .
5. A formulation according to claim 1 wherein said water soluble cellulose derivative is soluble in said aqueous formulation at a viscosity of about 100,000 cps at about 20°C and sheer rate of 1s^{-1} .
6. A formulation according to claim 1 wherein said amount of gelling agent is an amount of from about 0.5 to 5 wt. %.
7. A formulation according to claim 1 wherein said amount of gelling agent is an amount of from about 1 to 5 wt. %.
8. A formulation according to claim 1, further comprising at least one member selected from the group consisting of sodium chloride, cetrinide, thimerosal, benzalkonium chloride, boric acid, sodium carbonate, potassium chloride, propylene glycol, polyoxyethylene, polyoxypropylene, polyoxyl 40 stearate, polyvinyl alcohol, poloxamer 188, sodium citrate, sodium thiosulfate, sodium bisulfite, dextran 70, acetic acid, polyethylene glycol, povidone, dextrose, magnesium chloride, alginic acid, sodium acetate, sodium borate, edetate disodium, sodium hydroxide, and hydrochloric acid.
9. A formulation according to claim 1 wherein said gelling agent is at least one member selected from the group consisting of hydroxypropyl methylcellulose, methyl cellulose, hydroxypropyl cellulose, carboxymethyl cellulose, hydroxyethyl cellulose, and cellulose gum.
10. A formulation according to claim 1 wherein said gelling agent is hydroxypropyl methylcellulose.

11. An ophthalmic delivery system containing the formulation of claim 1.

12. The ophthalmic delivery system of claim 11 comprising an ophthalmic tube having an ophthalmic tip and containing said aqueous gel.

5 13. A method of treating myopia comprising administering the formulation of claim 1 to the eye of a human individual, whereby myopia is treated.

14. The method of claim 13 wherein said human individual is a pediatric subject.

15. Use of the formulation of claim 1 in the preparation of a medicament for the treatment of myopia.

10 16. Use of the formulation of claim 1 in the preparation of a medicament for the treatment of myopia in a pediatric subject.

17. A method of making the formulation of claim 1 comprising autoclaving a mixture comprised of said gelling agent and water, sterile filtering a solution comprising said pirenzepine and water, and aseptically admixing them.

15 18. The method of the claim 17 wherein said autoclaving step is conducted under nitrogen.

19. A formulation according to claim 1 wherein the formulation is selected from the group consisting of the formulations of Table 1.

20. A formulation according to claim 1 in sterile form.

20

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International application No.

PCT/US02/13823

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US CL : 514/374, 912

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/374, 912

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEAST

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Database USPT on WEST, US 5,637,604 A, (PENNSYLVANIA), LATIES et al, 10 June 1997, see the entire document.	1-20

☐

Further documents are listed in the continuation of Box C.

☐

See patent family annex.

* Special categories of cited documents:		"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

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(54) Title: INHALANT FORMULATION CONTAINING SULFOALKYL ETHER CYCLODEXTRIN AND CORTICOSTEROID

(57) Abstract: An inhalable formulation containing SAE-CD and corticosteroid is provided. The formulation is adapted for administration to a subject by nebulization with any known nebulizer. The formulation can be included in a kit. The formulation is administered as an aqueous solution, however, it can be stored as a dry powder, ready-to-use solution, or concentrated composition. The formulation is employed in an improved nebulization system for administering corticosteroid by inhalation. SAE-CD present in the formulation significantly enhances the chemical stability of budesonide. A method of administering the formulation by inhalation is provided. The formulation can also be administered by conventional nasal delivery apparatus.



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Inhalant Formulation Containing Sulfoalkyl Ether Cyclodextrin and Corticosteroid

BY:

James D. Pipkin, Rupert O. Zimmerer, Diane O. Thompson, Gerold L. Mosher

CROSS-REFERENCE TO EARLIER FILED APPLICATIONS

The present application is a continuation-in-part of and claims the priority of U.S. application No. 11/479,979 filed June 30, 2006.

FIELD OF THE INVENTION

5 The present invention relates to methods of and systems for administering formulations of sulfoalkyl ether cyclodextrin and corticosteroid, such as budesonide, by nebulization and inhalation. The invention also relates to methods of treating respiratory diseases or disorders of the air passageways.

BACKGROUND OF THE INVENTION

10 The delivery of a drug by inhalation allows deposition of the drug in different sections of the respiratory tract, e.g., throat, trachea, bronchi and alveoli. Generally, the smaller the particle size, the longer the particle will remain suspended in air and the farther down the respiratory tract the drug can be delivered. Corticosteroids are delivered by inhalation using nebulizers, metered dose inhalers, or dry powder inhalers. The principle
15 advantages of nebulizers over other methods of pulmonary installation are that patient cooperation is not required and the delivery of higher doses of medication is easier. The main concerns about nebulizers, however, are their increased cost, reduced portability and the inconvenience of needing to prepare medication beforehand and the increased time requirement for administering a treatment. A method of improving the administration of
20 drugs, such as corticosteroids by nebulization would be desired.

Budesonide ((R,S)-11 β , 16 α , 17, 21-tetrahydroxypregna-1, 4-diene-3, 20-dione cyclic 16, 17-acetal with butyraldehyde; C₂₅H₃₄O₆; Mw: 430.5) is well known. It is provided commercially as a mixture of two isomers (22R and 22S). Budesonide is an anti-inflammatory corticosteroid that exhibits potent glucocorticoid activity. Administration of

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budesonide is indicated for maintenance treatment of asthma and as prophylactic therapy in children.

Commercial formulations of budesonide are sold by AstraZeneca LP (Wilmington, DE) under the trademarks ENTOCORT™ EC, PULMICORT RESPULES®, Rhinocort Aqua®, Rhinocort® Nasal Inhaler and Pulmicort Turbuhaler®, and under its generic name. PULMICORT RESPULES® suspension, which is a sterile aqueous suspension of micronized budesonide, is administered by inhalation using a nebulizer, in particular a compressed air driven jet nebulizer that delivers from 2 to 18% of the drug mass contained in the nominal charge. The general formulation for a unit dose of the PULMICORT RESPULES is set forth in U.S. Patent No. 6,598,603, and it is an aqueous suspension in which budesonide is suspended in an aqueous medium comprising about 0.05 to 1.0 mg of budesonide, 0.05 to 0.15 mg of NaEDTA, 8.0 to 9.0 mg of NaCl, 0.15 to 0.25 mg of polysorbate, 0.25 to 0.30 mg of anhydrous citric acid, and 0.45 to 0.55 mg of sodium citrate per one ml of water. RHINOCORT® NASAL INHALER™ is a metered-dose pressurized aerosol unit containing a suspension of micronized budesonide in a mixture of propellants. RHINOCORT® AQUA™ is an unscented metered-dose manual-pump spray formulation containing a suspension of micronized budesonide in an aqueous medium. The suspensions should not be administered with an ultrasonic nebulizer.

A wide variety of nebulizers differing in mode of operation are available, e.g. jet nebulizers (optionally sold with compressors), ultrasonic nebulizers, vibrating membrane, vibrating mesh nebulizers, vibrating plate nebulizers, vibrating cone nebulizers, and others. The vibrating mesh, vibrating cone or vibrating plate nebulizers are of particular interest since they do not require the use of an air compressor for delivery, have a minimal residual volume in the reservoir after delivery of a unit dose, and can be used to deliver low volumes of inhalable solutions. Exemplary vibrating membrane, mesh or plate nebulizers are described by R. Dhand (*Respiratory Care*, (Dec. 2002), 47(12), p. 1406-1418). Keller et al. (ATS 99th International Conference, Seattle, May 16th-21st, 2003; poster 2727) disclose the results of a study on the administration of AZTREONAM with a PARI eFlow nebulizer and report low oropharyngeal deposition, nebulization efficiency unaffected by fill volume, a respirable fraction of 82±1.7%, constant dose administration per inhalation cycle time, and an excellent correlation between delivered dose, fill volume and nebulization time, and expected high lung deposition.

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The desired properties of a liquid for nebulization generally include: 1) reduced viscosity; 2) sterile medium; 3) reduced surface tension; 4) stability toward the mechanism of the nebulizer; 5) moderate pH of about 4-10; 6) ability to form droplets with an MMAD of <5 μm or preferably <3 μm ; 7) absence of irritating preservatives and stabilizing agents; 8) suitable tonicity. On the one hand, suspensions possess some advantages but on the other hand solutions possess other advantages.

Smaldone et al. (*J. Aerosol Med.* (1998), 11, 113-125) disclose the results of a study on the *in vitro* determination of inhaled mass and particle distribution of a budesonide suspension. They conclude that 2%-18% of the nebulizer's charge of budesonide was delivered from the suspension, meaning that budesonide delivery was incomplete resulting in a significant waste of drug. In the thirteen most efficient systems, the suspension can be nebulized sufficiently well for lower respiratory tract delivery.

Another study further demonstrated the highly variable efficiency of nebulization from one nebulizer to another. Barry et al. (*J. Allergy Clin. Immunol.* (1998), 320-321) state that this variability should be taken into account when treating patients with nebulized budesonide. Berg et al. (*J. Aerosol Sci.* (1998), 19(7), 1101-1104) also report the highly variable efficiency of nebulization of PULMICORT™ suspension from one nebulizer to the next. Moreover, the mass mean aerodynamic diameter (MMAD) of the nebulized droplets is highly variable from one nebulizer to the next. In general, suspensions are less efficiently nebulized than solutions, O'Riordan (*Respiratory Care*, (2002), 1305-1313). Inhaled corticosteroids are utilized in the treatment of asthma and are of significant benefit because they are delivered directly to the site of action, the lung. The goal of an inhaled corticosteroid is to provide localized therapy with immediate drug activity in the lungs. Inhaled corticosteroids are well absorbed from the lungs. In fact, it can be assumed that all of the drug available at the receptor site in the lungs will be absorbed systemically. However, it is well known that using current methods and formulations the greater part of an inhaled corticosteroid dose is swallowed and becomes available for oral absorption, resulting in unwanted systemic effects. For inhaled corticosteroids, high pulmonary availability is more important than high oral bioavailability because the lung is the target organ. A product with high pulmonary availability has greater potential to exert positive effects in the lung. The ideal inhaled corticosteroid formulation would provide minimum oral delivery thereby reducing the likelihood of systemic adverse effects.

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The majority of the corticosteroid dose delivered to the lung is absorbed and available systemically. For the portion of the inhaled corticosteroid dose delivered orally, bioavailability depends upon absorption from the GI tract and the extent of first pass metabolism in the liver. Since this oral component of corticosteroid drug delivery does not provide any beneficial therapeutic effect but can increase systemic side effects, it is desirable for the oral bioavailability of inhaled corticosteroid to be relatively low.

Both particle size and formulation influence the efficacy of an inhaled corticosteroid. The formulation of a drug has a significant impact on the delivery of that drug to the lungs, and therefore its efficacy. Most important in the delivery of drug to the lung are the aerosol vehicle and the size of the particles delivered. Additionally, a reduced degree of pulmonary deposition suggests a greater degree of oropharyngeal deposition. Due to a particular formulation employed, some corticosteroids are more likely to be deposited in the mouth and throat and may cause local adverse effects.

While receptor distribution is the major determinant of bronchodilator efficacy, particle size appears to be more important in determining the efficacy of an inhaled corticosteroid. The smallest airways have an internal diameter of 2 micrometers (mcm) or less. Thus, an inhaler with particles having a mean aerodynamic diameter of 1 mcm should have a greater respirable fraction than an inhaler with particles that have an average diameter of 3.5 to 4 mcm. For patients with obstructive lung disease, all particles should ideally be no greater than 2 to 3 mcm. A particle that is small (less than 5 mcm) is more likely to be inhaled into the smaller airways of the lungs, thus improving efficacy. In contrast, particles that are larger than 5 mcm can be deposited in the mouth and throat, both reducing the proportion of particles that reach the lungs and potentially causing local adverse effects such as oral candidiasis and hoarseness (dysphonia). Particles having a mass median aerodynamic diameter (MMAD) of close to 1 mcm are considered to have a greater respirable fraction per dose than those with a diameter of 3.5 mcm or greater.

A further disadvantage to the nebulization of budesonide suspensions is the need to generate very small droplets, MMAD of about $<3\text{ }\mu\text{m}$. Since the nebulized droplets are so small, then the micronized budesonide must be even smaller or in the range of $0.5\text{-}2.0\text{ }\mu\text{m}$ and the particles should have a narrow particle size distribution. Generation of such particles is difficult.

Even so, efforts have been made to improve the nebulization of budesonide suspensions with ultrasonic nebulizers by using submicron-sized particles (Keller et al. in

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Respiratory Drug Delivery VIII (2002), 197-206). A suspension of nanoparticles (0.1-1.0 μm) of the corticosteroid might be used to increase the proportion of respirable particles as compared to a coarser suspension as in the PULMICORT™ suspension. No improvement over PULMICORT™ suspension (about 4.4 μm budesonide particle size in suspension) was observed. Moreover, concerns exist regarding the use of nanosuspensions in that the small particles (<0.05 μm) may induce an allergic response in a subject. Sheffield Pharmaceuticals, Inc. (St. Louis, MO; "The Pharmacokinetics of Nebulized Nanocrystal Budesonide Suspension in Healthy Volunteers", Kraft, et al. in *J. Clin. Pharmacol.*, (2004), 44:67-72) has disclosed the preparation and evaluation of UDB (unit dose budesonide), which is a suspension-based formulation containing nanoparticles of budesonide dispersed in a liquid medium. This product is being developed by MAP Pharmaceuticals, Inc. (Mountain View, California). Seeman et al. (ATS 99th International Conference, Seattle, May 16th-21st, 2003; poster 2727) disclose the results of a study evaluating the performance of the PARI eFlow nebulizer with a budesonide suspension (PULMICORT RESPULES, 500 $\mu\text{g}/\text{ml}$, in a 2 ml ampoule) and report achieving a MMAD of 3.6-4.2 μm and a respirable fraction of greater than 67%.

The inhalation of drug particles as opposed to dissolved drug is known to be disadvantageous. Brain et al. (Bronchial Asthma, 2nd Ed. (Ed. E.B. Weis et al., Little Brown & Co. (1985), pp. 594-603) report that less soluble particles that deposit on the mucous blanket covering pulmonary airways and the nasal passages are moved toward the pharynx by the cilia. Such particles would include the larger drug particles deposited in the upper respiratory tract. Mucus, cells and debris coming from the nasal cavities and the lungs meet at the pharynx, mix with saliva, and enter the gastrointestinal tract upon being swallowed. Reportedly, by this mechanism, particles are removed from the lungs with half-times of minutes to hours. Accordingly, there is little time for solubilization of slowly dissolving drugs, such as budesonide. In contrast, particles deposited in the nonciliated compartments, such as the alveoli, have much longer residence times. Since it is difficult to generate very small particles of budesonide for deep lung deposition, much of the inhaled suspension would likely be found in the upper to middle respiratory tract. However, it is much easier to generate small droplets from a solution than it is from a suspension of solids. For these reasons, nebulization of a budesonide-containing solution should be preferred over that of a suspension.

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O’Riordan (*Respiratory Care* (2002 Nov), 47(11), 1305-1313) states that drugs can be delivered by nebulization of either solutions or suspensions, but that in general, nebulization of a solution is preferred over that of a suspension. He states that ultrasonic nebulizers should not be used on suspensions and should be used only on solutions.

5 O’Callaghan (*Thorax*, (1990), 45, 109-111), Storr et al. (*Arch. Dis. Child* (1986), 61, 270-273), and Webb et al. (*Arch. Dis. Child* (1986), 61, 1108-1110) suggest that nebulization of corticosteroid (in particular beclomethasone) solutions may be preferred over that of suspensions because the latter may be inefficient if the nebulized particles are too large to enter the lung in therapeutically effective amounts. However, data presented
10 by O’Callaghan (*J. Pharm. Pharmacol.* (2002), 54, 565-569) on the nebulization of flunisolide solution versus suspension showed that the two performed similarly. Therefore, it cannot be generalized that nebulization of a solution is preferred over that of a suspension.

Accordingly, there is a widely recognized need for a non-suspension formulation
15 comprising a corticosteroid for administration via nebulization. However, the PULMICORT® suspension unit dose formulation is widely available and accepted in the field of inhalation therapy. It would be of great benefit to this field of therapy to provide a method of improving the administration of the PULMICORT® suspension unit dose formulation, or more generally, of a suspension unit dose formulation containing a
20 corticosteroid.

However, the current focus in nebulizer therapy is to administer higher concentrations of drug, use solution, preferably predominantly aqueous-based solutions in preference to non-aqueous or alcoholic or non-aqueous alcoholic solutions or suspensions if possible, minimize treatment time, synchronize nebulization with inhalation, and
25 administer smaller droplets for deeper lung deposition of drug.

Corticosteroid-containing solutions for nebulization are known. There are a number of different ways to prepare solutions for nebulization. These generally have been prepared by the addition of a cosolvent, surfactant, or buffer. However, cosolvents, such as ethanol, polyethylene glycol and propylene glycol are only tolerated in low amounts
30 when administered by inhalation due to irritation of the respiratory tract. There are limits to acceptable levels of these cosolvents in inhaled products. Typically, the cosolvents make up less than about 35% by weight of the nebulized composition, although it is the total dose of cosolvent as well as its concentration that determines these limits. The limits

are set by the propensity of these solvents either to cause local irritation of lung tissue, to form hyperosmotic solutions that would draw fluid into the lungs, and/or to intoxicate the patient. In addition, most potential hydrophobic therapeutic agents are not sufficiently soluble in these cosolvent mixtures.

5 Saidi et al. (U.S. Patent No. 6,241,969) disclose the preparation of corticosteroid-containing solutions for nasal and pulmonary delivery. The dissolved corticosteroids are present in a concentrated, essentially non-aqueous form for storage or in a diluted, aqueous-based form for administration.

10 Keller et al. (in Respiratory Drug Delivery IX (2004) 221-231) disclose the deposition of solution formulations containing budesonide and surfactants to children.

 Lintz et al. (AAPS Annual Meeting and Exposition, Baltimore, Nov. 8, 2004; Poster M1128) disclose the preparation and aerosol characterization of liquid formulations containing budesonide, water, citrate salt, sodium chloride and alcohol, propylene glycol and/or surfactant, such as Tween, Pluronic, or phospholipids with HLB-values between 10
15 and 20. The aerosol characterization of such a combination surfactant solution in a vibrating membrane/mesh nebulizer (the Pari eFlow) compared to a suspension was studied using adult and child breath simulation. They reported a respirable fraction of 83.3% for the PARI eFlow with the solution and 61% for the PULMICORT RESPULES with the PARI LC+. They also reported the results regarding the respirable drug delivery
20 rate (% drug < 5µm/min), delivered dose (% of drug charged to device), drug delivery rate (% drug/min), and respirable dose (% < 5µm).

 Schueepp et al. (ATS 99th International Conference, Seattle, May 16th-21st, 2003; poster 1607) disclose assessment of the aerosol performance of a customized eFlow Baby Functional Model with an experimental budesonide solution (100 µg in 0.5 ml) utilizing a
25 baby cast model and applying different breathing patterns. .

 An alternative approach to administration of the PULMICORT™ suspension is administration of a liposome formulation. Waldrep et al. (*J. Aerosol Med.* (1994), 7(2), 135-145) reportedly succeeded in preparing a liposome formulation of budesonide and phosphatidylcholine derivatives.

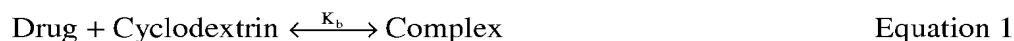
30 None of the above-identified formulations has provided a method of improving the administration of a suspension-based unit dose formulation containing a corticosteroid. Instead, the general focus of the art has been to completely circumvent formulating a

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suspension by first preparing a liquid formulation that is then divided into multiple unit doses that are packaged for marketing and then sold for use.

Solubilization of drugs by cyclodextrins and their derivatives is well known. Cyclodextrins are cyclic carbohydrates derived from starch. The unmodified
5 cyclodextrins differ by the number of glucopyranose units joined together in the cylindrical structure. The parent cyclodextrins contain 6, 7, or 8 glucopyranose units and are referred to as α -, β -, and γ -cyclodextrin respectively. Each cyclodextrin subunit has secondary hydroxyl groups at the 2 and 3 positions and a primary hydroxyl group at the 6-position. The cyclodextrins may be pictured as hollow truncated cones with hydrophilic
10 exterior surfaces and hydrophobic interior cavities. In aqueous solutions, these hydrophobic cavities provide a haven for hydrophobic organic compounds that can fit all or part of their structure into these cavities. This process, known as inclusion complexation, may result in increased apparent aqueous solubility and stability for the complexed drug. The complex is stabilized by hydrophobic interactions and does not
15 involve the formation of any covalent bonds.

This dynamic and reversible equilibrium process can be described by Equations 1 and 2, where the amount in the complexed form is a function of the concentrations of the drug and cyclodextrin, and the equilibrium or binding constant, K_b . When cyclodextrin formulations are administered by injection into the blood stream, the complex rapidly
20 dissociates due to the effects of dilution and non-specific binding of the drug to blood and tissue components.



$$25 \quad K_b = \frac{[\text{Complex}]}{[\text{Drug}][\text{Cyclodextrin}]} \quad \text{Equation 2}$$

Binding constants of cyclodextrin and an active agent can be determined by the equilibrium solubility technique (T. Higuchi et al. in "Advances in Analytical Chemistry and Instrumentation Vol. 4"; C.N. Reilly ed.; John Wiley & Sons, Inc, 1965, pp. 117-212).
30 Generally, the higher the concentration of cyclodextrin, the more the equilibrium process of Equations 1 and 2 is shifted to the formation of more complex, meaning that the

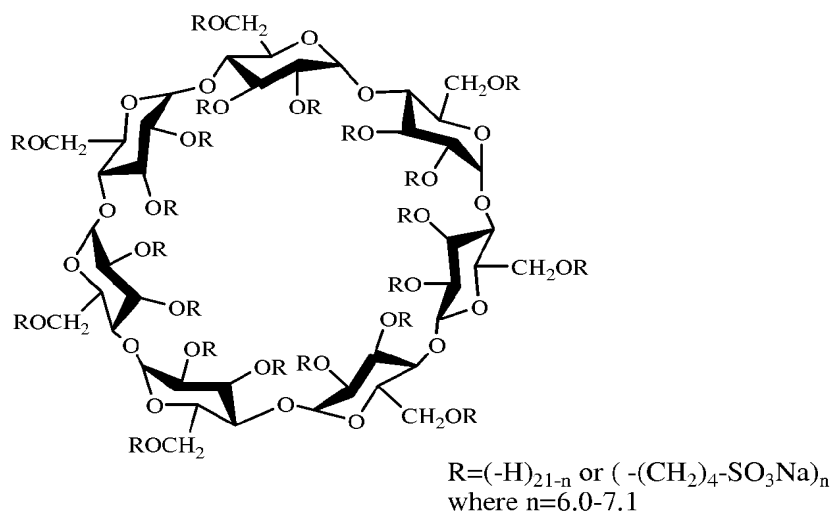
- 9 -

concentration of free drug is generally decreased by increasing the concentration of cyclodextrin in solution.

The underivatized parent cyclodextrins are known to interact with human tissues and extract cholesterol and other membrane components, particularly upon accumulation in the kidney tubule cells, leading to toxic and sometimes fatal renal effects.

The parent cyclodextrins often exhibit a differing affinity for any given substrate. For example, γ -cyclodextrin often forms complexes with limited solubility, resulting in solubility curves of the type Bs. This behavior is known for a large number of steroids which imposes serious limitations towards the use of γ -CD in liquid preparations. β -CD, however, does not complex well with a host of different classes of compounds. It has been shown for β -CD and γ -CD that derivatization, e.g. alkylation, results in not only better aqueous solubility of the derivatives compared to the parent CD, but also changes the type of solubility curves from the limiting Bs-type to the more linear A-type curve (Bernd W. Muller and Ulrich Brauns, "Change of Phase-Solubility Behavior by Gamma-Cyclodextrin Derivatization", *Pharmaceutical Research* (1985) p 309-310).

Chemical modification of the parent cyclodextrins (usually at the hydroxyls) has resulted in derivatives with improved safety while retaining or improving the complexation ability. Of the numerous derivatized cyclodextrins prepared to date, only two appear to be commercially viable: the 2-hydroxypropyl derivatives (HP-CD; neutral



Sulfobutyl Ether- β -Cyclodextrin (Captisol[®])

cyclodextrins being commercially developed by Janssen and others), and the sulfoalkyl ether derivatives, such as sulfobutyl ether, (SBE-CD; anionic cyclodextrins being

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developed by CyDex, Inc.) However, the HP- β -CD still possesses toxicity that the SBE-CD does not.

U.S. Patents No. 5,376,645 and No. 5,134,127 to Stella et al., U.S. Patent No. 3,426,011 to Parmerter et al., Lammers et al. (*Recl. Trav. Chim. Pays-Bas* (1972), 91(6), 733-742); *Staerke* (1971), 23(5), 167-171) and Qu et al. (*J. Inclusion Phenom. Macro. Chem.*, (2002), 43, 213-221) disclose sulfoalkyl ether derivatized cyclodextrins. The references suggest that SAE-CD should be suitable for solubilizing a range of different compounds. However, Stella discloses that the molar ratio of sulfoalkyl ether derivatized cyclodextrin to active ingredient suitable for solubilization of the active ingredient, even a corticosteroid, in water ranges from 10:1 to 1:10.

A sulfobutyl ether derivative of beta cyclodextrin (SBE- β -CD), in particular the derivative with an average of about 7 substituents per cyclodextrin molecule (SBE7- β -CD), has been commercialized by CyDex, Inc. as CAPTISOL[®]. The anionic sulfobutyl ether substituent dramatically improves the aqueous solubility of the parent cyclodextrin. In addition, the presence of the charges decreases the ability of the molecule to complex with cholesterol as compared to the hydroxypropyl derivative. Reversible, non-covalent, complexation of drugs with CAPTISOL[®] cyclodextrin generally allows for increased solubility and stability of drugs in aqueous solutions. While CAPTISOL[®] is a relatively new but known cyclodextrin, its use in the preparation of corticosteroid-containing solutions for nebulization has not previously been evaluated.

Hemolytic assays are generally used in the field of parenteral formulations to predict whether or not a particular formulation is likely to be unsuitable for injection into the bloodstream of a subject. If the formulation being tested induces a significant amount of hemolysis, that formulation will generally be considered unsuitable for administration to a subject. It is generally expected that a higher osmolality is associated with a higher hemolytic potential. As depicted in FIG. 1 (Thompson, D.O., *Critical Reviews in Therapeutic Drug Carrier Systems*, (1997), 14(1), 1-104), the hemolytic behavior of the CAPTISOL[®] is compared to the same for the parent β -cyclodextrin, the commercially available hydroxypropyl derivatives, ENCAPSIN[™] cyclodextrin (degree of substitution~3-4) and MOLECUSOL[®] cyclodextrin (degree of substitution~7-8), and two other sulfobutyl ether derivatives, SBE1- β -CD and SBE4- β -CD. Unlike the other cyclodextrin derivatives, sulfoalkyl ether (SAE-CD) derivatives, in particular those such

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as the CAPTISOL[®] (degree of substitution~7) and SBE4- β -CD (degree of substitution~4), show essentially no hemolytic behavior and exhibit substantially lower membrane damaging potential than the commercially available hydroxypropyl derivatives at concentrations typically used to solubilize pharmaceutical formulations. The range of concentrations depicted in the figure includes the concentrations typically used to solubilize pharmaceutical formulations when initially diluted in the blood stream after injection. After oral administration, SAE-CD does not undergo significant systemic absorption.

The osmolality of a formulation is generally associated with its hemolytic potential: the higher the osmolality (or the more hypertonic), the greater the hemolytic potential. Zannou et al. ("Osmotic properties of sulfobutyl ether and hydroxypropyl cyclodextrins", *Pharma. Res.* (2001), 18(8), 1226-1231) compared the osmolality of solutions containing SBE-CD and HP-CD. As depicted in FIG. 2, the SBE-CD containing solutions have a greater osmolality than HP-CD containing solutions comprising similar concentrations of cyclodextrin derivative. Thus, it is surprising that SAE-CD exhibits lower hemolysis than does HP-CD at equivalent concentrations, even though HP-CD has a lower osmolality.

Methylated cyclodextrins have been prepared and their hemolytic effect on human erythrocytes has been evaluated. These cyclodextrins were found to cause moderate to severe hemolysis (Jodal et al., *Proc. 4th Int. Symp. Cyclodextrins*, (1988), 421-425; Yoshida et al., *Int. J. Pharm.*, (1988), 46(3), 217-222).

Administration of cyclodextrins into the lungs of a mammal may not be acceptable. In fact, literature exists on the potential or observed toxicity of native cyclodextrins and cyclodextrin derivatives. The NTP Chemical Repository indicates that α -cyclodextrin may be harmful by inhalation. Nimbalkar et al. (*Biotechnol. Appl. Biochem.* (2001), 33, 123-125) cautions on the pulmonary use of an HP- β -CD/diacetyldapsone complex due to its initial effect of delaying cell growth of lung cells.

Even so, a number of studies regarding the use of cyclodextrins for inhalation have been reported although none have been commercialized. The studies suggest that different drug-cyclodextrin combinations will be required for specific optimal or even useful inhaled or intra-nasal formulations. Attempts have been made to develop cyclodextrin-containing powders and solutions for buccal, pulmonary and/or nasal delivery.

U.S. Patent No. 5,914,122 to Otterbeck et al. discloses the preparation of stable budesonide-containing solutions for rectal administration as a foam. They demonstrate the use of cyclodextrin, such as β -CD, γ -CD or HP- β -CD, and/or EDTA as a stabilizer. Cyclodextrin is also suggested as a solubilizer for increasing the concentration of budesonide in solution. In each case, the greatest shelf-life they report for any of their formulations is, in terms of acceptable retention of the active ingredient, only three to six months.

U.S. Pregrant Patent Publication No. 20020055496 to McCoy et al. discloses essentially non-aqueous intra-oral formulations containing HP- β -CD. The formulations may be administered with an aerosol, spray pump or propellant.

Russian Patent No. 2180217 to Chuchalin discloses a stable budesonide-containing solution for inhalation. The solution comprises budesonide, propylene glycol, poly(ethylene oxide), succinic acid, Trilon B, nipazole, thiourea, water, and optionally HP- β -CD.

Müller et al. (*Proceed. Int'l. Symp. Control. Rel. Bioact. Mater.* (1997), 24, 69-70) discloses the results of a study on the preparation of budesonide microparticles by an ASES (Aerosol Solvent Extraction System) supercritical carbon dioxide process for use in a dry powder inhaler. HP- β -CD is suggested as a carrier for a powder.

Müller et al. (U.S. Patent No. 6,407,079) discloses pharmaceutical compositions containing HP- β -CD. They suggest that nasal administration of a solution containing the cyclodextrin is possible.

The art recognizes that it may be necessary to evaluate structurally related variations of a particular type of cyclodextrin derivative in order to optimize the binding of a particular compound with that type of cyclodextrin derivative. However, it is often the case that there are not extreme differences in the binding of a particular compound with a first embodiment versus a second embodiment of a particular cyclodextrin derivative. For example, cases where there are extreme differences in the binding of a particular therapeutic agent for a first cyclodextrin derivative versus a structurally related second cyclodextrin derivative are uncommon. When such situations do exist, they are unexpected. Worth et al. (*24th International Symposium on Controlled Release of Bioactive Materials* (1997)) disclose the results of a study evaluating the utility of steroid/cyclodextrin complexes for pulmonary delivery. In side-by-side comparisons, β -CD,

SBE7- β -CD, and HP- β -CD were evaluated according to their ability to form inclusion complexes with beclomethasone dipropionate (BDP) and its active metabolite beclomethasone monopropionate (BMP). BMP was more easily solubilized with a cyclodextrin, and the observed order of solubilizing power was: HP- β -CD (highest) > β -CD > SBE7- β -CD. Thus, the artisan would expect that SAE-CD derivatives would not be as suitable for use in solubilizing corticosteroids such as BMP or BDP. Although no results regarding actual utility in an inhaled formulation were disclosed, they suggest that BMP rather than BDP would be a better alternative for development of a nebulizer solution.

Kinnarinen et al. (*11th International Cyclodextrin Symposium* CD, (2002)) disclose the results of a study of the *in vitro* pulmonary deposition of a budesonide/ γ -CD inclusion complex for dry powder inhalation. No advantage was observed by complexation with γ -CD. Vozzone et al. (*11th International Cyclodextrin Symposium* CD, (2002)) disclose the results of a study on the complexation of budesonide with γ -cyclodextrin for use in dry powder inhalation. No difference was observed within emitted doses of the cyclodextrin complex or a physical mixture of budesonide and the CD. But, a difference observed in the fine particle fraction of both formulations suggested that use of a cyclodextrin complex for pulmonary drug delivery might increase the respirable fraction of the dry powder.

Pinto et al. (*S.T.P. Pharma. Sciences* (1999), 9(3), 253-256) disclose the results of a study on the use of HP- β -CD in an inhalable dry powder formulation for beclomethasone. The HP- β -CD was evaluated as a complex or physical mixture with the drug in a study of *in vitro* deposition of the emitted dose from a MICRO-HALERTM inhalation device. The amount of respirable drug fraction was reportedly highest with the complex and lowest with the micronized drug alone.

Rajewski et al. (*J. Pharm. Sci.* (1996), 85(11), 1142-1169) provide a review of the pharmaceutical applications of cyclodextrins. In that review, they cite studies evaluating the use of cyclodextrin complexes in dry powder inhalation systems.

Shao et al. (*Eur. J. Pharm. Biopharm.* (1994), 40, 283-288) reported on the effectiveness of cyclodextrins as pulmonary absorption promoters. The relative effectiveness of cyclodextrins in enhancing pulmonary insulin absorption, as measured by pharmacodynamics, and relative efficiency was ranked as follows: dimethyl- β -cyclodextrin > α -cyclodextrin > β -cyclodextrin > γ -cyclodextrin > hydroxypropyl- β -

cyclodextrin. In view of this report, the artisan would expect the water soluble derivative of γ -CD to be less suitable for delivering compounds via inhalation than the respective derivative of β -CD because the underivatized β -CD is more suitable than the underivatized γ -CD.

5 Williams et al. (*Eur. J. Pharm. Biopharm.* (1999 Mar), 47(2), 145-52) reported the results of a study to determine the influence of the formulation technique for 2-hydroxypropyl-beta-cyclodextrin (HP- β -CD) on the stability of aspirin in a suspension-based pressurized metered-dose inhaler (pMDI) formulation containing a hydrofluoroalkane (HFA) propellant. HP- β -CD was formulated in a pMDI as a lyophilized
10 inclusion complex or a physical mixture with aspirin. Aspirin in the lyophilized inclusion complex exhibited the most significant degree of degradation during the 6-months storage, while aspirin alone in the pMDI demonstrated a moderate degree of degradation. Aspirin formulated in the physical mixture displayed the least degree of degradation. Reportedly, HP- β -CD may be used to enhance the stability of a chemically labile drug, but the drug
15 stability may be affected by the method of preparation of the formulation.

Gudmundsdottir et al. (*Pharmazie* (2001 Dec), 56(12), 963-6) disclose the results of a study in which midazolam was formulated in aqueous sulfobutylether-beta-cyclodextrin buffer solution. The nasal spray was tested in healthy volunteers and compared to intravenous midazolam in an open crossover trial. The nasal formulation
20 reportedly approaches the intravenous form in speed of absorption, serum concentration and clinical sedation effect. No serious side effects were observed.

Srichana et al. (*Respir. Med.* (2001 Jun), 95(6), 513-9) report the results of a study to develop a new carrier in dry powder aerosols. Two types of cyclodextrin were chosen; gamma cyclodextrin (γ -CD) and dimethyl-beta-cyclodextrin (DMCD) as carriers in dry
25 powder formulations. Salbutamol was used as a model drug and a control formulation containing lactose and the drug was included. A twin-stage impinger (TSI) was used to evaluate in delivery efficiency of those dry powder formulations. From the results obtained, it was found that the formulation containing γ -CD-enhanced drug delivery to the lower stage of the TSI (deposition = 65%) much greater than that of both formulations
30 containing DMCD (50%) and the control formulation (40%) ($P < 0.05$). The haemolysis of red blood cells incubated with the DMCD complex was higher than that obtained in the

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γ -CD complex. The drug release in both formulations containing γ -CD and DMCD was fast (over 70% was released in 5 min) and nearly all the drug was released within 30 min.

van der Kuy et al. (*Eur. J. Clin. Pharmacol.* (1999 Nov), 55(9), 677-80) report the results of the pharmacokinetic properties of two intranasal preparations of dihydroergotamine mesylate (DHEM) -containing formulation using a commercially available intranasal preparation. The formulations also contained randomly methylated β -cyclodextrin (RAMEB). No statistically significant differences were found in maximum plasma concentration (C_{max}), time to reach C_{max} (t_{max}), area under plasma concentration-time curve ($AUC_{0-8\text{ h}}$), $F_{rel}(t = 8\text{ h})$ and $C_{max}/AUC(t = 8\text{ h})$ for the three intranasal preparations. The results indicate that the pharmacokinetic properties of the intranasal preparations are not significantly different from the commercially available nasal spray.

U.S. patents 5,942,251 and 5,756,483 to Merkus cover pharmaceutical compositions for the intranasal administration of dihydroergotamine, apomorphine and morphine comprising one of these pharmacologically active ingredients in combination with a cyclodextrin and/or a disaccharide and/or a polysaccharide and/or a sugar alcohol.

U.S. patent 5,955,454 discloses a pharmaceutical preparation suitable for nasal administration containing a progestogen and a methylated β -cyclodextrin having a degree of substitution of between 0.5 and 3.0.

U.S. 5,977,070 to Piazza et al. discloses a pharmaceutical composition for the nasal delivery of compounds useful for treating osteoporosis, comprising an effective amount of a physiologically active truncated analog of PTH or PTHrp, or salt thereof and an absorption enhancer selected from the group consisting of dimethyl- β -cyclodextrin.

U.S. 6,436,902 to Backstrom et al. discloses compositions and methods for the pulmonary administration of a parathyroid hormone in the form of a dry powder suitable for inhalation in which at least 50% of the dry powder consists of (a) particles having a diameter of up to 10 microns; or (b) agglomerates of such particles. A dry powder inhaler device contains a preparation consisting of a dry powder comprising (i) a parathyroid hormone (PTH), and (ii) a substance that enhances the absorption of PTH in the lower respiratory tract, wherein at least 50% of (i) and (ii) consists of primary particles having a diameter of up to 10 microns, and wherein the substance is selected from the group consisting of a salt of a fatty acid, a bile salt or derivative thereof, a phospholipid, and a cyclodextrin or derivative thereof.

U.S. patent 6,518,239 to Kuo et al. discloses a dispersible aerosol formulation comprising an active agent and a dipeptide or tripeptide for aerosolized administration to the lung. The compositions reportedly may also include polymeric excipients/additives, e.g., polyvinylpyrrolidones, derivatized celluloses such as hydroxymethylcellulose, hydroxyethylcellulose, and hydroxypropyl methylcellulose, Ficolls (a polymeric sugar), hydroxyethylstarch, dextrates (e.g., cyclodextrins, such as 2-hydroxypropyl- β -cyclodextrin and sulfobutylether- β -cyclodextrin), polyethylene glycols, and pectin.

Nakate et al. (*Eur. J. Pharm. Biopharm.* (2003 Mar), 55(2), 147-54) disclose the results of a study to determine the improvement of pulmonary absorption of the cyclopeptide FK224 (low aqueous solubility) in rats by co-formulating it with beta-cyclodextrin. The purpose of the study was to investigate the effect of pulmonary delivery on the systemic absorption of FK224 in comparison with other administration routes, and to determine the bioavailability (BA) of FK224 following pulmonary administration in rats using various dosage forms. After administration of an aqueous suspension, the bioavailability was reduced to 2.7% compared with 16.8% for the solution. However, β -cyclodextrin (β -CD) was found to be an effective additive as far as improving the solubility of FK224 was concerned. The bioavailability of the aqueous suspension containing β -CD was increased to 19.2%. It was observed that both the C(max) and AUC of FK224 were increased as the amount of β -CD increased. The plasma profiles showed sustained absorption. They suggest that β -CD is an extremely effective additive as far as improving the pulmonary absorption of FK224 is concerned. They also suggest that β -CD or derivatives with various degrees of aqueous solubility are potential drug carriers for controlling pulmonary absorption.

Kobayashi et al. (*Pharm. Res.* (1996 Jan), 13(1), 80-3) disclose the results of a study on pulmonary delivery of salmon calcitonin (sCT) dry powders containing absorption enhancers in rats. After intratracheal administration of sCT dry powder and liquid (solution) preparations to rats, plasma sCT levels and calcium levels were measured. Reportedly, sCT in the dry powder and in the liquid were absorbed nearly to the same degree. Absorption enhancers (oleic acid, lecithin, citric acid, taurocholic acid, dimethyl- β -cyclodextrin, octyl- β -D-glucoside) were much more effective in the dry powder than in the solution.

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Adjei et al. (*Pharm. Res.* (1992 Feb), 9(2), 244-9) disclose the results of a study on the bioavailability of leuprolide acetate following nasal and inhalation delivery to rats and healthy humans. Systemic delivery of leuprolide acetate, a luteinizing hormone releasing hormone (LHRH) agonist, was compared after inhalation (i.h.) and intranasal (i.n.) administration. The i.n. bioavailability in rats was significantly increased by α -cyclodextrin (CD), EDTA, and solution volume. Absorption ranged from 8 to 46% compared to i.v. controls. Studies in healthy human males were conducted with leuprolide acetate i.n. by spray, or inhalation aerosol (i.h.), and subcutaneous (s.c.) and intravenous (i.v.) injection. The s.c. injection was 94% bioavailable compared with i.v. The i.n. bioavailability averaged 2.4%, with significant subject-to-subject variability. Inhalation delivery gave a slightly lower intersubject variability. Mean C_{max} with a 1-mg dose of solution aerosol was 0.97 ng/ml, compared with 4.4 and 11.4 ng/ml for suspension aerosols given at 1- and 2-mg bolus dosages, respectively. The mean bioavailability of the suspension aerosols (28% relative to s.c. administration) was fourfold greater than that of the solution aerosol (6.6%).

CyDex (*Cyclopedia* (2002), 5(1), 3) discloses that SBE-CD is non-toxic to rats in an inhaled aerosol composition when present alone. They do not disclose a nebulizable composition comprising a drug, in particular a corticosteroid, and SBE-CD. Studies comparing the nebulization of PULMICORT RESPULES and an aqueous inhalable solution of budesonide and SAE-CD in various nebulizers have been disclosed (Zimmerer et al. *Respiratory Drug Delivery IX* (2004) 461 – 464).

In deciding whether to administer a suspension versus solution, one must also consider the type of nebulizer to be used. The two most common types of nebulizers are the ultrasonic nebulizer and the air driven jet nebulizer. There are significant differences between the two. For example, jet nebulizers cool rather than heat the liquid in the reservoir, whereas ultrasonic nebulizers heat the liquid. While heating of the solution in reservoir can reduce the viscosity of the solution and enhance formation of droplets, excessive heating could lead to drug degradation. The ultrasonic nebulizer is quieter and provides faster delivery than the jet nebulizer, but ultrasonic nebulizers are more expensive and are not advised for the administration of the currently available steroid for nebulization. Most importantly, however, ultrasonic nebulizers generally provide a significantly higher rate of administration than do jet nebulizers.

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Patients with asthma are often treated with inhaled short acting or long acting β 2-agonists, inhaled anticholinergics, and inhaled corticosteroids alone, sequentially or in combination. Combinations of inhaled corticosteroids and long acting β 2-agonists are known, for example budesonide plus formoterol or fluticasone plus salmeterol are available in a dry powder inhaler. However, there is no example of such combinations that are available as a solution for nebulization. Combining the medications into one solution would reduce the time required to administer the medications separately.

For inhaled corticosteroids, high pulmonary availability is more important than high oral bioavailability because the lung is the target organ. A product with high pulmonary availability has greater potential to exert positive effects in the lung. The ideal ICS would have minimum oral bioavailability, reducing the likelihood of systemic adverse effects.

Although extremely effective in the treatment of asthma, inhaled corticosteroids can have a number of adverse side effects such as oral candidiasis, hoarseness (dysphonia), and pharyngitis. Therefore, inhaled corticosteroids are best delivered by a method that minimizes the oral and/or pharyngeal deposition of the corticosteroid and instead maximizes pulmonary delivery.

Some corticosteroids possess a hydroxyl group at position 21 of the corticosteroid. Those compounds include budesonide, flunisolide, triamcinolone acetonide, beclomethasone monopropionate, and the active form of ciclesonide (desisobutyryl-ciclesonide). It is known that ciclesonide is inhaled as an inactive compound and converted by esterases in the lung to its active form, desisobutyryl-ciclesonide (des-CIC). Budesonide conjugates to form intracellular fatty acid esters, which are highly lipophilic. Budesonide forms conjugates with 5 fatty acids: oleate, palmitate, linoleate, palmitoleate, and arachidonate.

In summary, the art suggests that, in some cases, nebulization of solutions may be preferred over that of suspensions and that, in some cases, an ultrasonic nebulizer, vibrating mesh, electronic or other mechanism of aerosolization may be preferred over an air driven jet nebulizer depending upon the nebulization liquid formulations being compared. Even though the art discloses inhalable solution formulations containing a corticosteroid and cyclodextrin, the results of the art are unpredictable. In other words, the combination of one cyclodextrin with one drug does not suggest that another cyclodextrin may be suitable. Neither does the art suggest that one cyclodextrin-corticosteroid

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inhalable formulation will possess advantages over another cyclodextrin-corticosteroid inhalable formulation.

A need remains in the art for a stabilized aqueous solution budesonide-containing inhalable formulation that does not require the addition of preservatives and that provides
5 significant advantages over other stabilized aqueous solution budesonide-containing inhalable formulations. A need also remains for a method of improving the administration of budesonide-containing suspension formulations by nebulization by converting the suspension to a solution.

There is also a need to develop improved systems that can solubilize water-
10 insoluble drugs for nebulization, and to minimize the levels of cosolvent necessary to accomplish this. The ideal system would consist of non-toxic ingredients and be stable for long periods of storage at room temperature. When nebulized, it would produce respirable droplets in the less than 10 micron or less than 5 micron or less than 3 micron and a substantial portion of extra-fine aerosol in the less than about 1 micron size range.

The need continues to remain for a method of improving the administration, by
15 nebulization, of a suspension-based unit dose formulation. Such a method would reduce the overall time of administration, increase the overall amount of drug administered, reduce the amount of drug left in the reservoir of the nebulizer, increase the portion of pulmonary deposition relative to oropharyngeal deposition of corticosteroid, and/or
20 enhance deep lung penetration of the corticosteroid as compared to such administration, absent the improvement, of the suspension-based unit dose formulation.

SUMMARY OF THE INVENTION

The present invention seeks to overcome the disadvantages present in known formulations. As such, a derivatized cyclodextrin-based, e.g., sulfoalkyl ether
25 cyclodextrin (SAE-CD)-based, inhalable formulation is provided. The present formulation includes a corticosteroid as a principle active agent. The present formulation may provide enhanced solubility and/or enhanced chemical, thermochemical, hydrolytic and/or photochemical stability of the active agent or other ingredients in the formulation. Moreover, the present formulation may possess other advantages, e.g. enhanced drug
30 delivery, increased rate of drug administration, reduced treatment time, reduced toxicity, ease of manufacture, assurance of sterility, improved stability, enhanced bioabsorption, no requirement of particle size control, increased output rate, increased total output, no

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concern for solid particle growth, enhanced pharmacokinetic profile, reduced corticosteroid-related side effects, enhanced pulmonary deposition, reduced oropharyngeal deposition, improved nebulization performance, and/or no need to confirm formation of a suspension, over other inhalable solution or suspension formulations containing a corticosteroid, such as budesonide.

The invention provides a formulation, system and method for treating a respiratory disease or disorder that is therapeutically responsive to corticosteroid therapy. The system of the invention comprises a nebulizer, and a liquid formulation of the invention. The liquid formulation of the invention comprises a dose of corticosteroid, an aqueous liquid carrier and a solubility enhancer.

The invention provides a method of treating, preventing or ameliorating in a subject a corticosteroid-responsive disease or disorder, meaning a disease or disorder in a subject that can be treated with a therapeutically effective amount of corticosteroid to provide a clinical or therapeutic benefit to the subject. The method comprises: providing an aqueous inhalable formulation comprising a corticosteroid, an aqueous liquid carrier, and solubility enhancer; and delivering or administering via nebulization to a subject in need thereof a dose of about at least 25 μg , 40 μg , at least 45 μg , at least 48 μg , 45-1000 μg , about 1 μg to 20 mg, or 1 μg to 10 mg, 0.01 mg to 10 mg, 0.025 mg to 10 mg, 0.05 mg to 5 mg, 0.1 mg to 5 mg, 0.125 mg to 5 mg, 0.25 mg to 5 mg, 0.5 mg to 5 mg, 0.05 mg to 2 mg, 0.1 mg to 2 mg, 0.125 mg to 2 mg, 0.25 mg to 2 mg, 0.5 mg to 2 mg, 1 μg , 10 μg , 25 μg , 50 μg , 100 μg , 125 μg , 200 μg , 250 μg , 25 to 66 μg , 48 to 81 μg , 73 to 125 μg , 40 μg , 64 μg , 95 μg , 35 to 95 μg , 25 to 125 μg , 60 to 170 μg , 110 μg , 170 μg , 45 to 220 μg , 45 to 85 μg , 48 to 82 μg , 85 to 160 μg , 140 to 220 μg , 120 to 325 μg , 205 μg , 320 μg , 325 μg , 90 to 400 μg , 95 to 170 μg , 165 to 275 μg , or 275 to 400 μg of the corticosteroid. In some embodiments, the corticosteroid is budesonide.

In some embodiments: 1) the inhalable formulation has a volume of about 10 μl to 100 ml, 50 μl to 50 ml, 50 μl to 10 ml, 0.1 to 10 ml, 0.1 ml to less than 10 ml, 0.1 ml to 7.5 ml, 0.1 ml to 5 ml, 0.1 ml to 3 ml, 0.1 ml to 2 ml, 0.1 ml to 1 ml, 0.05 ml to 7.5 ml, 0.05 ml to 5 ml, 0.05 ml to 3 ml, 0.05 ml to 2 ml, or 0.05 ml to 1 ml; 2) the step of delivering or administering is conducted over a period of less than 30 min, less than 20 min, less than 10 min, less than 7 min, less than 5 min, less than 3 min, or less than 2 min, or the time is about 0.05 to 10 min, about 0.1 to 5 min, about 0.1 to 3 min, about 0.1 to 2

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min, about 0.1 to 1.5 min, about 0.5 min to about 1.5 min, or about 1 min, or the time is about the time it takes for a subject to take a single breath (about 1 to 3 or 1 to 5 sec); 3) 20% to 85%, or 30% to 80%, or at least 30%, at least 40%, at least 56%, at least 59%, at least 62% of the dose of corticosteroid is delivered to the lungs of the subject; 4) the
5 formulation, system or method provides an enhanced pharmacokinetic profile of 1.5 to 8 fold, 1.5 to 6 fold, 1.5 to 4 fold, 1.5 to 2 fold, 1.64 to 3.55 fold for C_{max} , 1.48 to 6.25 fold for C_{max} , 1.59 to 3.55 or 1.19 to 6.11 fold for AUC_{inf} , 1.69-3.67 or 1.21 to 7.66 fold for AUC_{last} , on normalized dose, as compared to administration of a suspension formulation comprising approximately the same amount of corticosteroid and delivered under
10 substantially the same conditions; 5) no more than, 15% to 85%, 15% to 70%, no more than 20% to 70%, no more than 50%, no more than 40%, or no more than 60% of the dose of corticosteroid is delivered to the subject outside the lung.

The present inventors have unexpectedly discovered that SAE-CD is systemically absorbed following administration via inhalation. It is also eliminated from the lungs.
15 SAE-CD also complexes with corticosteroids in aqueous inhalable liquid formulations. Coadministration of the corticosteroid with SAE-CD may result in increased output rate and total drug delivery as compared to a control excluding SAE-CD. Moreover, an inhalable solution of the invention administered by nebulization provides other advantages set forth herein as compared to a control/reference sample of a suspension comprising
20 substantially the same amount of corticosteroid in substantially the same volume of liquid when administered under substantially the same conditions.

The inhalable solution can be used in more types of nebulizers as suspensions can clog vibrating mesh nebulizers and do not perform well in ultrasonic nebulizers. The nebulizers would be more easily cleaned of residual corticosteroid resulting in less
25 contamination. As drug in solution will be distributed equally in the large and small droplets leaving the nebulizer, the fine particle fraction will contain more corticosteroid resulting in a greater respirable dose that can reach the peripheral lung and smaller airways. This may result in less of the total drug depositing in the oropharyngeal cavity and being swallowed, thus reducing the potential for undesired side effects while
30 enhancing bioavailability and maximizing the desired effect. The inhalable solution will also allow reducing the amount of drug in the unit dose and/or reducing the time of nebulization to achieve the desired amount of drug in the lung. The inhalable solution is more easily manufactured and filled into unit doses and product performance does not

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depend on very tight control of the particle size of ingoing corticosteroid and maintenance of a homogeneous suspension during filling. However, smaller particle size will, in general, facilitate more rapid dissolution during manufacturing. It is not necessary to maintain tight control as described above. It may be terminally sterilized without risk of changing the corticosteroid's particle size of the suspended corticosteroid. The inhalable solution is not subject to change due to particle growth via Ostwald ripening or agglomeration thus the amount of drug in the respirable fraction will not change over time. It does not need to be resuspended prior to administration with carefully scripted procedures to reduce foaming so as not to create large variation in dose. It may be more accurately diluted to achieve the desired amount of drug in the volume placed in the nebulizer. Alternatively, dose adjustment may be controlled more easily by adjusting the time of nebulization as the concentration of the inhalable solution does not change as fast in the nebulizer as does concentration of the suspension, especially in air-jet nebulizers.

An SAE-CD-containing formulation can be prepared with sufficient active agent solubility and stability for a commercial product. If needed, the SAE-CD-containing formulation can be prepared as a clear aqueous solution that can be sterile filtered through a filter having a pore size of 0.45 μm or less and that is stable and preserved under a variety of storage conditions. The invention thus provides a filtration-sterilized liquid formulation comprising a solution of the invention and a method of sterilizing a solution of the invention by sterile filtration through a filter. Sterile filtration can be done without substantial mass loss of solubilized corticosteroid, meaning less than 5% mass loss.

Any corticosteroid suitable for administration via inhalation can be used according to the invention. Exemplary suitable corticosteroids are listed herein. Some embodiments of the invention comprise a corticosteroid having a lipophilicity approximating or exceeding that of flunisolide. Some embodiments of the invention exclude a corticosteroid having a lipophilicity less than flunisolide, i.e., embodiments excluding hydrocortisone, prednisolone, prednisone, dexamethasone, betamethasone, methylprednisolone, triamcinolone, fluocortolone.

One aspect of the invention provides a liquid formulation comprising an effective amount of corticosteroid, such as budesonide, and an SAE-CD, wherein the SAE-CD is present in an amount sufficient to dissolve and stabilize the corticosteroid during storage.

Another aspect of the invention provides a method of improving the administration of corticosteroid to a subject by nebulization, the method comprising the steps of:

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providing in a unit dose an aqueous suspension formulation comprising water and corticosteroid suspended therein;

combining the suspension with an amount of SAE-CD sufficient to and for a period of time sufficient to solubilize the corticosteroid and form a solution; and

5 administering the solution to the subject, wherein the amount of time required to administer a therapeutic dose of corticosteroid with the solution is less than the amount of time required to administer the same therapeutic dose of corticosteroid with the suspension under similar, or otherwise comparable, nebulization conditions.

When administered with a nebulizer, a suspension for nebulization will provide a
10 first corticosteroid output rate under a first set of nebulization conditions. However, when SAE-CD is added to the suspension and mixed therein, a sufficient amount of the corticosteroid is dissolved to form a liquid formulation for nebulization that provides a greater corticosteroid output rate as compared to the formulation excluding the SAE-CD when administered under substantially the same conditions. In one embodiment, the drug
15 output rate of the formulation is increased over that of the suspension even though the total volume of nebulized composition, i.e., the total volume of solution emitted by the nebulizer, has not increased. In another embodiment, SAE-CD is present in an amount sufficient to solubilize at least 50%, at least 75%, at least 90%, at least 95% or substantially all of the corticosteroid. Some embodiments of the invention include those
20 wherein at least 50% wt., at least 75% wt., at least 90% wt., at least 95% wt., at least 98% wt., or all of the corticosteroid is dissolved in the inhalable liquid formulation.

In some embodiments, SAE-CD is present in an amount sufficient to decrease the amount of unsolubilized corticosteroid in the suspension formulation and to improve the administration of the suspension formulation via nebulization. In some embodiments,
25 SAE-CD is present in an amount sufficient to solubilize enough corticosteroid such that the suspension formulation to which the SAE-CD was added is converted to a solution, substantially clear solution (containing less than 5% precipitate or solid), or a clear solution. It is possible that other components of the suspension formulation will not completely dissolve in, or may separate out from, the solution formulation containing
30 SAE-CD.

According to some embodiments, a nebulizer charged with a corticosteroid/SAE-CD-containing solution generates smaller droplets than does the same nebulizer charged with a corticosteroid/HP- β -CD-containing solution operated under otherwise

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similar conditions. As a result of generating smaller droplets, the system comprising SAE-CD is improved over an otherwise similar system comprising HP- β -CD, since the SAE-CD based system will generate a greater proportion of respirable droplets and permit deeper lung penetration.

5 Some aspects of the invention provides for the use of SAE-CD in a nebulizable unit dose liquid formulation. In one embodiment, the invention provides use of SAE-CD for converting a nebulizable corticosteroid-containing suspension-based unit dose formulation to a nebulizable corticosteroid-containing liquid unit dose formulation.

 Some embodiments of the invention include those wherein: 1) the corticosteroid to
10 SAE-CD molar ratio is 0.5 to 0.0001 (1:2 to 1:10,000), 1:1 to 1:100, 1:1 to 1:10,000, or 0.1 (1:10) to 0.03 (1:33.33). The molar ratio of SAE-CD to corticosteroid in the formulation or system is generally greater than 10:1, greater than about 11:1, greater than 13:1, or greater than 14:1. Depending upon the corticosteroid used in the formulation, the molar ratio of corticosteroid to SAE-CD can vary in order to obtain a solution suitable for
15 administration via inhalation for the treatment of a respiratory disease or disorder. In some embodiments, the nebulization composition comprises at least $4.8 \pm 0.5\%$ wt./vol of SAE-CD to provide a self-preserved formulation for a period predetermined period of time. In some embodiments, the nebulization composition comprises less than or about $21.5 \pm 2\%$ wt./wt. of SAE-CD. In some embodiments, the SAE-CD is present in an
20 amount sufficient to provide a clear solution. For example, the nebulization composition can be visibly clear as viewed by the unaided eye.

 Some suitable SAE-CD's include, for example, sulfobutyl ether 4- β -CD or sulfobutyl ether 7- β -CD, sulfobutyl ether 6- γ -CD, sulfobutyl ether 4- γ -CD, sulfobutyl ether 3 to 8- γ -CD, or a sulfobutyl ether 5- γ -CD, or a compound of the formula 1 or a
25 mixture thereof.

 A composition of the invention can further comprise a conventional preservative, an antioxidant, a buffering agent, an acidifying agent, a solubilizing agent, a complexation enhancing agent, saline, an electrolyte, another therapeutic agent, an alkalizing agent, a tonicity modifier, surface tension modifier, viscosity modifier, surfactant, density
30 modifier, volatility modifier, or a combination thereof. If desired, the composition further comprises a liquid carrier other than water. If a conventional preservative is included in the composition, the corticosteroid, such as budesonide, may have a greater binding with

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the SAE-CD than does a conventional preservative. A composition can be purged with an inert gas prior to storage to remove substantially all of the oxygen contained in the formulation. In general, the formulation or composition of the invention has a shelf-life of at least 6 months depending upon the intended use.

5 The formulation can be prepared at a temperature at or above 5°C, at or above 25°C, at or above 35°C, at or above 45°C or at or above 50°C. Specific embodiments of the methods of preparing a liquid formulation include those wherein: 1) the method further comprises the step of sterile filtering the formulation through a filtration medium having a pore size of 0.1 microns or larger; 2) the liquid formulation is sterilized by
10 irradiation or autoclaving; 3) the nebulization solution is purged with nitrogen or argon or other inert pharmaceutically acceptable gas prior to storage such that a substantial portion of the oxygen dissolved in, and/or in surface contact with the solution is removed.

 The invention provides a method of stabilizing corticosteroid in an aqueous corticosteroid-containing formulation comprising the step of adding SAE-CD to an
15 aqueous corticosteroid-containing suspension or solution formulation in an amount sufficient to reduce the rate of degradation of corticosteroid as compared to a control sample excluding SAE-CD.

 The invention also provides a method of improving the administration of an inhalable aqueous corticosteroid-containing suspension unit dose formulation by
20 nebulization, the method comprising the step of adding SAE-CD to an aqueous corticosteroid-containing suspension unit dose formulation in an amount sufficient to solubilize the corticosteroid to form an inhalable aqueous corticosteroid-containing solution unit dose formulation, the improvement comprising increasing the output rate and/or extent of nebulized corticosteroid.

25 The invention provides a method of reducing the amount of time required to provide a therapeutically effective amount of corticosteroid to a subject by inhalation of an corticosteroid-containing composition with a nebulizer, the method comprising the steps of: including SAE-CD in the composition in an amount sufficient to solubilize the corticosteroid to form an inhalable aqueous corticosteroid-containing solution; and
30 administering the solution to the subject by inhalation with a nebulizer, wherein the amount of time required to provide a therapeutically effective amount of corticosteroid to the subject with the solution is reduced as compared to the amount of time required to provide a therapeutically effective amount of corticosteroid to the subject with a

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corticosteroid-containing suspension comprising the same amount or concentration of corticosteroid when the suspension and solution are administered under otherwise similar nebulization conditions. In some embodiments, the time required to administer or deliver a dose of corticosteroid is less than 30 min, less than 20 min, less than 10 min, less than 7 min, less than 5 min, less than 3 min, or less than 2 min, or the time is about 0.05 to 10 min, about 0.1 to 5 min, about 0.1 to 3 min, about 0.1 to 2 min, about 0.1 to 1.5 min, about 0.5 min to about 1.5 min, or about 1 min, or about the time it takes for a subject to take a single breath (about 1 to 3 sec, or 1 to 5 sec). The time will vary according to the dose of corticosteroid in, the concentration of corticosteroid in, and the volume of the inhalable composition in the reservoir of a nebulizer, and it will also depend upon the nebulizer format, nebulization efficiency, and reservoir volume. In a given nebulizer, the lower the volume of the inhalable composition, the more quickly a corresponding dose of corticosteroid is nebulized. The higher the concentration of corticosteroid in an inhalable composition, the faster a dose of the corticosteroid can be administered or delivered.

The output rate (the rate at which the dose corticosteroid is administered or delivered) will vary according to the performance parameters of the nebulizer used to administer it. The higher the output rate of a given nebulizer, the lower the amount of time required to deliver or administer an inhalable composition, as defined herein, or dose of corticosteroid.

The invention also provides an inhalable composition comprising a water soluble γ -CD derivative, a corticosteroid (either esterified or unesterified) and an aqueous liquid medium. Another embodiment of the invention also provides an inhalable composition comprising a water soluble β -CD derivative, a corticosteroid (unesterified) and an aqueous liquid medium.

Also, the invention provides an improved system for administering a corticosteroid-containing inhalable formulation by inhalation, the improvement comprising including SAE-CD in the inhalable formulation such that SAE-CD is present in an amount sufficient to provide an increased rate of inhaled corticosteroid as compared to administration of a control inhalable formulation excluding SAE-CD but otherwise being administered under approximately the same conditions.

The invention can be used to provide a system for administration of a corticosteroid by inhalation, the system comprising an inhalation device, such as a nebulizer, and a drug composition comprising a therapeutically effective amount of

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corticosteroid, liquid carrier and SAE-CD present in an amount sufficient to solubilize the corticosteroid when presented to an aqueous environment, wherein the molar ratio of corticosteroid to SAE-CD is in the range of about 0.072 (1:13.89 or about 1:14) to 0.0001 (1:10,000) or 0.063 (1:15.873 or about 1:16) to 0.003 (1:333.33 or about 1:333). The molar ratio of SAE-CD to corticosteroid ranges from > 10:1 to about 1000:1, about from > 10:1 to about 100:1, from > 10:1 to about 50:1, from > 10:1 to about 30:1, from > 10:1 to about 500:1. During operation, the system forms droplets having a MMAD in the range of about 1-8 μ or 3-8 μ . The corticosteroid is delivered at a rate of at least about 20-50 μ g/min, wherein this range may increase or decrease according to the concentration of corticosteroid in the nebulization solution in the reservoir of the nebulizer.

As a result of using SAE-CD corticosteroid therapy with an inhalable nebulization solution, one can expect advantages such as enhanced drug delivery, enhanced delivery especially to the peripheral or small airways facilitated by the finer aerosol produced, potentially improved treatment of nocturnal, asymptomatic asthma and recovery from acute asthma attacks, increased rate of drug administration, enhanced pharmacokinetic profile, reduced treatment time, improved formulation stability and/or improved patient compliance as compared to comparable corticosteroid therapy with an inhalable nebulization suspension or suspension chlorofluorocarbon (CFC) or hydrofluoroalkanes (HFA) pressurized metered-dose inhaler (pMDI).

The invention can be employed in a kit comprising SAE-CD, an aqueous carrier, and corticosteroid, wherein the kit is adapted for the preparation of a nebulization solution. Embodiments of the kit are detailed below. The invention provides the potential to accommodate combination products to overcome incompatibilities with suspension by other solution dosage forms.

The liquid formulation and composition of the invention provide an enhanced bioavailability/bioabsorption of the corticosteroid as compared to a suspension-based aqueous formulation containing approximately the same amount of corticosteroid and excluding SAE-CD. The liquid formulation or composition provides a higher ratio of AUC_i (or AUC_t) to amount of budesonide delivered to a subject than does the suspension based formulation. Thus, the liquid formulation and/or composition provides an improved clinical benefit or therapeutic benefit over the suspension-based formulation.

The invention also provides a method of administering a corticosteroid to a subject comprising the step of administering via inhalation to a subject an aqueous liquid

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composition comprising a unit dose of corticosteroid dissolved therein, wherein the composition provides a mean AUC_t, normalized for dose of the corticosteroid, of at least 6 (pg*h/ml) per µg of corticosteroid administered. In some embodiments, the unit dose comprises at least 45 µg, at least 48 µg, 45-1000 µg, about 1 µg to 20 mg, about 1 µg to 10 mg, 0.01 mg to 10 mg, 0.025 mg to 10 mg, 0.05 mg to 5 mg, 0.1 mg to 5 mg, 0.125 mg to 5 mg, 0.25 mg to 5 mg, 0.5 mg to 5 mg, 0.05 mg to 2 mg, 0.1 mg to 2 mg, 0.125 mg to 2 mg, 0.25 mg to 2 mg, 0.5 mg to 2 mg, 1 µg, 10 µg, 25 µg, 50 µg, 100 µg, 125 µg, 200 µg, 250 µg, 25 to 66 µg, 48 to 81 µg, 73 to 125 µg, 40 µg, 64 µg, 95 µg, 35 to 95 µg, 25 to 125 µg, 60 to 170 µg, 110 µg, 170 µg, 45 to 220 µg, 45 to 85 µg, 48 to 82 µg, 85 to 160 µg, 140 to 220 µg, 120 to 325 µg, 205 µg, 320 µg, 325 µg, 90 to 400 µg, 95 to 170 µg, 165 to 275 µg, or 275 to 400 µg of corticosteroid. In some embodiments, the normalized AUC_t is based upon the dose to subject, the normalized AUC_t is based upon the dose to lung of subject, or the normalized AUC_t is based upon the emitted dose or nominal dose. As used herein, AUC_t (or AUC_{last}, AUC_{0-t}) is the area under the plasma level versus time curve to the last time point t for which there is a quantifiable value.

The invention also provides a method of administering a corticosteroid to a subject comprising the step of administering via inhalation to a subject an aqueous liquid composition comprising a unit dose of corticosteroid dissolved therein, wherein the composition provides a mean AUC_i, normalized for dose of the corticosteroid, of at least 8 (pg*h/ml) per µg of corticosteroid administered. In some embodiments, the unit dose comprises at least 45 µg, at least 48 µg, or 45 – 1000 µg, about 1 µg to 20 mg, about 1 µg to 10 mg, 0.01 mg to 10 mg, 0.025 mg to 10 mg, 0.05 mg to 5 mg, 0.1 mg to 5 mg, 0.125 mg to 5 mg, 0.25 mg to 5 mg, 0.5 mg to 5 mg, 0.05 mg to 2 mg, 0.1 mg to 2 mg, 0.125 mg to 2 mg, 0.25 mg to 2 mg, or 0.5 mg to 2 mg of corticosteroid. In some embodiments, the normalized AUC_i is based upon the dose to subject, the normalized AUC_i is based upon the dose to lung of subject, or the normalized AUC_i is based upon the emitted dose or nominal dose. As used herein, the AUC_i (AUC_{inf}, AUC_(0→∞)) is the AUC extrapolated to infinity.

When the aqueous liquid solution formulation of the invention is administered via inhalation it can provide at least a two-fold increase in the AUC_t or AUC_i, normalized for dose of the corticosteroid, per microgram of corticosteroid administered as compared to administration via inhalation of an aqueous liquid suspension formulation comprising

substantially the same amount of corticosteroid suspended therein. In some embodiments, the formulation provides at least a 1.6 to five-fold (range as determined on an individual subject basis or a mean of 3 with a standard deviation of +/- 1 as determined from the individual subject data) or a two to four-fold increase (as determined using the geometric mean across a patient population) in the AUC_t or AUC_i, normalized for dose of the corticosteroid, per microgram of corticosteroid administered. The basis for amount of “corticosteroid administered” can be “dose to subject” or “dose to lung” or “emitted dose” or “nominal dose” or “nominal available dose” or “loaded dose”.

The aqueous liquid formulation can be administered with any type of device suitable for administration of a liquid formulation to the lung. For example, the formulation can be administered with an air driven jet, ultrasonic, capillary, electromagnetic, pulsating membrane, pulsating plate (disc), pulsating mesh nebulizer, a nebulizer comprising a vibration generator and an aqueous chamber, and a nebulizer comprising a nozzle array.

In some embodiments, the invention provides a system comprising a nebulizer and an aqueous liquid formulation/composition as described herein. When charged with a liquid formulation of the invention comprising a dose of corticosteroid, the nebulizer can provide a decreasing rate of increasing concentration (RIC) of corticosteroid in the formulation in the reservoir of the nebulizer, in particular when comparing the performance of the liquid formulation to the performance of a suspension-based formulation, e.g. PULMICORT RESPULES, in the nebulizer. In some embodiments, the percentage decrease in the RIC ranges from 10% to 60%, 15% to 60%, 20% to 60%, 30% to 60%, or 40% to 60%. In some embodiments, the system may provide a RIC of 0 to 40, 1 to 40, 5 to 30, or 10 to 30 mcg of corticosteroid/mL of solution volume per min of operation/nebulization.

The pharmacokinetic enhancement provided by the system and formulation/composition of the invention can be determined by comparison of C_{max}, AUC and T_{max} parameters. In some embodiments, the formulation and system of the invention can provide a C_{max} (pg of corticosteroid/ml) of 90 to 900, 200 to 600, 200 to 550, 200 to 250, 400 to 450, 500 to 600, 225, 437, or 545 on a dose non-normalized basis. The formulation and system of the invention can provide a dose normalized C_{max} (pg/ml/μg) of: 1) 0.3 to 2, 0.35 to 2, 0.6 to 1.5, 0.5 to 1.2, 0.8 to 1, 0.8 to 0.9, 0.7 to 0.8, 0.4, 1.9, 0.6, 1.5, 0.5, 1.2, 0.35, 2, 0.7, 0.8, or 0.9 on a nominal available dose normalized

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basis; 2) 3.4 to 9.2, 3.5 to 8.5, 5.5 to 9.2, 4.5 to 7.5, 5.8 to 7, 3.4, 3.5, 4.5, 5.5, 9.2, 8.5, 7.5, 5.8, 5.9, 6, or 7 on a dose to lung normalized basis; 3) 1.7 to 7.5, 3.2 to 4.1, 1.9 to 6, 3.2 to 7.5, 1.7 to 5.2, 3.6, 4.1, 3.2, 1.9, 6, 3.2, 7.4, 7.5, 1.7, 5.2 or 5.3 on a dose to subject normalized basis; 4) 0.9 to 3.3, 1.7 to 2.2, 0.9 to 3, 1 to 3, 1.7 to 3.3, 1 to 2.7, 1.9, 2.1, 2.2, 1.7, 0.9, 1, 2, 3, 2.9, 3.2, 3.3, or 2.7 on an emitted dose normalized basis.

In some embodiments, on the basis of the non-normalized dose of corticosteroid, the C_{max} provided by the corticosteroid solution is 1.6 to 2, 1.5 to 3, 1.5 to 2.5, 1.5 to 2, 1.5, 1.6, 2, 2.5, or 3 fold higher than the C_{max} provided by the suspension-based formulation when the dose of corticosteroid in the solution and the suspension is approximately the same amount loaded. On the basis of normalization to the nominal available dose of corticosteroid, the C_{max} provided by the corticosteroid solution is 1.8 to 6.2, 1.5 to 6.5, 2 to 6.5, 1.5 to 5.5, 2 to 4, 1.5 to 4, 1.5 to 3, 2.7, 3.3, 3.4, 1.5, 6.5, 2, 5.5, 4, or 3 fold higher than the C_{max} provided by the suspension-based formulation. On the basis of normalization to the dose of corticosteroid to lung, the C_{max} provided by the corticosteroid solution is 1.4 to 4.3, 1.4 to 4.5, 1.5 to 4.5, 1.5 to 3.5, 1.5 to 3, 1.4 to 3, 1.5 to 2.5, 1.5 to 2, 2, 2.3, 1.4, 4.5, 3.5, 3, 1.5 or 2.5 fold higher than the C_{max} provided by the suspension-based formulation. On the basis of normalization to the dose of corticosteroid to subject, the C_{max} provided by the corticosteroid solution is 2 to 3.5, 2 to 5, 1.7 to 3.8, 1.7 to 5, 2.7, 3, 2.4, 2, 3.5, 5, 1.7, or 3.8 fold higher than the C_{max} provided by the suspension-based formulation. On the basis of normalization to the emitted dose of corticosteroid, the C_{max} provided by the corticosteroid solution is 1.9 to 6.3, 1.75 to 6.5, 2.2 to 4.2, 2.2 to 6.3, 1.9 to 4.2, 3.2, 3.5, 3.6, 2.8, 1.75, 6.5, 2.2, 4.2, or 6.3 fold higher than the C_{max} provided by the suspension-based formulation.

In some embodiments, the C_{max} provided by the corticosteroid solution is at least 1.5, 1.6, 2, 2.6, and 3 fold higher than the C_{max} provided by the suspension-based formulation when the dose of corticosteroid in the solution is about 2 fold lower than the dose in the suspension.

In some embodiments, the formulation and system of the invention can provide an AUC_{inf} (pg*h/ml) of 500 to 1700, 530 to 1650, 250 to 2500, 280 to 1300, 780 to 1300, 980 to 2450, 275, 775, 980, 2400, 2500, 1300, 1290, 530, 1650, 250, 280 or 780 on a dose non-normalized basis. The formulation and system of the invention can provide a dose normalized AUC_{inf} (pg/ml/μg) of: 1) 1 to 5.5, 2 to 2.2, 1 to 5.3, 1.1 to 5.2, 1.5 to 2.6, 1.3 to 3.3, 2, 2.1, 2.2, 1, 5.5, 5.3, 5.2, 1.5, 2.6, 1.3 or 3.3 on a nominal available dose normalized

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basis; 2) 10 to 25, 14 to 18, 10.2 to 20, 13.6 to 18.8, 11.2 to 24.7, 10.2, 20, 13.6, 14, 19, 18.8, 11, 11.2, 25, 24.7, 14.2, 16.2, 17.3 on a dose to lung normalized basis; 3) 4 to 16, 4.2 to 16.1, 8 to 12.2, 5.4 to 16, 5.4 to 17, 8.5 to 9.6, 8.5, 9.5, 9.6, 4.2, 16.1, 8, 12.2, 12, 5.4, 16, 17, or 16.5 on a dose to subject normalized basis; 4) 2.5 to 9, 2.6 to 8.5, 4.5 to 5.1, 2.5 to 8, 2.6 to 7.9, 4.2 to 6.7, 3.1 to 8.5, 3.2 to 8.5, 4.5, 4.6, 5, 5.1, 2.5, 2.6, 4.2, 3.1, 9, 8.5, 5.1, 8, 7.9, or 6.7 to on an emitted dose normalized basis.

In some embodiments, on the basis of the non-normalized dose of corticosteroid, the AUC_{inf} provided by the corticosteroid solution is 1.6 to 2.5, 1.6 to 3.1, 1.5 to 3.5, 1.5 to 3.3, 2.5 to 3.3, 3.1, 1.5, 3.3, 1.6, or 2.5 fold higher than the AUC_{inf} provided by the suspension-based formulation when the dose of corticosteroid in the solution and the suspension is approximately the same. On the basis of normalization to the nominal available dose of corticosteroid, the AUC_{inf} provided by the corticosteroid solution is 1.75 to 6.5, 1.75 to 6.1, 2 to 6.5, 2 to 6.1, 2 to 4.5, 2 to 4.4, 3.3, 3.2, 3.5, 3.4, 1.75, 6.5, 6.1, 2, 4.5, or 4.4 fold higher than the AUC_{inf} provided by the suspension-based formulation. On the basis of normalization to the dose of corticosteroid to lung, the AUC_{inf} provided by the corticosteroid solution is 1.2 to 3.5, 1.2 to 4, 1.2 to 3, 1.2 to 2.85, 1.5 to 3.5, 1.4 to 3.5, 2, 2.2, 2.3, 2.4, 1.2, 3, 4, 2.85, 1.5, 3.5, or 1.4 fold higher than the AUC_{inf} provided by the suspension-based formulation. On the basis of normalization to the dose of corticosteroid to subject, the AUC_{inf} provided by the corticosteroid solution is 1.6 to 4.9, 1.5 to 5, 1.6 to 5, 1.6 to 3.7, 1.6 to 3.6, 2 to 4.9, 1.9 to 4, 2.6, 1.5, 5, 1.6, 3.7, 3.6, 2, 4.9, 1.9, or 4 fold higher than the AUC_{inf} provided by the suspension-based formulation. On the basis of normalization to the emitted dose of corticosteroid, the AUC_{inf} provided by the corticosteroid solution is 1.5 to 6, 1.7 to 6, 1.9 to 6, 1.9 to 5.4, 2.3 to 5.8, 1.9 to 5.5, 1.9 to 5.8, 1.5, 6, 1.7, 1.9, 5.4, 2.3, 5.8, 5.8, 3.2, 3.5, or 3.6 fold higher than the AUC_{inf} provided by the suspension-based formulation.

In some embodiments, the AUC_{inf} provided by the corticosteroid solution is at least 1.5, 1.6, 2, 2.5, 3 and 3.1 fold higher than the AUC_{inf} provided by the suspension-based formulation when the dose of corticosteroid in the solution is about 2 fold lower than the dose in the suspension.

The formulation and system of the invention can provide an AUC_{0-8hr} (pg*h/ml) of 2000 to 3000, 2500 to 2700, 2000, 3000, or 2600 on a dose non-normalized basis for the corticosteroid. The formulation and system of the invention can provide a dose normalized AUC_{inf} (pg/ml/ μ g) of: 1) 2 to 3, 2.5 to 2.7, or 2.6 on a loaded dose nominal

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normalized basis for the corticosteroid; 2) 3 to 4, 3.4 to 3.5, or 3.4 on an emitted dose normalized basis for the corticosteroid.

In some embodiments, on the basis of the normalized nominal (loaded) dose of corticosteroid, the AUC_{0-8hr} provided by the corticosteroid solution is at least 1.7, 1.8, 1.9, or 2 fold higher than the AUC_{0-8hr} provided by the suspension-based formulation when the dose of corticosteroid in the solution and the suspension is approximately the same. On the basis of normalization to the emitted dose of corticosteroid, the AUC_{0-8hr} provided by the corticosteroid solution is at least 1.5, 1.6, to 2 fold higher than the AUC_{0-8hr} provided by the suspension-based formulation.

The formulation and system of the invention can provide a C_{max} (pg/ml) of 1600 to 1800, 1650 to 1750, or 1700 on a dose non-normalized basis. The formulation and system of the invention can provide a dose normalized C_{max} (pg/ml/ μ g) of: 1) 1 to 2, 1.6 to 1.8, or 1.7 on a loaded dose nominal normalized basis; 2) 2 to 2.5, or 2.2 on an emitted dose normalized basis.

In some embodiments, on the basis of the normalized nominal (loaded) dose of corticosteroid, the C_{max} provided by the corticosteroid solution is at least 1.7, 1.8, 1.9, or 2 fold higher than the C_{max} provided by the suspension-based formulation when the dose of corticosteroid in the solution and the suspension is approximately the same. On the basis of normalization to the emitted dose of corticosteroid, the C_{max} provided by the corticosteroid solution is at least 1.5, 1.6, to 2 fold higher than the C_{max} provided by the suspension-based formulation.

In some embodiments, greater than 20%, greater than 25%, greater than 29%, greater than 35% of the emitted dose may be absorbed into the bloodstream of the patient. In some embodiments, greater than 40%, greater than 50%, greater than 55% of the dose to subject may be absorbed into the bloodstream of the patient. In some embodiments, greater than 10%, greater than 12%, greater than 15% of the nominal available dose may be absorbed into the bloodstream of the patient.

Some aspects of the invention provide a method of administering or delivering a dose of corticosteroid to the air passageways of a subject in need thereof, the method comprising administering or delivering the corticosteroid with a nebulizer comprising a charge of an aqueous liquid formulation, the formulation comprising an aqueous liquid carrier, sulfoalkyl ether cyclodextrin, and the corticosteroid dissolved therein, wherein, during operation, the system provides enhanced drug delivery, increased rate of drug

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administration, reduced treatment time, reduced toxicity, improved stability, enhanced bioabsorption, increased output rate, increased total output, enhanced pharmacokinetic profile, reduced corticosteroid-related side effects, enhanced pulmonary deposition, reduced oropharyngeal deposition, and/or improved nebulization performance over
5 another system comprising the nebulizer and a suspension based formulation of the corticosteroid. The systems can be operated under similar conditions or dissimilar conditions.

The invention includes all combinations of the embodiments and aspects disclosed herein. Accordingly, the invention includes the embodiments and aspects specifically
10 disclosed, broadly disclosed, or narrowly disclosed herein, as well as combinations thereof and subcombinations of the individual elements of said embodiments and aspects.

These and other aspects of this invention will be apparent upon reference to the following detailed description, examples, claims and attached figures.

BRIEF DESCRIPTION OF THE FIGURES

15 The following drawings are given by way of illustration only, and thus are not intended to limit the scope of the present invention.

Figure 1 depicts a graph of the hemolytic behavior of the CAPTISOL[®] as compared to the same for the parent β -cyclodextrin, the commercially available hydroxypropyl derivatives, ENCAPSIN[™] (degree of substitution ~3-4) and
20 MOLECUSOL[®] (degree of substitution ~7-8), and two other sulfobutyl ether derivatives, SBE1- β -CD and SBE4- β -CD.

Figure 2 depicts a graph of the osmolality of SBE-CD containing solutions of various degrees of substitution and HP- β -CD containing solutions comprising similar concentrations of cyclodextrin derivative.

25 Figure 3 depicts a phase solubility graph of the concentration (molar) of cyclodextrin versus the concentration (molar) of budesonide for γ -CD, HP- β -CD and SBE7- β -CD.

Figure 4 depicts a chart of the estimated percentage of nebulization composition emitted from three different nebulizers (PARI LC PLUS, HUDSON UPDRAFT II
30 NEB-U-MIST, and MYSTIQUE) for each of four different nebulization compositions (PULMICORT RESPULES suspension, 5% w/v SBE7- β -CD solution, 10% w/v SBE7- β -CD solution and 20% w/v SBE7- β -CD solution).

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Figures 5a-5b depict droplet size data for nebulization of solutions with a PARI LC PLUS nebulizer.

Figure 6 depicts droplet size data for nebulization of solutions with a HUDSON UPDRAFT II NEBUMIST nebulizer.

5 Figure 7 depicts droplet size data for nebulization of solutions with a MYSTIQUE ultrasonic nebulizer.

Figure 8 depicts comparative Dv_{50} droplet size data for nebulization of composition with the three nebulizers PARI LC PLUS, HUDSON UPDRAFT II NEBUMIST, and MYSTIQUE.

10 Figure 9 is a graph depicting the relationship between concentration of SAE-CD versus output rate of SAE-CD in various different nebulizers.

Figures 10a-10b depict comparative droplet size data for nebulization solutions with the PARI LC PLUS and MYSTIQUE nebulizers of PULMICORT RESPULES suspension and a modified PULMICORT RESPULES-based SAE-CD solution.

15 Figure 11 depicts a semi-log plot of the % of initial concentration of the R- and S-isomers of budesonide in solutions with and without CAPTISOL versus time at 60° C in solution.

Figure 12 depicts a semi-log plot of the % of initial concentration of budesonide versus Lux hours when the samples are exposed to fluorescent lamps.

20 Figure 13 depicts a phase solubility diagram for fluticasone propionate in the presence of several different cyclodextrins.

Figure 14 depicts a phase solubility diagram for mometasone furoate in the presence of several different cyclodextrins.

25 Figure 15 depicts a phase solubility diagram for esterified and non-esterified fluticasone in the presence of SAE(5-6)- γ -CD.

Figure 16 depicts a bar chart summarizing the aqueous solubility of beclomethasone dipropionate in the presence of various SAE-CD derivatives.

30 Figure 17 depicts a plot of the geometric mean of dose (of corticosteroid, μ g) to subject versus the geometric mean of AUC ($\text{pg}\cdot\text{hr}/\text{ml}$) when budesonide is administered to subjects according to Example 17.

Figure 18 depicts a plot of the geometric mean of dose (of corticosteroid, μ g) to lung of subject versus the geometric mean of AUC ($\text{pg}\cdot\text{hr}/\text{ml}$) when budesonide is administered to subjects according to Example 17.

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Figure 19 depicts a plot of dose (of corticosteroid, μg) to subject versus the AUC ($\text{pg}\cdot\text{hr}/\text{ml}$) for each individual when budesonide is administered to subjects according to Example 17.

Figure 20 depicts a plasma concentration profile for budesonide for individual
5 subjects according to Example 17.

Figure 21 depicts a plasma concentration profile for budesonide for dogs according to Example 31.

Figure 22 is a chart detailing the approximate distribution of a dose of budesonide upon administration of a solution of the invention according the procedure of Example 17.

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DETAILED DESCRIPTION OF THE INVENTION

The presently claimed formulation overcomes many of the undesired properties of other known aqueous inhalable solution or suspension corticosteroid-containing formulations. By including SAE-CD, in an inhalable liquid formulation containing corticosteroid, the corticosteroid is dissolved. Unexpectedly, the nebulization of
15 corticosteroid is improved in both an air driven jet nebulizer and an ultrasonic nebulizer. Moreover, the corticosteroid exhibits greater stability in the presence of SAE-CD than it does in its absence.

The inhalable formulation and system of the invention provides an improved pharmacokinetic profile over a suspension formulation comprising approximately the
20 same amount of corticosteroid and delivered under substantially the same conditions. The term "enhanced pharmacokinetic profile" is taken to mean a higher AUC (e.g. AUC_{last} or $\text{AUC}_{(0\rightarrow\infty)}$) per μg of corticosteroid delivered or administered, a higher C_{max} per μg of corticosteroid delivered or administered, increased bioavailability, absorption or distribution of the corticosteroid at the site of delivery, a shorter T_{max} or a longer T_{max} .

Alternatively, the inhalable formulation and system of the invention provides
25 substantially the same pharmacokinetic profile or an enhanced pharmacokinetic profile over a suspension formulation comprising approximately a higher amount of corticosteroid and delivered under substantially the same conditions. The corticosteroid in the formulation can be present at a dose that is less than 80%, less than 70% less than 60%
30 less than 50%, less than 40%, less than 20%, and less than 10% of that in the suspension.

The corticosteroid can be present in an amount sufficient for single dose or multi-dose administration. SAE-CD can be present in an amount sufficient to solubilize the

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corticosteroid when the two are placed in the aqueous carrier. The aqueous carrier can be present in an amount sufficient to aid in dissolution of the corticosteroid and form a nebulization solution of sufficient volume and sufficiently low viscosity to permit single dose or multi-dose administration with a nebulizer. SAE-CD can be present in solid form or in solution in the aqueous carrier. The corticosteroid can be present in dry powder/particle form or in suspension in the aqueous carrier.

Commercially available air driven jet, ultrasonic or pulsating membrane nebulizers include the AERONEB™ (Aerogen, San Francisco, CA), AERONEB GO (Aerogen); PARI LC PLUS™, PARI BOY™ N, PARI eflow, PARI LC SINUS, PARI SINUSTAR™, PARI SINUNEb, and PARI DURANEb™ (PARI Respiratory Equipment, Inc., Monterey, CA); MICROAIR™ (Omron Healthcare, Inc, Vernon Hills, Illinois), HALOLITE™ (Profile Therapeutics Inc, Boston, MA), RESPIMAT™ (Boehringer Ingelheim Ingelheim, Germany) AERODOSE™ (Aerogen, Inc, Mountain View, CA), OMRON ELITE™ (Omron Healthcare, Inc, Vernon Hills, Illinois), OMRON MICROAIR™ (Omron Healthcare, Inc, Vernon Hills, Illinois), MABISMIST™ II (Mabis Healthcare, Inc, Lake Forest, Illinois), LUMISCOPE™ 6610, (The Lumiscope Company, Inc, East Brunswick, New Jersey), AIRSEP MYSTIQUE™, (AirSep Corporation, Buffalo, NY), ACORN-1 and ACORN-II (Vital Signs, Inc, Totowa, New Jersey), AQUATOWER™ (Medical Industries America, Adel, Iowa), AVA-NEB (Hudson Respiratory Care Incorporated, Temecula, California), AEROCURRENT™ utilizing the AEROCELL™ disposable cartridge (AerovectRx Corporation, Atlanta, Georgia), CIRBUS (Intersurgical Incorporated, Liverpool, New York), DART (Professional Medical Products, Greenwood, South Carolina), DEVILBISS™ PULMO AIDE (DeVilbiss Corp; Somerset, Pennsylvania), DOWNDRAFT™ (Marquest, Englewood, Colorado), FAN JET (Marquest, Englewood, Colorado), MB-5 (Mefar, Bovezzo, Italy), MISTY NEB™ (Baxter, Valencia, California), SALTER 8900 (Salter Labs, Arvin, California), SIDESTREAM™ (Medic-Aid, Sussex, UK), UPDRAFT-II™ (Hudson Respiratory Care; Temecula, California), WHISPER JET™ (Marquest Medical Products, Englewood, Colorado), AIOLOS™ (Aiolos Medicnnsk Teknik, Karlstad, Sweden), INSPIRON™ (Intertech Resources, Inc., Bannockburn, Illinois), OPTIMIST™ (Unomedical Inc., McAllen, Texas), PRODOMO™, SPIRA™ (Respiratory Care Center, Hameenlinna, Finland), AERx™ Essence™ and Ultra™, (Aradigm Corporation, Hayward, California), SONIK™ LDI Nebulizer (Evit Labs, Sacramento, California), and

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SWIRLER® Radioaerosol System (AMICI, Inc., Spring City, PA). Nebulizers that nebulize liquid formulations containing no propellant are suitable for use with the compositions provided herein. Any of these and other known nebulizers can be used to deliver the formulation of the invention including but not limited to the following:

5 nebulizers available from Pari GmbH (Starnberg, Germany), DeVilbiss Healthcare (Heston, Middlesex, UK), Healthdyne, Vital Signs, Baxter, Allied Health Care, Invacare, Hudson, Omron, Bmed, AirSep, Luminscope, Medisana, Siemens, Aerogen, Mountain Medical, Aerosol Medical Ltd. (Colchester, Essex, UK), AFP Medical (Rugby, Warwickshire, UK), Bard Ltd. (Sunderland, UK), Carri-Med Ltd. (Dorking, UK), Plaem

10 Nuiva (Brescia, Italy), Henleys Medical Supplies (London, UK), Intersurgical (Berkshire, UK), Lifecare Hospital Supplies (Leies, UK), Medic-Aid Ltd. (West Sussex, UK), Medix Ltd. (Essex, UK), Sinclair Medical Ltd. (Surrey, UK), and many other companies. The AERx and RESPIMAT nebulizers are described by D. E. Geller (*Respir. Care* (2002), 47 (12), 1392-1404), the entire disclosure of which is incorporated by reference.

15 Nebulizers for use herein include, but are not limited to, jet nebulizers (optionally sold with compressors), ultrasonic nebulizers, vibrating membrane, vibrating mesh nebulizers, vibrating plate nebulizers, vibrating cone nebulizer, and others. Exemplary jet nebulizers for use herein include Pari LC plus/ProNeb, Pari LC plus/ProNeb Turbo, Pari LC Plus/Dura Neb 1000 & 2000 Pari LC plus/Walkhaler, Pari LC

20 plus/Pari Master, Pari LC star, Omron CompAir XL Portable Nebulizer System (NE-C18 and JetAir Disposable nebulizer), Omron compare Elite Compressor Nebulizer System (NE-C21 and Elite Air Reusable Nebulizer, Pari LC Plus or Pari LC Star nebulizer with Proneb Ultra compressor, Pulmo-aide, Pulmo-aide LT, Pulmo-aide traveler, Invacare Passport, Inspiration Healthdyne 626, Pulmo-Neb Traverler, DeVilbiss 646, Whisper Jet,

25 Acorn II, Misty-Neb, Allied aerosol, Schuco Home Care, Lexan Plasic Pocet Neb, SideStream Hand Held Neb, Mobil Mist, Up-Draft, Up-Draft II, T Up-Draft, ISO-NEB, Ava-Neb, Micro Mist, and PulmoMate. Exemplary ultrasonic nebulizers for use herein include MicroAir, UltraAir, Siemens Ultra Nebulizer 145, CompAir, Pulmosonic, Scout, 5003 Ultrasonic Neb, 5110 Ultrasonic Neb, 5004 Desk Ultrasonic Nebulizer, Mystique

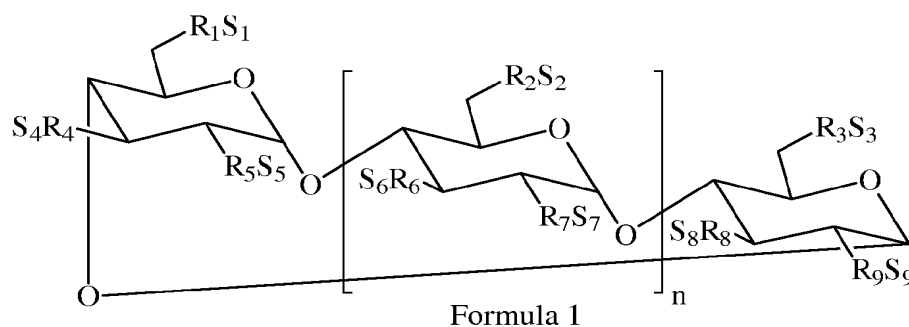
30 Ultrasonic, Lumiscope's Ultrasonic Nebulizer, Medisana Ultrasonic Nebulizer, Microstat Ultrasonic Nebulizer, and Mabismist Hand Held Ultrasonic Nebulizer. Other nebulizers for use herein include 5000 Electromagnetic Neb, 5001 Electromagnetic Neb 5002 Rotary Piston Neb, Lumineb I Piston Nebulizer 5500, Aeroneb Portable Nebulizer System,

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Aerodose™ Inhaler, and AeroEclipse Breath Actuated Nebulizer. Exemplary vibrating membrane, mesh or plate nebulizers are described by R. Dhand (*Respiratory Care*, (Dec. 2002), 47(12), p. 1406-1418), the entire disclosure of which is hereby incorporated by reference.

Other suitable nebulizers are described in U.S. Patents No. 5,954,047, No. 6,026,808, No. 6,095,141, and No. 6,527,151, the entire disclosures of which are hereby incorporated by reference.

The present invention provides SAE-CD based formulations, wherein the SAE-CD is a compound of the Formula 1:



wherein:

n is 4, 5 or 6;

R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈ and R₉ are each, independently, -O- or a -O-(C₂ - C₆ alkylene)-SO₃⁻ group, wherein at least one of R₁ to R₉ is independently a -O-(C₂ - C₆ alkylene)-SO₃⁻ group, preferably a -O-(CH₂)_mSO₃⁻ group, wherein m is 2 to 6, preferably 2 to 4, (e.g. -OCH₂CH₂CH₂SO₃⁻ or -OCH₂CH₂CH₂CH₂SO₃⁻); and

S₁, S₂, S₃, S₄, S₅, S₆, S₇, S₈ and S₉ are each, independently, a pharmaceutically acceptable cation which includes, for example, H⁺, alkali metals (e.g. Li⁺, Na⁺, K⁺), alkaline earth metals (e.g., Ca⁺², Mg⁺²), ammonium ions and amine cations such as the cations of (C₁ - C₆)-alkylamines, piperidine, pyrazine, (C₁ - C₆)-alkanolamine and (C₄ - C₈)-cycloalkanolamine.

Exemplary embodiments of the SAE-CD derivative of the invention include derivatives of the Formula II (SAEx-α-CD), wherein "x" ranges from 1 to 18; of the Formula III (SAEy-β-CD), wherein "y" ranges from 1 to 21; and of the Formula IV (SAEz-γ-CD), wherein "z" ranges from 1 to 24 such as:

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<u>SAEx-α-CD</u>	<u>SAEy-β-CD</u>	<u>SAEz-γ-CD</u>	<u>Name</u>
SEEx- α -CD	SEEy- β -CD	SE Ez- γ -CD	Sulfoethyl ether CD
SPEx- α -CD	SPEy- β -CD	SPEz- γ -CD	Sulfopropyl ether CD
SBEEx- α -CD	SBEy- β -CD	SBEz- γ -CD	Sulfobutyl ether CD
SPtEx- α -CD	SPtEy- β -CD	SPtEz- γ -CD	Sulfopentyl ether CD
SHEx- α -CD	SHEy- β -CD	SHEz- γ -CD	Sulfohexyl ether CD

“SAE” represents a sulfoalkyl ether substituent bound to a cyclodextrin. The values “x”, “y” and “z” represent the average degree of substitution as defined herein in terms of the number of sulfoalkyl ether groups per CD molecule.

5 The SAE-CD used is described in U.S. Patents No. 5,376,645 and No. 5,134,127 to Stella et al, the entire disclosures of which are hereby incorporated by reference. U.S. Patent No. 3,426,011 to Parmerter et al. discloses anionic cyclodextrin derivatives having sulfoalkyl ether substituents. Lammers et al. (*Recl. Trav. Chim. Pays-Bas* (1972), 91(6), 733-742); *Staerke* (1971), 23(5), 167-171) and Qu et al. (*J. Inclusion Phenom. Macro.*
10 *Chem.*, (2002), 43, 213-221) disclose sulfoalkyl ether derivatized cyclodextrins. U.S. Patent No. 6,153,746 to Shah et al. discloses a process for the preparation of sulfoalkyl ether cyclodextrin derivatives. An SAE-CD can be made according to the disclosures of Stella et al., Parmerter et al., Lammers et al. or Qu et al., and if processed to remove the major portion (>50%) of the underivatized parent cyclodextrin, used according to the
15 present invention. The SAE-CD can contain from 0% to less than 50% wt. of underivatized parent cyclodextrin.

The terms “alkylene” and “alkyl,” as used herein (e.g., in the -O-(C₂ - C₆-alkylene)SO₃⁻ group or in the alkylamines), include linear, cyclic, and branched, saturated and unsaturated (i.e., containing one double bond) divalent alkylene groups and
20 monovalent alkyl groups, respectively. The term “alkanol” in this text likewise includes both linear, cyclic and branched, saturated and unsaturated alkyl components of the alkanol groups, in which the hydroxyl groups may be situated at any position on the alkyl moiety. The term “cycloalkanol” includes unsubstituted or substituted (e.g., by methyl or ethyl) cyclic alcohols.

25 An embodiment of the present invention provides compositions containing a mixture of cyclodextrin derivatives, having the structure set out in formula (I), where the composition overall contains on the average at least 1 and up to 3n + 6 alkylsulfonic acid

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moieties per cyclodextrin molecule. The present invention also provides compositions containing a single type of cyclodextrin derivative, or at least 50% of a single type of cyclodextrin derivative. The invention also includes formulations containing cyclodextrin derivatives having a narrow or wide and high or low degree of substitution. These combinations can be optimized as needed to provide cyclodextrins having particular properties.

The present invention also provides compositions containing a mixture of cyclodextrin derivatives wherein two or more different types of cyclodextrin derivatives are included in the composition. By different types, is meant cyclodextrins derivatized with different types of functional groups e.g., hydroxyalkyl and sulfoalkyl. Each independent different type can contain one or more functional groups, e.g. SBE-CD where the cyclodextrin ring has only sulfobutyl functional groups, and hydroxypropyl-ethyl- β -CD where the cyclodextrin ring has both hydroxypropyl functional groups and ethyl functional groups. The amount of each type of cyclodextrin derivative present can be varied as desired to provide a mixture having the desired properties.

Exemplary SAE-CD derivatives include SBE4- β -CD, SBE7- β -CD, SBE11- β -CD, SBE3.4- γ -CD, SBE4.2- γ -CD, SBE4.9- γ -CD, SBE5.2- γ -CD, SBE6.1- γ -CD, SBE7.5- γ -CD, SBE7.8- γ -CD and SBE5- γ -CD which correspond to SAE-CD derivatives of the formula I wherein $n = 5, 5, 5$ and 6 ; m is 4 ; and there are on average $4, 7, 11$ and 5 sulfoalkyl ether substituents present, respectively. These SAE-CD derivatives increase the solubility of poorly water soluble active agents to varying degrees.

Since SAE-CD is a poly-anionic cyclodextrin, it can be provided in different salt forms. Suitable counterions include cationic organic atoms or molecules and cationic inorganic atoms or molecules. The SAE-CD can include a single type of counterion or a mixture of different counterions. The properties of the SAE-CD can be modified by changing the identity of the counterion present. For example, a first salt form of SAE-CD can have a greater corticosteroid stabilizing and/or solubilizing power than a different second salt form of SAE-CD. Likewise, an SAE-CD having a first degree of substitution can have a greater corticosteroid stabilizing and/or solubilizing power than a second SAE-CD having a different degree of substitution. The enhanced solubilization of a corticosteroid by one SAE-CD versus another is demonstrated by the data in the following tables which depict the molar solubility for fluticasone propionate with different SAE-CDs at about 0.03 to $0.12M$ concentrations such that the solubilizing power followed about this

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rank order over this concentration range of SAE-CD: SBE5.2- γ -CD > SPE5.4- γ -CD > SBE6.1- γ -CD > SBE9.7- γ -CD >> SBE7- α -CD > SBE6.7- β -CD > SPE7- β -CD. For mometasone furoate, the solubilizing power followed about this rank order over this concentration range of SAE-CD: SBE9.7- γ -CD > SBE6.1- γ -CD > SBE5.2- γ -CD >> SPE5.4- γ -CD > SBE7- α -CD > SBE6.7- β -CD > SPE7- β -CD. Differences were also observed for the binding of budesonide and triamcinolone with specific embodiments of SAE-CD. According to the invention, a SAE- γ -CD binds a corticosteroid better than a SAE- β -CD does. Also, a SAE- β -CD binds budesonide better than a SAE- α -CD does. The data is summarized in FIGS. 13-14.

10

-CD	[CD] M	[Fluticasone] x10 ⁵ M		[Mometasone] x10 ⁵ M		[Budesonide] x10 ⁵ M	[Triamcinolone acetoneide] x10 ⁵ M
		as propionate	non esterified	as furoate	non esterified		
H ₂ O	NA	0.39	0.16	1.82	0.00	6.59	3.56
β	0.015M			1.36	12.9	81.3	
(SBE) _{6.7} β	0.0465	5.41	126.4	16.4	121.7	254.8	457.0
	0.0950	7.99	215.9	31.1	226.1	428.1	1023.3
(SBE) _{2.4} β	0.04	1.70	12.8				
	0.08	2.46					
(SPE) ₇ β	0.04	1.05	93.9	7.23	122.4		
	0.08	2.12	151.2	10.8	223.3	241.6	

Solubility of selected steroids enhanced by alpha-cyclodextrins

-CD	[CD] M	[Fluticasone] x10 ⁵ M		[Mometasone] x10 ⁵ M		[Budesonide] x10 ⁵ M	[Triamcinolone acetoneide] x10 ⁵ M
		as propionate	non esterified	as furoate	non esterified		
H ₂ O	NA	0.39	0.16	1.82	0.00	6.59	3.56
A	0.04			0.00	8.4		
	0.08			0.27	28.5		
(SBE) ₇ α	0.04	8.37		30.1	55.0	348.1	
	0.08	11.4		35.5	116.9	597.9	

Solubility of selected steroids enhanced by gamma-cyclodextrins

-CD	[CD] M	[Fluticasone] x10⁵M		[Mometasone] x10⁵M		[Budesonide] x10⁵M	[Triamcin- olone acetone] x10⁵M
		as propionate	non esterified	as furoate	non esterified		
H ₂ O	NA	0.39	0.16	1.82	0.00	6.59	3.56
Γ	0.035	73.5		14.1	2.71	10.1	197.8
	0.1	22.1	82.2	65.8	0.09	4.1	138.6
(SBE) _{5.2}	0.04	79.12				375.8	
γ	0.1	215.3	1440.4	93.9	889.2	861.6	
	0.04	51.82	575.6	41.5	841.1	306.6	1059.5
(SBE) _{6.1}	0.08	120.8	949.0	92.9	1423.1	698.8	2386.1
γ	0.04	54.5					
	0.075	103.1	895.0	94.0	889.6	453.4	
(SPE) _{5.4}	0.04	71.7	759.5	28.7		400.9	
γ	0.08	140.1	1387.8	51.3	1467.1	774.2	

The inventors have also discovered that SAE-γ-CD is particularly suitable for use in complexing esterified and non-esterified corticosteroids as compared to complexation of the same corticosteroids with SAE-β-CD or SAE-α-CD. The table above also summarizes the phase solubility data depicted in FIG. 15 for fluticasone and fluticasone propionate with various different SAE-γ-CD species having a degree of substitution in the range of 5-10.

The present inventors have discovered that SAE-γ-CD is also much more effective at binding with a particular regioisomer of esterified corticosteroids than is SAE-β-CD or SAE-α-CD. The procedure set forth in Example 18 details the comparative evaluation of the binding of SAE-γ-CD and SAE-β-CD with a series of structurally related corticosteroid derivatives. The table below summarizes the results of a study comparing the binding of SBEx-γ-CD, wherein x represents the average degree of substitution, derivatives and SBE-β-CD derivative with different forms of beclmethasone.

CD	Beclomethasone dipropionate ($\mu\text{g/mL}$)	Beclomethasone 17-mono- propionate ($\mu\text{g/mL}$)	Beclomethasone 21-mono- propionate ($\mu\text{g/mL}$)	Beclomethasone (unesterified) ($\mu\text{g/mL}$)
SBE _{3.4} γ -CD	0.04M $\rightarrow$ 336.8	0.04M $\rightarrow$ 10621.6	0.04M $\rightarrow$ 172.6	0.04M $\rightarrow$ 11360.2
SBE _{5.24} γ -CD	0.04M $\rightarrow$ 267.0	0.04M $\rightarrow$ 9500.8	0.04M $\rightarrow$ 139.8	0.04M $\rightarrow$ 10949.9
SBE _{6.1} γ -CD	0.04M $\rightarrow$ 243.8	0.04M $\rightarrow$ 11666.9	0.04M $\rightarrow$ 153.8	0.04M $\rightarrow$ 11007.0
SBE _{7.5} γ -CD	0.04M $\rightarrow$ 168.5	0.04M $\rightarrow$ 8539.1	0.04M $\rightarrow$ 122.4	0.04M $\rightarrow$ 9635.2
SBE _{6.7} β -CD	0.04M $\rightarrow$ 60.4	0.04M $\rightarrow$ 6799.6	0.04M $\rightarrow$ 50.6	0.04M $\rightarrow$ 6927.0
γ -CD	0.04M $\rightarrow$ 105.8	0.04M $\rightarrow$ 136.9	0.04M $\rightarrow$ 9.4	0.04M $\rightarrow$ 114.8

The survey study shows that in the presence of SBE(3.4) γ -CD (0.04M), all of the forms of beclomethasone were at or near their highest solubilities. B17P, the active metabolite of BDP, has the highest solubility of the esterified beclomethasone forms in any of the derivatized CDs. The results indicate that SBE- γ -CD complexes with beclomethasone dipropionate better than Captisol or γ -CD alone. Of the SAE-CD derivatives evaluated, the optimal degree of substitution of the SBE γ -CD that provides the greatest enhancement in solubility of BDP is DS = 3.4, and solubility decreases almost linearly as the degree of substitution increases. This is true for both the 24 hr and 5 day equilibration times. So in terms of BDP solubilization with SAE-CD: SBE(3.4) γ -CD > SBE(5.2) γ -CD > SBE(6.1) γ -CD > SBE(7.5) γ -CD > γ -CD > Captisol (SBE7- β -CD). The data is summarized in FIG. 16. Therefore, the present inventors have discovered that SAE- γ -CD cyclodextrin derivatives are unexpectedly better at solubilizing corticosteroids than are SAE- β -CD derivatives. Moreover, the formulations based upon SAE- γ -CD are suitable for use in inhalable formulations contrary to the disclosure of Worth et al. (above), which suggests that SAE-CD derivatives are not.

By “complexed” is meant “being part of a clathrate or inclusion complex with”, i.e., a complexed therapeutic agent is part of a clathrate or inclusion complex with a cyclodextrin derivative. By “major portion” is meant at least about 50% by weight. Thus,

a formulation according to the present invention may contain an active agent of which more than about 50% by weight is complexed with a cyclodextrin. The actual percent of active agent that is complexed will vary according to the complexation equilibrium constant characterizing the complexation of a specific cyclodextrin to a specific active agent. The invention also includes embodiments wherein the active agent is not complexed with the cyclodextrin or wherein a minor portion of the active agent is complexed with the derivatized cyclodextrin. It should be noted that an SAE-CD, or any other anionic derivatized cyclodextrin, can form one or more ionic bonds with a positively charged compound. This ionic association can occur regardless of whether the positively charged compound is complexed with the cyclodextrin either by inclusion in the cavity or formation of a salt bridge.

The binding of a drug to the derivatized cyclodextrin can be improved by including an acid or base along with the drug and cyclodextrin. For example, the binding of a basic drug with the cyclodextrin might be improved by including an acid along with the basic drug and cyclodextrin. Likewise, the binding of an acidic drug with the cyclodextrin might be improved by including a base (alkaline material) along with the acidic drug and cyclodextrin. The binding of a neutral drug might be improved by including a basic, acidic or other neutral compound along with the neutral drug and cyclodextrin. Suitable acidic compounds include inorganic and organic acids. Examples of inorganic acids are mineral acids, such as hydrochloric and hydrobromic acid. Other suitable acids include sulfuric acid, sulfonic acid, sulfenic acid, and phosphoric acid. Examples of organic acids are aliphatic carboxylic acids, such as acetic acid, ascorbic acid, carbonic acid, citric acid, butyric acid, fumaric acid, glutaric acid, glycolic acid, α -ketoglutaric acid, lactic acid, malic acid, mevalonic acid, maleic acid, malonic acid, oxalic acid, pimelic acid, propionic acid, succinic acid, tartaric acid, or tartronic acid. Aliphatic carboxylic acids bearing one or more oxygenated substituents in the aliphatic chain are also useful. A combination of acids can be used.

Suitable basic compounds include inorganic and organic bases. Suitable inorganic bases include ammonia, metal oxide and metal hydroxide. Suitable organic bases include primary amine, secondary amine, tertiary amine, imidazole, triazole, tetrazole, pyrazole, indole, diethanolamine, triethanolamine, diethylamine, methylamine, tromethamine (TRIS), aromatic amine, unsaturated amine, primary thiol, and secondary thiol. A combination of bases can be used.

An anionic derivatized cyclodextrin can complex or otherwise bind with an acid-ionizable agent. As used herein, the term acid-ionizable agent is taken to mean any compound that becomes or is ionized in the presence of an acid. An acid-ionizable agent comprises at least one acid-ionizable functional group that becomes ionized when exposed to acid or when placed in an acidic medium. Exemplary acid-ionizable functional groups include a primary amine, secondary amine, tertiary amine, quaternary amine, aromatic amine, unsaturated amine, primary thiol, secondary thiol, sulfonium, hydroxyl, enol and others known to those of ordinary skill in the chemical arts.

The degree to which an acid-ionizable agent is bound by non-covalent ionic binding versus inclusion complexation formation can be determined spectrophotometrically using methods such as ^1H NMR, ^{13}C NMR, or circular dichroism, for example, and by analysis of the phase solubility data for the acid-ionizable agent and anionic derivatized cyclodextrin. The artisan of ordinary skill in the art will be able to use these conventional methods to approximate the amount of each type of binding that is occurring in solution to determine whether or not binding between the species is occurring predominantly by non-covalent ionic binding or inclusion complex formation. An acid-ionizable agent that binds to derivatized cyclodextrin by both means will generally exhibit a bi-phasic phase solubility curve. Under conditions where non-covalent ionic bonding predominates over inclusion complex formation, the amount of inclusion complex formation, measured by NMR or circular dichroism, will be reduced even though the phase solubility data indicates significant binding between the species under those conditions; moreover, the intrinsic solubility of the acid-ionizable agent, as determined from the phase solubility data, will generally be higher than expected under those conditions.

As used herein, the term non-covalent ionic bond refers to a bond formed between an anionic species and a cationic species. The bond is non-covalent such that the two species together form a salt or ion pair. An anionic derivatized cyclodextrin provides the anionic species of the ion pair and the acid-ionizable agent provides the cationic species of the ion pair. Since an anionic derivatized cyclodextrin is multi-valent, an SAE-CD can form an ion pair with one or more acid-ionizable agents.

The parent cyclodextrins have limited water solubility as compared to SAE-CD and HPCD. Underivatized α -CD has a water solubility of about 14.5% w/v at saturation. Underivatized β -CD has a water solubility of about 1.85% w/v at saturation.

Underivatized γ -CD has a water solubility of about 23.2% w/v at saturation. Dimethyl-beta-cyclodextrin (DMCD) forms a 43% w/w aqueous solution at saturation. The SAE-CD can be combined with one or more other cyclodextrins or cyclodextrin derivatives in the inhalable solution to solubilize the corticosteroid.

5 Other water soluble cyclodextrin derivatives that can be used according to the invention include the hydroxyethyl, hydroxypropyl (including 2- and 3-hydroxypropyl) and dihydroxypropyl ethers, their corresponding mixed ethers and further mixed ethers with methyl or ethyl groups, such as methylhydroxyethyl, ethyl-hydroxyethyl and ethyl-hydroxypropyl ethers of alpha-, beta- and gamma-cyclodextrin; and the maltosyl, glucosyl
10 and maltotriosyl derivatives of alpha, beta- and gamma-cyclodextrin, which may contain one or more sugar residues, e.g. glucosyl or diglucosyl, maltosyl or dimaltosyl, as well as various mixtures thereof, e.g. a mixture of maltosyl and dimaltosyl derivatives. Specific cyclodextrin derivatives for use herein include hydroxypropyl-beta-cyclodextrin, hydroxyethyl-beta-cyclodextrin, hydroxypropyl-gamma-cyclodextrin, hydroxyethyl-
15 gamma-cyclodextrin, dihydroxypropyl-beta-cyclodextrin, glucosyl-alpha-cyclodextrin, glucosyl-beta-cyclodextrin, diglucosyl-beta-cyclodextrin, maltosyl-alpha-cyclodextrin, maltosyl-beta-cyclodextrin, maltosyl-gamma-cyclodextrin, maltotriosyl-beta-cyclodextrin, maltotriosyl-gamma-cyclodextrin and dimaltosyl-beta-cyclodextrin, and mixtures thereof such as maltosyl-beta-cyclodextrin/dimaltosyl-beta-cyclodextrin, as well as methyl-beta-
20 cyclodextrin. Procedures for preparing such cyclodextrin derivatives are well-known, for example, from Bodor United States Patent No. 5,024,998 dated June 18, 1991, and references cited therein. Other cyclodextrins suitable for use in the present invention include the carboxyalkyl thioether derivatives such as ORG 26054 and ORG 25969 made by ORGANON (AKZO-NOBEL), hydroxybutenyl ether derivatives made by EASTMAN, sulfoalkyl-hydroxyalkyl ether derivatives, sulfoalkyl-alkyl ether derivatives, and other
25 derivatives as described in US Pregrant Patent Application Publications No. 2002/0128468, No. 2004/0106575, No. 2004/0109888, and No. 2004/0063663, or U.S. Patents No. 6,610,671, No. 6,479,467, No. 6,660,804, or No. 6,509,323.

The HP- β -CD can be obtained from Research Diagnostics Inc. (Flanders, NJ).
30 HP- β -CD is available with different degrees of substitution. Exemplary products include ENCAPSIN™ (degree of substitution~4; HP4- β -CD) and MOLECUSOL™ (degree of substitution~8; HP8- β -CD); however, embodiments including other degrees of substitution are also available. Since HPCD is non-ionic, it is not available in salt form.

Dimethyl cyclodextrin is available from FLUKA Chemie (Buchs, CII) or Wacker (Iowa). Other derivatized cyclodextrins suitable in the invention include water soluble derivatized cyclodextrins. Exemplary water-soluble derivatized cyclodextrins include carboxylated derivatives; sulfated derivatives; alkylated derivatives; hydroxyalkylated derivatives; methylated derivatives; and carboxy- β -cyclodextrins, e.g. succinyl- β -cyclodextrin (SCD), and 6^A-amino-6^A-deoxy-N-(3-carboxypropyl)- β -cyclodextrin. All of these materials can be made according to methods known in the prior art. Suitable derivatized cyclodextrins are disclosed in Modified Cyclodextrins: Scaffolds and Templates for Supramolecular Chemistry (Eds. Christopher J. Easton, Stephen F. Lincoln, Imperial College Press, London, UK, 1999) and New Trends in Cyclodextrins and Derivatives (Ed. Dominique Duchene, Editions de Santé, Paris, France, 1991).

Sulfobutyl ether β -cyclodextrin (CAPTISOL, CyDex Inc., degree of substitution = 6.6), 2-hydroxypropyl β -cyclodextrin (HP- β -CD, CERESTAR, degree of substitution = 5.5), succinylated- β -cyclodextrin (S-CD, Cyclolab), and 2,6-di-o-methyl- β -cyclodextrin (DM-CD, Fluka) %w/w solutions were prepared at their native pH or buffered as needed. Sulfoalkyl ether γ -CD and sulfoalkyl ether α -CD derivatives were obtained from CyDex, Inc. (Lenexa, KS) and The University of Kansas (Lawrence, KS).

The amount of derivatized cyclodextrin required to provide the desired effect will vary according to the materials comprising the formulation.

Different cyclodextrins are able to solubilize a corticosteroid to different extents. FIG. 3 depicts a molar phase solubility curve for budesonide with HP- β -CD, SBE7- β -CD, and γ -CD as compared to water. The inventors have found that SAE-CD is superior to other cyclodextrins and cyclodextrin derivatives at solubilizing budesonide. On a molar basis, SBE- β -CD is a better solubilizer of budesonide than HP- β -CD. In addition, the solubilizing power among the SAE-CD derivatives followed about this rank order for budesonide over a SAE-CD concentration range of 0.04 to 0.1 M: SBE5.2- γ -CD ~ SPE5.4- γ -CD > SBE6.1- γ -CD > SBE7- α -CD > SBE9.7- γ -CD ~ SBE6.7- β -CD > SPE7- β -CD. For example, a 0.1 M concentration of SBE7- β -CD was able to solubilize a greater amount of budesonide than either γ -CD or HP- β -CD. Moreover, SAE-CD-containing nebulizable formulations provide a greater output rate for corticosteroid by nebulization as compared to γ -CD or HP- β -CD administered under otherwise similar conditions.

It was unexpectedly discovered that the nebulization of Captisol solutions provides several advantages with respect to other cyclodextrins. The droplets leaving the nebulizer are of a more advantageous size and the Captisol solutions are nebulized faster than similar solutions of other Cyclodextrins. The table below shows that the average particle size (Dv50) of Captisol solutions is smaller than that of HP- β -CD or γ -CD. More importantly, as seen in the table below, the Dv90 shows that the other cyclodextrins had significant number of very large droplets. The data (Malvern particle size) was obtained for each formulation as emitted from a PARI LC PLUS nebulizer equipped with a PARI PRONEB ULTRA air compressor. The smaller droplet size is favored for an inhalable composition as it permits deeper lung delivery of active agents such as a corticosteroid.

Formulation	Dv10 (μm)	Dv 50 (μm)	Dv 90 (μm)
5% Captisol	1.9 ± 0.04	3.84 ± 0.08	10.52 ± 0.2
10% Captisol	1.82 ± 0.05	3.61 ± 0.25	11.18 ± 1.92
20% Captisol	1.78 ± 0.04	3.12 ± 0.11	10.02 ± 0.23
5% Hydroxypropyl β -Cyclodextrin	1.89 ± 0.04	3.99 ± 0.13	14.89 ± 2.45
10% Hydroxypropyl β -Cyclodextrin	1.95 ± 0.03	4.62 ± 0.34	120.1 ± 172.67
20% Hydroxypropyl β -Cyclodextrin	1.91 ± 0.02	4.26 ± 0.16	13.77 ± 1.00
5% γ -Cyclodextrin	1.94 ± 0.05	3.99 ± 0.36	205.62 ± 222.10
10% γ -Cyclodextrin	2.03 ± 0.05	4.84 ± 0.49	451.55 ± 25.92
20% γ -Cyclodextrin	1.96 ± 0.04	4.97 ± 0.12	286.46 ± 235.13

This advantage is further shown in the output rate of these solutions. The table below shows that Captisol is emitted from the nebulizer faster and also to a greater extent than the other cyclodextrins, thus the output rate of the nebulizer is greater when Captisol is nebulized.

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Formulation	Percent Emitted	Sputter Time (min)	Output Rate mg/min
5% Captisol	56.42	3.81	296
10% Captisol	55.13	3.84	287
20% Captisol	50.56	4.06	249
5% Hydroxypropyl β -Cyclodextrin	43.32	4.14	209
10% Hydroxypropyl β -Cyclodextrin	46.22	4.27	216
20% Hydroxypropyl β -Cyclodextrin	46.90	4.01	234
5% γ -Cyclodextrin	52.74	5.41	195
10% γ -Cyclodextrin	53.75	4.98	216
20% γ -Cyclodextrin	51.91	4.81	216

Nebulization is stopped when the sound changes (time to sputter) or visible particles are no longer produced. Sputter occurs at substantial completion of delivery of solution in the reservoir of the nebulizer.

The advantage of Captisol was further demonstrated by preparing solutions containing budesonide dissolved in various cyclodextrins and comparing their performance in nebulization to the performance of commercial PULMICORT® RESPULES®, a commercially available suspension-based unit dose formulation. The suspension obtained from several unit dose ampoules of PULMICORT™ was pooled to form a multi-use suspension based unit dose formulation, and and SAE-CD (specifically, CAPTISOL), HP- β - or γ - cyclodextrin powder was added to achieve a 0.25mg/ml budesonide solution concentration. These budesonide-containing solutions contained 5%w/v Captisol (P5C), 1%w/v gamma-CD (P1 γ CD) and 5%w/v hydroxypropyl-beta-cyclodextrin (P5HP β CD). Each was prepared at least 30 minutes prior to all testing. All three formulations were clear, colorless solutions. (Note: a 250 mg/mL solution of budesonide cannot be achieved in a 5% w/v solution of γ -cyclodextrin as it exhibits “B” type solubility behavior) A 2 ml aliquot of the suspension or solution was placed in the same Pari LC Plus nebulizer setup and the amount of budesonide in the emitted droplets was determined by collecting them onto a filter and measuring the budesonide using HPLC. As shown in the table below, the total output rate (μ g budesonide collected/time to sputter) for each suspension or solution.

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Sample ID	Total Output Rate ($\mu\text{g}/\text{min}$)	SD ($\mu\text{g}/\text{min}$)
Pulmicort	33.85	3.85
Pulmicort + 5% Captisol	44.04	1.42
Pulmicort + 5% HP- β -CD	21.37	2.44
Pulmicort + 1% γ -CD	40.36	5.73

The output rate is highest for the Captisol solution indicating that an equivalent amount of drug can be delivered in a shorter period of time. Under the conditions used, β -CD is unable to solubilize an equivalent amount of corticosteroid due to the limited solubility of β -CD in water.

The present invention can be used with other suspension-based aqueous formulations, which formulations may be adapted for nasal delivery or pulmonary delivery. Exemplary suspension-based aqueous formulations include the UDB formulation (Sheffield Pharmaceuticals, Inc.), VANCENASE™ AQ (beclomethasone dipropionate aqueous suspension; Schering Corporation, Kenilworth, NJ), ATOMASE™ (beclomethasone dipropionate aqueous suspension; Douglas Pharmaceuticals Ltd., Auckland, Australia), BECONASE™ (beclomethasone dipropionate aqueous suspension; Glaxo Wellcome, NASACORT AQ™ (triamcinolone acetonide nasal spray, Aventis Pharmaceuticals), TRI-NASAL™ (triamcinolone acetonide aqueous suspension; Muro Pharmaceuticals Inc.) and AEROBID-M™, (flunisolide inhalation aerosol, Forest Pharmaceuticals), NASALIDE™ and NASAREL™ (flunisolide nasal spray, Ivax Corporation), FLONASE™ (fluticasone propionate, GlaxoSmithKline), and NASONEX™ (mometasone furoate, Schering-Plough Corporation).

The suspension formulation can comprise corticosteroid present in particulate, microparticulate, nanoparticulate or nanocrystalline form. Accordingly, an SAE-CD can be used to improve the administration of a corticosteroid suspension-based unit dose formulation. Moreover, the SAE-CD outperforms other cyclodextrin derivatives.

According to one embodiment, SAE-CD (in solid or liquid form) and a suspension-based unit dose formulation comprising corticosteroid are mixed. The SAE-CD is present in an amount sufficient to increase the amount of solubilized corticosteroid, i.e. decrease the amount of unsolubilized corticosteroid, therein. Prior to administration, the liquid may be optionally aseptically filtered or terminally sterilized. The liquid is then administered to a subject by inhalation using a nebulizer. As a result, the amount of drug that the

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subject receives is higher than the subject would have received had the unaltered suspension formulation been administered.

According to another embodiment, SAE-CD (in liquid form, as ready-to-use liquid or as a concentrate) and a solid unit dose formulation comprising corticosteroid are mixed to form a liquid formulation. The SAE-CD is present in an amount sufficient to solubilize a substantial portion of the corticosteroid. The liquid is then administered via inhalation using a nebulizer.

According to another embodiment, SAE-CD (in solid form) and a solid unit dose formulation comprising corticosteroid are mixed to form a solid mixture to which is added an aqueous liquid carrier in an amount sufficient to form a nebulizable formulation. Mixing and/or heating are optionally employed upon addition of the liquid carrier to form the formulation. The SAE-CD is present in an amount sufficient to solubilize a substantial portion of the corticosteroid. The formulation is then administered via inhalation using a nebulizer.

The size of the reservoir varies from one type of nebulizer to another. The volume of the liquid formulation may be adjusted as needed to provide the required volume for loading into the reservoir of a particular type or brand of nebulizer. The volume can be adjusted by adding additional liquid carrier or additional solution containing SAE-CD. In general, the reservoir volume of a nebulizer is about 10 μ l to 100 ml. Low volume nebulizers, such as ultrasonic and vibrating mesh/vibrating plate/vibrating cone/vibrating membrane nebulizers, pre-filled reservoir strips inclusive of delivery nozzle typically have a reservoir volume of 10 μ l to 6 ml or 10 μ l to 5 ml. The low volume nebulizers provide the advantage of shorter administration times as compared to large volume nebulizers.

Example 28 details a procedure for preparation of a solution of the invention to be used with a low volume (low reservoir volume and/or low reservoir residual volume) nebulizer, such as an AERx nebulizer. The solutions of the invention can be nebulized with any nebulizer; however, with an AERx delivery system that coordinates both the inhalation and delivery processes to optimize deep lung penetration, an initial sample volume of about 10 to 100, or 50 μ l can be used to load AERx Strip unit dose container. Administration of this solution with the system makes it feasible for a therapeutic dose to be administered to a subject in a single puff (a single full inhalation by a subject, i.e. 3 – 5 seconds) via nebulization. Based on general performance expectations of such devices where the emitted dose is 60 – 65% and the subsequent lung doses are 65 – 85%, about 10

µg to 60 µg or 10 µg to 110 µg of budesonide may be expected to be delivered to the lung in a single dosing event using budesonide solutions prepared with SAECD.

Example 32 details a procedure for the comparison of nebulization parameters in four different nebulizers using a formulation of the invention and PULMICORT
5 RESPULES (suspension-based formulation). In each case, the formulation of the invention out performs the suspension-based formulation. The solution of the invention provide a 1.25, 1.4, 2.1, 3.3, 3.67, 1.25 to 3.7, or 1.25 to 4 fold increase in the amount of budesonide delivered. Under the conditions tested, the AIRSEP MYSTIQUE was most efficient at emitting/nebulizing the SAE-CD / budesonide formulation.

10 Unless otherwise specified, the term “dose”, which is understood to include an effective dose, is taken to mean a nominal dose, emitted dose, nominal available dose, dose to subject, dose to lung or other such term of art. The term “nominal dose” refers to an amount of corticosteroid placed in the reservoir of a nebulizer, wherein the volume of liquid in the reservoir is determined according the size of the reservoir. The term
15 “nominal available dose” refers to the amount of corticosteroid that is determined could be or should have been available to a subject when administered a formulation of the invention by nebulization but formulation is/was not administered in its entirety. The term “emitted dose” refers to the amount of corticosteroid emitted from a nebulizer. The term “dose to subject” refers to the amount of corticosteroid delivered to and retained by a
20 subject following administration of a formulation of the invention by nebulization. The term “dose to lung” refers to the amount of corticosteroid delivered to and retained by the lungs of a subject following administration of a formulation of the invention by nebulization.

In general, a single-use suspension-based unit dose formulation of corticosteroid
25 contains about 0.125, 0.25, 0.5, 1, 2, or about 0.125 to about 2 mg of corticosteroid suspended in about 50 µl to 10 ml of liquid carrier. Alternatively, the corticosteroid is present at a concentration of about 20 mcg to about 30 mg of corticosteroid per ml of suspension. As a result, about 10 to 500 mg of SAE-CD, or 10 to 250 mg of SAE-CD, or 10 to 300 mg of SAE-CD, be it in solid form or dissolved in a liquid carrier, is added to
30 each ml the suspension in order to dissolve a substantial portion of the corticosteroid and form a nebulizable unit dose liquid formulation that is then administered to a subject.

The formulation, method or system can employ a dose of about 1 µg to 20 mg, 1 µg to 10 mg, 0.01 mg to 10 mg, 0.025 mg to 10 mg, 0.05 mg to 5 mg, 0.1 mg to 5 mg,

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0.125 mg to 5 mg, 0.25 mg to 5 mg, 0.5 mg to 5 mg, 0.05 mg to 2 mg, 0.1 mg to 2 mg, 0.125 mg to 2 mg, 0.25 mg to 2 mg, 0.5 mg to 2 mg of corticosteroid. These dose amounts are suitable for the corticosteroids of the invention, in particular corticosteroids that are as lipophilic as or more lipophilic than flunisolide, which corticosteroids include
5 beclomethasone, beclomethasone dipropionate, beclomethasone monopropionate, budesonide, ciclesonide, desisobutryl-ciclesonide, flunisolide, fluticasone, fluticasone propionate, mometasone, mometasone furoate, triamcinolone acetonide.

The formulation of the invention comprises a dose of corticosteroid in an approximate solution volume of 10 μ l to 100 ml, 50 μ l to 50 ml, 50 μ l to 10 ml, 0.1 to 10
10 ml, 0.1 ml to less than 10 ml, 0.1 ml to 7.5 ml, 0.1 ml to 5 ml, 0.1 ml to 3 ml, 0.1 ml to 2 ml, 0.1 ml to 1 ml, 0.05 ml to 7.5 ml, 0.05 ml to 5 ml, 0.05 ml to 3 ml, 0.05 ml to 2 ml, or 0.05 ml to 1 ml.

Due to the wide range of nebulizer reservoir volumes available and of varying dose requirements among the corticosteroids, a formulation of the invention can comprise 1 μ g
15 to 20 mg of corticosteroid in 0.01 ml to 100 ml of solution volume.

In general, a multi-use suspension-based unit dose formulation of corticosteroid contains approximately 0.125 to 2 mg of corticosteroid suspended in 1 to 100 ml of liquid carrier. A multi-use formulation actually contains two or more unit doses of corticosteroid. Single unit dose aliquots are taken from a multi-use unit dose formulation,
20 and the single unit dose are typically administered one-at-a-time to a subject. As a result, about 10 to 500 mg of SAE-CD, be it in solid form or dissolved in a liquid carrier, is added to each ml the suspension in order to dissolve a substantial portion of the corticosteroid and form a multi-use unit dose liquid formulation that is then administered to a subject in single unit dose aliquots.

One aspect of the invention is that a suspension-based unit dose formulation is converted to a liquid unit dose formulation prior to pulmonary administration via inhalation (of a nebulized mist) to a subject. The conversion can take place in the same container in which the suspension is provided, in a different container, or in the reservoir of a nebulizer. In order to form a liquid formulation, a substantial portion of the
30 corticosteroid must be dissolved. As used in reference to the amount of dissolved corticosteroid, a "substantial portion" is at least 20% wt., at least 30% wt., at least 40% wt., or at least 20% wt and less than 50% wt. of the corticosteroid. As used in reference to

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the amount of dissolved corticosteroid, a “major portion” is at least 50% wt. of the corticosteroid.

It is well known that pharmacists working in compounding pharmacies can and do prepare a suspension-based unit dose formulation comprising corticosteroid. Such pharmacists will now be able to prepare a single use or multi-use liquid unit dose formulation by employing a method described herein. Alternatively, a subject (patient) undergoing corticosteroid treatment may convert the suspension-based formulation to a liquid formulation of the invention by employing a method described herein. Instead of preparing the liquid formulation from the suspension at the pharmacy, a kit containing the suspension formulation and SAE-CD can be prepared.

The concentration of SAE-CD in solution can be expressed on a weight to weight or weight to volume basis; however, these two units are interconvertible. When a known weight of cyclodextrin is dissolved in a known weight of water, the %w/w cyclodextrin concentration is determined by dividing the cyclodextrin weight in grams by the total weight (cyclodextrin + water weight) in like units and multiplying by 100. When a known weight of cyclodextrin is dissolved to a known total volume, the %w/v cyclodextrin concentration is determined by dividing the cyclodextrin weight in grams by the total volume in milliliters and multiplying by 100. The correlation between the two cyclodextrin concentration percentages was experimentally determined by preparing various %w/w cyclodextrin solutions and measuring the density of each with a pycnometer at 25°C. The density (g/mL) of each %w/w CAPTISOL solution is presented in the table below.

Captisol % w/w	Density (g/mL)	Viscosity (Cp, 25C)
59.4	1.320	527.0
49.4	1.259	51.9
39.7	1.202	17.0
29.8	1.149	5.91
19.7	1.095	2.78
8.5	1.041	1.75
0.0	1.002	1
slope = 0.0053 y-intercept = 0.995 correlation = 0.9989		

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The resulting linear relationship readily enables the conversion of CAPTISOL concentrations expressed in %w/w to that of %w/v by the following equation:

$$\%w/v = ((\%w/w * \text{slope}) + \text{y-intercept}) * \%w/w$$

5 where the slope and intercept values are determined from a linear regression of the density data in the table. For example, by using the above equation, a 40%w/w CAPTISOL solution would be equivalent to a ~48.3% w/v CAPTISOL solution.

The performance of an inhalable solution of the invention in a nebulizer may depend upon the viscosity of the solution in its reservoir, the nebulization solution. The
10 viscosity of an aqueous solution of SBE7- β -CD changes with respect to concentration approximately as indicated in the table above. Viscosity of the inhalable composition can have an impact on percentage of nebulization composition emitted from a nebulizer, output rate of nebulized corticosteroid and droplet size distribution.

The amount of residual nebulization inhalable composition left in the reservoir of
15 the nebulizer may be greater for solutions containing SAE-CD than for a budesonide-containing suspension. For example, Figure 4 depicts a chart of the estimated percentage of nebulization composition emitted from three different nebulizers (PARI LC PLUS, HUDSON UPDRAFT II NEB-U-MIST, and MYSTIQUE) for each of four different nebulization compositions (PULMICORT RESPULES suspension, 5% w/w SBE7- β -CD
20 solution, 10% w/w SBE7- β -CD solution and 20% w/w SBE7- β -CD solution). The PULMICORT RESPULES suspension was used as the control. The PARI LC PLUS, MYSTIQUE and HUDSON nebulizers were used for the comparison. The MYSTIQUE nebulizer was unable to nebulize the suspension and concentrated SAE-CD solution (20% w/w) efficiently so they were not evaluated with that nebulizer. The results suggest that,
25 under the conditions tested, nebulization of PULMICORT RESPULES suspension results in a greater percentage of nebulized composition, meaning that, with the suspension, less nebulization composition is left in the reservoir of the nebulizer upon completion of nebulization as compared to with the solution. In some cases, nebulization of the suspension resulted in the greatest percentage by weight of total composition emitted by a
30 nebulizer. In other words, under similar nebulization conditions, the PARI LC PLUS and HUDSON nebulizers more efficiently reduced the volume of nebulization suspension than of nebulization solution; however, this did not correspond with the total amount of drug emitted by the nebulizer.

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Output rate of an SAE-CD nebulization solution versus that of a suspension, each containing budesonide, was compared. A modified version of the method of Example 10 was followed to determine output rate. The tables below summarize the data observed.

Sample ID	2 minutes Bud recvr'd (ug)	Total Bud Recovered (ug)	Total Neb Time (min:sec)	Total Neb Time (min)	Out Put Rate 2 minutes (ug/min)	Total Out Put Rate (ug/min)
1-PUL-1	84.021	164.199	5:34	5.57	42.01	29.48
1-PUL-2	90.395	175.63	4:58	4.97	45.20	35.34
1-PUL-3	82.046	174.546	4:45	4.75	41.02	36.75
	Mean	171.458		Mean	42.74	33.85
	SD	6.310		SD	2.18	3.85
	CV	3.680		CV	5.10	11.38
2-P5C-1	131.412	258.894	5:42	5.7	65.71	45.42
2-P5C-2	126.945	246.987	5:36	5.6	63.47	44.10
2-P5C-3	128.464	236.371	5:33	5.55	64.23	42.59
	Mean	247.417		Mean	64.47	44.04
	SD	11.268		SD	1.14	1.42
	CV	4.554		CV	1.76	3.22

Data obtained using a PARI LC PLUS nebulizer equipped with a PARI PRONEB

5 ULTRA air compressor.

Sample ID	2 minutes Bud recvr'd (ug)	Total Bud Recovered (ug)	Total Neb Time (min:sec)	Total Neb Time (min)	Out Put Rate 2 minutes (ug/min)	Total Out Put Rate (ug/min)
10-PUL-1	11.200	27.926	5:20	5.33	5.60	5.24
10-PUL-2	29.015	40.11	4:15	4.25	14.51	9.44
10-PUL-3	25.363	30.516	4:17	4.28	12.68	7.13
	Mean	32.851		Mean	10.93	7.27
	SD	6.419		SD	4.71	2.10
	CV	19.539		CV	43.05	28.93
11-P5C-1	41.049	98.155	5:47	5.78	20.52	16.98
11-P5C-2	44.495	131.8	6:00	6	22.25	21.97
11-P5C-3	53.374	132.31	5:55	5.92	26.69	22.35
	Mean	120.755		Mean	23.15	20.43
	SD	19.574		SD	3.18	2.99
	CV	16.210		CV	13.73	14.66

Data obtained using a MYSTIQUE ultrasonic nebulizer.

All of the above formulations contain approximately 250 µg/mL of budesonide.

The samples identified as "P5C" contain 50 mg/mL (or about 5%) SBE7-β-CD.

The table below shows the nebulizer output rate for solutions containing various
10 levels of SAE-CD.

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Sample ID	Viscosity (Cp)	Nebulizer Volume (ml)	% Emitted (By Weight Difference)	Nebulization Time (Minutes: Seconds)	Output Rate
21.5% w/w SBE7- β -CD	3.06	3	47.47	10:51	4.52
10.75% w/w SBE7- β -CD	1.84	3	51.36	8:53	6.02
5.15% w/w SBE7- β -CD	1.23	3	55.47	9:59	5.78
H ₂ O		3	50.36	9:21	5.47

Surprisingly, nebulization of the SAE-CD-containing solution provided a higher budesonide output rate than nebulization of the PULMICORT RESPULES suspension even though the nebulizer emitted a greater total amount of the suspension. Higher SAE-CD concentrations, above 25% w/v led to slightly longer nebulization times and lower output rates once the viscosity exceeded an approximate upper limit.

Without being held bound to a particular mechanism, it is believed that the nebulizer preferentially nebulizes the water (supernatant) of the suspension rather than the particles of the suspension thereby causing an increase in the molar concentration of budesonide in the suspension in the reservoir. Since the corticosteroid is dissolved in the solution of the invention, this type of separation does not occur to a significant degree. As a result, the rate of increasing concentration of corticosteroid in the reservoir is lower for a solution than it is with a suspension. Further support for this phenomenon is detailed in the following table.

	Initial Conc	Final Conc	RIC over 2 min. mcg/mL per min	RIC over TTS, mcg/mL per min
Pulmicort Respules	250	572	31.26	64.37
CEB	250	398	14.96	26.69

The data above was obtained by comparing the nebulization of a solution of the invention (CEB, SAE-CD, 5 %wt./v, 2 ml; budesonide, 250 μ g/ml) and PULMICORT RESPULES suspension 2 ml; budesonide, 250 μ g/ml) using a PARI LC+ equipped with a PARI PRONEB ULTRA air compressor. The concentration of budesonide in the reservoir was measured at two minutes and at time to sputter (TTS). The rate of increasing

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concentration (RIC) of the corticosteroid in the solution in the reservoir was then calculated for each formulation over each interval of time. The RIC of the solution of the invention provided an approximately 48% decrease in the RIC over two minutes and an approximately 42% decrease in the RIC over the TTS. In some embodiments, the percentage decrease in the RIC ranges from 10% to 60%, 15% to 60%, 20% to 60%, 30% to 60%, or 40% to 60%. In some embodiments, the system may provide a RIC of 0 to 40, 1 to 40, 5 to 30, or 10 to 30 mcg of corticosteroid/mL of solution volume per min of operation/nebulization.

Accordingly, the invention provides a system for administration of a corticosteroid by nebulization comprising: a nebulizer comprising a reservoir; and an inhalable aqueous composition comprising SAE-CD and a dose of corticosteroid, whereby the system provides a reduced RIC for the corticosteroid in the reservoir as compared to the RIC provided by a suspension comprising approximately the same amount of corticosteroid and volume of aqueous composition operated under approximately the same conditions.

Based on data above, $21.5 \pm 5\%$ w/w SBE7- β -CD concentration was identified as the approximate upper acceptable level for the nebulizer tested, "acceptable" being defined as the upper concentration of SBE7- β -CD that can be used without building up excessive viscosity, which may adversely affect the nebulization time and output rate. The practical upper limit for concentration of SAE-CD will vary among the nebulizer formats. The upper acceptable concentration of SAE-CD in a liquid formulation for use in a nebulizer may vary according to the DS of the derivative, the alkyl chain length of the sulfoalkyl functional group, and/or the CD ring size of the SAE-CD.

For administration to the respiratory tract, particularly the lungs, a nebulizer is used to produce appropriately sized droplets. Typically, the particle size of the droplet produced by a nebulizer for inhalation is in the range between about 0.5 to about 5 microns. If it is desired that the droplets reach the lower regions of the respiratory tract, i.e., the alveoli and terminal bronchi, the particle size range can be between about 0.5 and about 2.5 microns. If it is desired that the droplets reach the upper respiratory tract, the particle size range can be between 2.5 microns and 5 microns.

As noted above, viscosity of the nebulization composition can impact droplet size and droplet size distribution. For example, the present formulations tend to form larger droplets, in terms of Dv_{50} , at the lower concentrations, and thereby lower viscosity, of SAE-CD in the absence of budesonide. FIGS. 5a-5b depict droplet size data for

nebulization of inhalable compositions with a PARI LC PLUS nebulizer. For each of the figures, a MALVERN laser light scattering device (Mastersizer S, Malvern Instruments Ltd, Malvern, Worcs, U.K.) was used to measure MMAD. FIG. 5a depicts the results obtained using γ -CD solutions of varying concentrations (5% w/v, 10% w/v and 20% w/v) in the absence of budesonide. The results indicate that γ -CD on its own would not behave acceptably in a nebulizer, since almost all of the mass of the solution is of an unacceptable droplet size range. Even with extensive recycling and droplet size selection by a nebulizer, a γ -CD based nebulization solution containing corticosteroid would require an extremely long dosing period due to the low percentage of mass that is of the appropriate droplet size range, especially since γ -CD is not an effective solubilizer of budesonide at the concentrations tested.

In comparison, FIG. 5b depicts the results obtained using the same nebulizer with PULMICORT RESPULES suspension or a modified PULMICORT RESPULES solution containing SAE-CD of different concentrations (5% w/v, 10% w/v and 20% w/v). With each of these samples, a significant portion of the nebulized mass is of a respirable size range. Moreover, the solutions containing SAE-CD apparently form droplets that are comparable in size to those of the nebulized suspension.

Figure 6 depicts droplet size data for nebulization of inhalable compositions with a HUDSON UPDRAFT II NEBUMIST nebulizer charged with PULMICORT RESPULES suspension or a solution containing SAE-CD at different concentrations. (5% w/v, 10% w/v and 20% w/v). As compared to the PARI LC PLUS nebulizer, the NEB-U-MIST forms a slightly larger particle size distribution, a significant portion of the nebulized mass is still in the appropriate size range. Accordingly, the nebulization solution made from the suspension and containing SAE-CD is suitable for use in a variety of different air driven jet nebulizers.

The package insert for PULMICORT RESPULES suspension states that the suspension should not be nebulized with an ultrasonic nebulizer. Figure 7 depicts droplet size data for nebulization of inhalable compositions with a MYSTIQUE ultrasonic nebulizer. The compositions include three different SAE-CD containing solutions. Unlike the suspension, the SAE-CD containing solution can be nebulized with an ultrasonic nebulizer. Thus, the invention provides a method of improving the pulmonary delivery of corticosteroid in a suspension-based unit dose formulation from an ultrasonic nebulizer, the method comprising the step of including SAE-CD in the formulation in an amount

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sufficient to decrease the amount of undissolved corticosteroid in the suspension-based unit dose formulation.

The performance of nebulization compositions across a range of nebulizers is typically compared by comparing the Dv50 of the droplet size distribution for the respective compositions. Figure 8 depicts comparative Dv50 droplet size data for nebulization of an inhalable composition with the three above-mentioned nebulizers. In each case, the SAE-CD containing solutions are suitable for administration by nebulization across a range of concentrations. Moreover, the droplet size distribution can be partially controlled by adjusting the concentration of SAE-CD.

Figure 9 is a graph depicting the relationship between concentration of SAE-CD versus output rate of SAE-CD in various different nebulizers with different sources of compressed air required for the specific setup: the RAINDROP-Rat, RAINDROP-Dog, PARI LC STAR-UNC, PARI LC STAR-Rat PARI LC PLUS and DEVILBISS PULMO AIDE air jet driven nebulizers. The nebulizers were used in a variety of setups including free standing as well as animal exposure chambers and/or individual exposure masks. In general, the data demonstrate that output of SAE-CD increases with increasing SAE-CD concentration. Depending upon the nebulizer used, the conditions under which the nebulizer is operated and the concentration of SAE-CD in solution, different maximum output rates can be achieved. For example, the maximum output rate in the Raindrop-Dog setup is from a 250 mg/mL CAPTISOL concentration.

Even though nebulization of PULMICORT RESPULES suspension with an ultrasonic nebulizer is not recommended, it can be achieved. Figures 10a-10b depict comparative droplet size data for nebulization solutions with the PARI LC PLUS and MYSTIQUE nebulizers of PULMICORT RESPULES suspension and a modified PULMICORT RESPULES-based SAE-CD solution. PULMICORT RESPULES suspension with and without 5% w/v SBE7- β -CD were used as the test samples. The procedure of Example 12 was followed. FIG. 10a depicts the Dv10 and Dv50 data for the solutions run on the PARI LC PLUS air driven jet nebulizer and FIG. 10b depicts the Dv10 and Dv50 data for the solutions run on the MYSTIQUE ultrasonic nebulizer. In each case, the droplet size data for the two different solutions is comparable. However, the budesonide output rate for the two solutions was significantly different. Use of SAE-CD in a nebulization composition, however, results in an increased output rate of budesonide regardless of the format of the nebulizer. The invention, thus, provides a method of

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increasing the output rate of a corticosteroid-containing suspension-based unit dose formulation being delivered by a nebulizer, the method comprising the step of including SAE-CD in the formulation in an amount sufficient to increase the amount of dissolved corticosteroid in the formulation to form an altered formulation, whereby the output rate of corticosteroid for the altered formulation is greater than the output rate of corticosteroid for the suspension formulation.

Two pulsating membrane nebulizers (AERONEB GO and the nebulizer of U.S. Patent No. 6,962,151) were evaluated according to Examples 21 and 22, respectively, to determine the performance of the devices with a solution of the invention and to demonstrate the utility of using concentrated solutions of corticosteroids in an efficient electronic nebulizer. Several *in vitro* parameters, important for the clinical use of corticosteroids, were determined and compared to these parameters of the solution formulation described in Example 6 and the 2mL commercial reference suspension both in an air jet nebulizer. The total delivery/nebulization time (time to sputter where production of aerosol is no longer visible), MMAD droplet size and fine particle fraction parameters were obtained for each device. A cascade impactor was engaged with the output of each device to determine performance during nebulization and characterize the *in vitro* aerosol drug output.

The solution used in the AERONEB GO device was compared side-by-side with a RAINDROP air jet nebulizer employing a solution (0.5 ml) comprising budesonide (500 µg/0.5 ml), CAPTISOL (10% w/v), water, and Tween 80 (optional), wherein the solution was made by adding CAPTISOL to a suspension of budesonide particles (RESPULES) as detailed below. In each device, the control suspension-based sample was the RESPULES suspension (2 ml containing 500 µg of budesonide). The RAINDROP nebulizer was equipped with a PARI PRONEB ULTRA compressor. The AERONEB GO micropump nebulizer was equipped with the OnQ Aerosol Generator. The total output of budesonide at cessation of aerolization (at sputter) was quantified. The emitted dose and fine particle dose were measured with the cascade impactor. The solution in the nebulizer of the '151 Patent comprised budesonide, CAPTISOL, water, and technetium-99 radiolabel in the form of diethylenetriaminepenta-acetic acid as a surrogate marker for budesonide. The data from the two studies is summarized in the table below.

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Aerosol Performance Using NGI Cascade Impactor				
Nebulizer device	AeroNeb Go		Raindrop/ Pari Proneb Ultra	
Formulation	CEB #1	Pulmicort	CEB #2	Pulmicort
Amount of drug (μg)*	500.00	500.00	500.00	500.00
Nebulization Time (min)+	1.0	4.0	5.0	5.5
Amt of drug exiting nebulizer (μg) *	405.93	266.06	150.27	92.42
Percent of drug exiting nebulizer (%)	81.26	53.24	30.03	18.49
Amt of drug exiting nebulizer in fine particle fraction (% < 5 μm) (μg) *	262.17	131.73	131.44	76.27
Percent of drug exiting nebulizer in fine particle fraction (< 5 μm,)	66.33	49.54	87.43	82.55
Percent of total drug in fine particle fraction (< 5 μm, %)	53.91	26.38	26.27	15.26
MMAD (μm, estimated by linear regression)	4.10	5.54	2.22	2.86
GSD (sigma g, estimated by linear regression)	2.02	1.55	2.37	2.21

* Data normalized to 500 μg budesonide in each nebulizer using total of recovered drug.

+Note: Nebulizers were run 30 seconds past end of nebulization time to insure complete emptying into the NGI impactor.

- 5 Using the electronic pulsating membrane nebulizers (AERONEB GO nebulizer and the nebulizer of the '151 Patent), delivery of a unit dose of corticosteroid was completed within less than one minute. The RAINDROP nebulizer completed delivery of a unit dose in just over 5.5 min with the RESPULES and just over 5 min with a solution of the invention. In addition, the pulsating membrane nebulizers delivered a substantially
- 10 greater percentage of corticosteroid in the FPF, which is generally defined as the fraction of particles less than 5 μ or the fraction of particles on cascade impactor stages with a cut-off of less than 6 μ . Accordingly, the total nebulization time of the AERONEB GO is one fourth the time to sputter for the Pari LC+ air jet nebulizer. As a result, treatment time would be reduced with the pulsating membrane nebulizer as compared to the air jet
- 15 nebulizer, and the amount of budesonide emitted from the pulsating membrane nebulizer is 2 to 3 times more than from the air jet nebulizer. It was also determined that the percent of drug exiting the nebulizer (the emitted dose) was 81% of the amount initially loaded

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into the reservoir (the nominal dose). Hence, less drug would need to be loaded into the pulsating membrane nebulizer to treat the patient in need thereof to provide the same “dose to subject” as provided by an air jet nebulizer.

Aerosol droplet size was determined using a Malvern Spraytec instrument. The Dv10, Dv50, and Dv90 from the pulsating membrane nebulizer are very similar to those for the reference product (PULMICORT RESPULES) and a CAPTISOL solution of the same concentration in an air jet nebulizer. This suggests that the formulation would be similarly distributed within the patient after inhalation.

A clinical study according to Example 17 was conducted to compare the pulmonary disposition of budesonide from a radiolabeled liquid formulation and the clearance of budesonide from the lung as determined by appearance of budesonide in the plasma. A solution formulation of the invention was compared to a suspension-based formulation of budesonide. At various times up to 24 hours, plasma samples were collected and assayed for budesonide and various pharmacokinetic parameters determined. The area under the plasma concentration - time curve (AUC) is a measure of the delivery of budesonide to the lung, since oral absorption of the corticosteroid was blocked by the administration of charcoal. A comparison of the AUC data was made by consideration of the dose delivered to each subject (“dose to subject”) or dose delivered to the lungs of each subject (“dose to lung”) or dose emitted by the nebulizer or device (“emitted dose”) or dose available for nebulization or delivery (“nominal dose” or “nominal available dose” or “loaded dose”). The AUC data ($_{0-t}$, and $_{0-\infty}$) was normalized in terms of the budesonide (μg) delivered to each subject by dividing the AUC data by the corresponding dose to subject. As used herein the term “dose to subject” is taken to mean the amount of corticosteroid delivered to a subject following completion of a dosing cycle with a nebulizer and is calculated by subtracting the sum of drug remaining in the nebulizer, drug removed from the mouth of a subject, and the amount collected from the exhalation filter from the amount of drug initially present in the reservoir of the nebulizer. The following expression can be used to calculate the dose to subject (D_s): $D_s = D_{r_{\text{init}}} - (D_{\text{mw}} + D_{\text{dev}})$, wherein $D_{r_{\text{init}}}$ denotes the amount of drug initially present in the reservoir of the nebulizer, D_{mw} denotes the amount of drug removed from the mouth of a subject by using a mouthwash, D_{dev} denotes the amount of drug remaining in the device following completion of an administration dosing cycle. The term D_{dev} includes drug remaining in the reservoir after completion of dosing, and drug remaining in the remainder of the device

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after completion of dosing. As used herein, the term “dose to lung” is taken to mean the amount of drug delivered to the lungs of a subject, which amount is a subset of “dose to subject”. The dosing parameters used in this example are detailed below and are provided on the basis of arithmetic means.

<i>Normalization</i>	<i>Parameter or Ratio</i>	Arithmetic Means			
		Captisol Enabled Budesonide			Pulmicort Respules
		<i>25% TTS</i>	<i>50% TTS</i>	<i>75% TTS</i>	<i>100% TTS</i>
Intended Nominal Loaded Dose	μg	1000	1000	1000	1000
	Ratio CEB/PR	1	1	1	1
Nominal Available Dose	μg	250	500	750	1000
	Ratio CEB/PR	0.25	0.50	0.75	1
Dose to Lung	μg	39.56 Range= 24.61→65.65	63.85 Range= 48.27→80.70	94.38 Range= 72.81→123.79	90.17 Range= 76.21→100.75
	Ratio CEB/PR	0.44 Range= 0.27→0.78	0.72 Range= 0.57→0.95	1.05 Range= 0.87→1.46	1
Dose to Subject	μg	64.1 Range= 47.57→81.75	109.1 Range= 85.43→156.67	169.8 Range= 142.48→219.03	199.9 Range= 159.58→249.88
	Ratio CEB/PR	0.33 Range= 0.23→0.48	0.55 Range= 0.41→0.64	0.86 Range= 0.74→1.04	1
Emitted Dose	μg	119.5 Range= 93.40→166.2	205.9 Range= 164.0→272.9	321.8 Range= 277.9→391.0	440.2 Range= 396.7→481.10
	Ratio CEB/PR	0.27 Range= 0.20→0.37	0.47 Range= 0.41→0.57	0.73 Range= 0.66→0.87	1

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The pharmacokinetic data obtained from the study of Example 17 is summarized below. The data is presented on a geometric mean or individual subject basis. The data in the table below is based upon the “dose to subject”.

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		Captisol Enabled Budesonide			Pulmicort Respules
	Parameter	Geometric Means			
		25% TTS ¹ Dose to Subject = 62.7 µg Range ³ = 47.6 → 81.8	50% TTS ¹ Dose to Subject = 107.1 µg Range ³ = 85.4 → 156.7	75% TTS ¹ Dose to Subject = 168.2 µg Range ³ = 142.5 → 219.0	100% TTS Dose to Subject = 197.7 µg Range ³ = 159.6 → 249.9
Non- normalized	AUC _{0-t} (pg.h/ml)	370 Range= 162 → 1209	875 Range= 647 → 1122	1130 Range= 865 → 1588	516 Range= 378 → 631
	AUC _{0-∞} (pg.h/ml)	533 Range= 279 → 1309	1022 Range= 778 → 1285	1617 Range= 978 → 2351	644 Range= 421 → 864
Normalized for dose to subject	AUC _{0-t} (pg.h/ml)/ µg	6 Range= 3 → 15	8 Range= 7 → 11	7 Range= 4 → 11	3 Range= 2 → 5
	AUC _{0-∞} (pg.h/ml)/ µg	8 Range= 4 → 16	10 Range= 8 → 12	10 Range= 5 → 16	3 Range= 2 → 5

¹ TTS = Time to Sputter. The percentage value can be used to calculate a nominal available dose for the corticosteroid in the study conducted according to Example 17.

² Using the added radioactive tracer, the dose of budesonide delivered may be calculated by subtracting the amount of budesonide remaining in the nebulizer post-dose, on the exhalation filter, and in the mouthwash from the amount initially added to the nebulizer.

³ The ranges in the table above are based upon the geometric means for a respective value as determined with each individual.

Based upon the above data, the formulation of the invention provides a normalized AUC_t of 3-15 (or about at least 6) (pg*h/ml)/µg of budesonide when about 60-65 µg of budesonide were delivered, a normalized AUC_t of 7-11 (or about at least 8) (pg*h/ml)/µg of budesonide when about 105-110 µg of budesonide were delivered, and a normalized AUC_t of 4-11 (or about at least 7) (pg*h/ml)/µg of budesonide when 165-170 µg of budesonide were delivered, based upon the “dose to subject”. In addition, the formulation of the invention provided a normalized AUC_i of 4-16 (or about at least 8) (pg*h/ml)/µg of budesonide when about 60-65 µg of budesonide were delivered, a normalized AUC_i of 8-12 (or about at least 10) (pg*h/ml)/µg of budesonide when about 105-110 µg of

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budesonide were delivered, and a normalized AUC_i of 5-16 (or about at least 10) (pg*h/ml)/μg of budesonide when 165-170 μg of budesonide were delivered, based upon the “dose to subject”. The data is also summarized in FIG. 17, which is a plot of the geometric mean of “dose to subject” (μg budesonide) versus the geometric mean of AUC (pg*h/ml), and FIG. 18, which is a plot of the geometric mean of “dose to lung” (μg budesonide) versus the geometric mean of AUC (pg*h/ml).

In some embodiments, the invention includes a method of providing in a subject a mean plasma AUC_t, normalized for dose of corticosteroid to subject, of at least 6 (pg*h/ml)/μg of corticosteroid delivered, as dose to subject, comprising: administering to the subject via nebulization a unit dose comprising at least 45 μg, at least 48 μg, or 45 μg to 1000 μg, about 1 μg to 20 mg, about 1 μg to 10 mg, 0.01 mg to 10 mg, 0.025 mg to 10 mg, 0.05 mg to 5 mg, 0.1 mg to 5 mg, 0.125 mg to 5 mg, 0.25 mg to 5 mg, 0.5 mg to 5 mg, 0.05 mg to 2 mg, 0.1 mg to 2 mg, 0.125 mg to 2 mg, 0.25 mg to 2 mg, or 0.5 mg to 2 mg of corticosteroid dissolved in an aqueous liquid carrier comprising sulfoalkyl ether cyclodextrin.

FIG. 19 is a plot of the “dose to subject” (μg budesonide) versus the corresponding AUC (pg*h/ml) for each individual subject of the study. The slope of the substantially linear solid line, taken from data across three different doses delivered, defines a dose response curve for a patient receiving the corticosteroid. As a result, the slope can be used to predict the dose a patient would need to provide a target plasma level at a second dose if the patient has received a first dose and the patient’s AUC per μg of corticosteroid has been determined. The slope ranges from 5.7 to 16, or more specifically from 9 to 10, when the data is viewed on an individual subject basis

The plasma concentration profile for budesonide for the subjects of the clinical study is depicted in FIG. 20.

This normalized AUC data and associated radiolabel distribution data show that more of the dose delivered to the subject made it into the lung and from there into systemic circulation when budesonide was administered as a solution than when the budesonide was administered as a suspension. Assuming that an equivalent dose deposited in the lung results in a similar efficacy and systemic absorption, these results suggest that 1.6 to 5 times or 2 to 4 times less dose to subject is required when administered in solution to be as effective as the reference suspension product. Depending

upon whether it is determined on an individual basis or a geometric mean basis, administration of a solution of the invention provides a 1.6-fold increase, 2.2-fold increase, a 2.5-fold increase, a five-fold increase, a 1.6 to five fold increase, a two to four-fold increase, a two to 3.5-fold increase, a two to 3.3-fold increase or at least a two-fold increase in the AUC_t or AUC_i per µg of budesonide delivered as compared to administration of the PULMICORT RESPULES suspension-based aqueous formulation. The AUC_t or AUC_i per µg of budesonide delivered was observed as varying per individual, with the value being higher than the above-noted values for some individuals and lower than the above-noted values for other individuals.

The data in the table below is based upon the various different doses measured or determined during the study and summarizes the level (factor) of enhancement in the pharmacokinetic profile parameter C_{max} non-normalized or normalized on the basis of nominal available dose, dose to lung, dose to subject, or emitted dose.

<i>Normalization</i>	<i>Parameter or Ratio</i>	Geometric Means			
		Captisol Enabled Budesonide			Pulmicort Respules
		<i>25% TTS</i>	<i>50% TTS</i>	<i>75% TTS</i>	<i>100% TTS</i>
Non-normalized	C_{max} (pg/mL)	225.8 Range= 91.6→483.3	437.2 Range= 297.1→739.1	545.5 Range= 376.6→891.6	266.3 Range= 155.8→331.5
	Ratio CEB/PR	0.85 Range= 0.55→1.55	1.64 Range= 0.90→2.60	2.05 Range= 1.50→2.86	1
Normalized for nominal available dose¹	(pg/mL) µg	0.90 Range= 0.37→1.93	0.87 Range= 0.59→1.48	0.73 Range= 0.50→1.19	0.27 Range= 0.16→0.33
	Ratio CEB/PR	3.39 Range= 2.20→6.21	3.28 Range= 1.79→5.20	2.73 Range= 2.01→3.82	1
Normalized for dose to lung²	(pg/mL) µg	6.03 Range= 3.49→8.51	6.93 Range= 5.49→9.16	5.86 Range= 4.52→7.45	2.97 Range= 1.59→3.95
	Ratio CEB/PR	2.03 Range= 1.48→2.59	2.33 Range= 1.56→4.30	1.97 Range= 1.38→3.17	1
Normalized for dose to subject³	(pg/mL) µg	3.60 Range= 1.93→5.94	4.08 Range= 3.22→7.44	3.24 Range= 1.72→5.23	1.35 Range= 0.75→1.85

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	Ratio CEB/PR	2.67 Range= 1.99→3.21	3.03 Range= 2.17→5.01	2.41 Range= 1.72→3.76	1
Normalized for emitted dose⁴	(pg/mL) μg	1.93 Range= 0.98→2.91	2.15 Range= 1.71→3.25	1.71 Range= 1.00→2.68	0.61 Range= 0.34→0.84
	Ratio CEB/PR	3.18 Range= 2.19→4.20	3.55 Range= 2.17→6.26	2.82 Range= 1.93→4.23	1

According to the table above, the formulation and system of the invention can provide a $C_{\max}$ (pg/ml) of 90 to 900, 200 to 600, 200 to 550, 200 to 250, 400 to 450, 500 to 600, 225, 437, or 545 on a dose non-normalized basis. The formulation and system of the invention can provide a dose normalized $C_{\max}$ (pg/ml/μg) of: 1) 0.3 to 2, 0.35 to 2, 0.6 to 1.5, 0.5 to 1.2, 0.8 to 1, 0.8 to 0.9, 0.7 to 0.8, 0.4, 1.9, 0.6, 1.5, 0.5, 1.2, 0.35, 2, 0.7, 0.8, or 0.9 on a nominal available dose normalized basis; 2) 3.4 to 9.2, 3.5 to 8.5, 5.5 to 9.2, 4.5 to 7.5, 5.8 to 7, 3.4, 3.5, 4.5, 5.5, 9.2, 8.5, 7.5, 5.8, 5.9, 6, or 7 on a dose to lung normalized basis; 3) 1.7 to 7.5, 3.2 to 4.1, 1.9 to 6, 3.2 to 7.5, 1.7 to 5.2, 3.6, 4.1, 3.2, 1.9, 6, 3.2, 7.4, 7.5, 1.7, 5.2 or 5.3 on a dose to subject normalized basis; 4) 0.9 to 3.3, 1.7 to 2.2, 0.9 to 3, 1 to 3, 1.7 to 3.3, 1 to 2.7, 1.9, 2.1, 2.2, 1.7, 0.9, 1, 2, 3, 2.9, 3.2, 3.3, or 2.7 on an emitted dose normalized basis.

The solution of the invention provides an enhanced pharmacokinetic profile over the suspension-based formulation. On the basis of the non-normalized dose of corticosteroid, the $C_{\max}$ provided by the corticosteroid solution is 1.6 to 2, 1.5 to 3, 1.5 to 2.5, 1.5 to 2, 1.5, 1.6, 2, 2.5, or 3 fold higher than the $C_{\max}$ provided by the suspension-based formulation when the dose of corticosteroid in the solution and the suspension is approximately the same amount loaded. On the basis of normalization to the nominal available dose of corticosteroid, the $C_{\max}$ provided by the corticosteroid solution is 1.8 to 6.2, 1.5 to 6.5, 2 to 6.5, 1.5 to 5.5, 2 to 4, 1.5 to 4, 1.5 to 3, 2.7, 3.3, 3.4, 1.5, 6.5, 2, 5.5, 4, or 3 fold higher than the $C_{\max}$ provided by the suspension-based formulation. On the basis of normalization to the dose of corticosteroid to lung, the $C_{\max}$ provided by the corticosteroid solution is 1.4 to 4.3, 1.4 to 4.5, 1.5 to 4.5, 1.5 to 3.5, 1.5 to 3, 1.4 to 3, 1.5 to 2.5, 1.5 to 2, 2, 2.3, 1.4, 4.5, 3.5, 3, 1.5 or 2.5 fold higher than the $C_{\max}$ provided by the suspension-based formulation. On the basis of normalization to the dose of corticosteroid to subject, the $C_{\max}$ provided by the corticosteroid solution is 2 to 3.5, 2 to 5, 1.7 to 3.8, 1.7 to 5, 2.7, 3, 2.4, 2, 3.5, 5, 1.7, or 3.8 fold higher than the $C_{\max}$ provided by the

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suspension-based formulation. On the basis of normalization to the emitted dose of corticosteroid, the C_{max} provided by the corticosteroid solution is 1.9 to 6.3, 1.75 to 6.5, 2.2 to 4.2, 2.2 to 6.3, 1.9 to 4.2, 3.2, 3.5, 3.6, 2.8, 1.75, 6.5, 2.2, 4.2, or 6.3 fold higher than the C_{max} provided by the suspension-based formulation.

5 Alternatively, the C_{max} provided by the corticosteroid solution is at least 1.5, 1.6, 2, 2.6, and 3 fold higher than the C_{max} provided by the suspension-based formulation when the dose of corticosteroid in the solution is about 2 fold lower than the dose in the suspension.

10 The data in the table below is based upon the various different doses measured or determined during the study and summarize the level (factor) of enhancement in the pharmacokinetic profile parameter AUC_{inf} non-normalized or normalized on the basis of nominal available dose, dose to lung, dose to subject, or emitted dose.

<i>Normalization</i>	<i>Parameter or Ratio</i>	Geometric Means			
		Captisol Enabled Budesonide			Pulmicort Respules
		<i>25% TTS</i>	<i>50% TTS</i>	<i>75% TTS</i>	<i>100% TTS</i>
Non-normalized	AUC _{inf} (pg*h/mL)	533.0 Range= 278.6→1309	1022.2 Range= 777.5→1285	1616.8 Range= 977.5→2438.2	643.6 Range= 420.6→864.1
	Ratio CEB/PR	0.83 Range= 0.44→1.51	1.59 Range= 1.00→3.06	2.51 Range= 1.53→3.28	1
Normalized for nominal available dose	<u>(pg*h/mL)</u> μg	2.13 Range= 1.11→5.23	2.04 Range= 1.55→2.57	2.16 Range= 1.30→3.25	0.64 Range= 0.42→0.86
	Ratio CEB/PR	3.31 Range= 1.77→6.06	3.18 Range= 2.00→6.11	3.35 Range= 2.04→4.37	1
Normalized for dose to lung	<u>(pg*h/mL)</u> μg	14.24 Range= 10.17→19.94	16.20 Range= 13.59→18.77	17.37 Range= 11.16→24.69	7.17 Range= 5.52→10.22
	Ratio CEB/PR	1.98 Range= 1.19→2.85	2.26 Range= 1.47→3.40	2.42 Range= 1.37→3.52	1
Normalized for dose to subject	<u>(pg*h/mL)</u> μg	8.50 Range= 4.23→16.08	9.54 Range= 8.01→12.23	9.61 Range= 5.41→16.5	3.26 Range= 1.68→5.14
	Ratio CEB/PR	2.61 Range= 1.60→3.64	2.93 Range= 2.00→4.87	2.95 Range= 1.93→3.93	1

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Normalized for emitted dose	(pg*h/mL)	4.55 Range= 2.62→7.87	5.04 Range= 4.23→6.66	5.07 Range= 3.16→8.48	1.43 Range= 0.81→2.31
	Ratio CEB/PR	3.18 Range= 1.92→5.37	3.52 Range= 2.31→5.82	3.55 Range= 1.91→5.47	1

According to the table above, the formulation and system of the invention can provide an AUC_{inf} (pg*h/ml) of 500 to 1700, 530 to 1650, 250 to 2500, 280 to 1300, 780 to 1300, 980 to 2450, 275, 775, 980, 2400, 2500, 1300, 1290, 530, 1650, 250, 280 or 780 on a dose non-normalized basis. The formulation and system of the invention can provide a dose normalized AUC_{inf} (pg/ml/ μ g) of: 1) 1 to 5.5, 2 to 2.2, 1 to 5.3, 1.1 to 5.2, 1.5 to 2.6, 1.3 to 3.3, 2, 2.1, 2.2, 1, 5.5, 5.3, 5.2, 1.5, 2.6, 1.3 or 3.3 on a nominal available dose normalized basis; 2) 10 to 25, 14 to 18, 10.2 to 20, 13.6 to 18.8, 11.2 to 24.7, 10.2, 20, 13.6, 14, 19, 18.8, 11, 11.2, 25, 24.7, 14.2, 16.2, 17.3 on a dose to lung normalized basis; 3) 4 to 16, 4.2 to 16.1, 8 to 12.2, 5.4 to 16, 5.4 to 17, 8.5 to 9.6, 8.5, 9.5, 9.6, 4.2, 16.1, 8, 12.2, 12, 5.4, 16, 17, or 16.5 on a dose to subject normalized basis; 4) 2.5 to 9, 2.6 to 8.5, 4.5 to 5.1, 2.5 to 8, 2.6 to 7.9, 4.2 to 6.7, 3.1 to 8.5, 3.2 to 8.5, 4.5, 4.6, 5, 5.1, 2.5, 2.6, 4.2, 3.1, 9, 8.5, 5.1, 8, 7.9, or 6.7 to on an emitted dose normalized basis.

Accordingly, the solution of the invention provides an enhanced pharmacokinetic profile over the suspension-based formulation. On the basis of the non-normalized dose of corticosteroid, the AUC_{inf} provided by the corticosteroid solution is 1.6 to 2.5, 1.6 to 3.1, 1.5 to 3.5, 1.5 to 3.3, 2.5 to 3.3, 3.1, 1.5, 3.3, 1.6, or 2.5 fold higher than the AUC_{inf} provided by the suspension-based formulation when the dose of corticosteroid in the solution and the suspension is approximately the same. On the basis of normalization to the nominal available dose of corticosteroid, the AUC_{inf} provided by the corticosteroid solution is 1.75 to 6.5, 1.75 to 6.1, 2 to 6.5, 2 to 6.1, 2 to 4.5, 2 to 4.4, 3.3, 3.2, 3.5, 3.4, 1.75, 6.5, 6.1, 2, 4.5, or 4.4 fold higher than the AUC_{inf} provided by the suspension-based formulation. On the basis of normalization to the dose of corticosteroid to lung, the AUC_{inf} provided by the corticosteroid solution is 1.2 to 3.5, 1.2 to 4, 1.2 to 3, 1.2 to 2.85, 1.5 to 3.5, 1.4 to 3.5, 2, 2.2, 2.3, 2.4, 1.2, 3, 4, 2.85, 1.5, 3.5, or 1.4 fold higher than the AUC_{inf} provided by the suspension-based formulation. On the basis of normalization to the dose of corticosteroid to subject, the AUC_{inf} provided by the corticosteroid solution is 1.6 to 4.9, 1.5 to 5, 1.6 to 5, 1.6 to 3.7, 1.6 to 3.6, 2 to 4.9, 1.9 to 4, 2.6, 1.5, 5, 1.6, 3.7, 3.6, 2, 4.9, 1.9, or 4 fold higher than the AUC_{inf} provided by the suspension-based

formulation. On the basis of normalization to the emitted dose of corticosteroid, the AUC_{inf} provided by the corticosteroid solution is 1.5 to 6, 1.7 to 6, 1.9 to 6, 1.9 to 5.4, 2.3 to 5.8, 1.9 to 5.5, 1.9 to 5.8, 1.5, 6, 1.7, 1.9, 5.4, 2.3, 5.8, 5.8, 3.2, 3.5, or 3.6 fold higher than the AUC_{inf} provided by the suspension-based formulation.

- 5 Alternatively, the AUC_{inf} provided by the corticosteroid solution is at least 1.5, 1.6, 2, 2.5, 3 and 3.1 fold higher than the AUC_{inf} provided by the suspension-based formulation when the dose of corticosteroid in the solution is about 2 fold lower than the dose in the suspension.

- 10 The data in the table below is based upon the various different doses measured or determined during the study and summarize the level (factor) of enhancement in the pharmacokinetic profile parameter AUC_{last} non-normalized or normalized on the basis of nominal available dose, dose to lung, dose to subject, or emitted dose.

<i>Normalization</i>	<i>Parameter or Ratio</i>	Geometric Means			
		Captisol Enabled Budesonide			Pulmicort Respules
		<i>25% TTS</i>	<i>50% TTS</i>	<i>75% TTS</i>	<i>100% TTS</i>
Non-normalized	AUC_{last}	396.7	874.5	1130	516.2
	(pg*h/mL)	Range = 162.3→1209	Range = 646.7→1122	Range = 864.6→1588	Range = 377.6→630.9
	Ratio CEB/PR	0.77 0.32→1.92	1.69 1.14→2.97	2.19 1.55→2.93	1
Normalized for nominal available dose¹	(pg*h/mL)	1.59	1.75	1.51	0.52
	μg	0.65→4.84	1.29→2.24	1.15→2.12	0.38→0.63
	Ratio CEB/PR	3.07 1.29→7.66	3.39 2.28→5.94	2.92 2.07→3.90	1
Normalized for dose to lung²	(pg*h/mL)	10.60	13.86	12.14	5.75
	μg	6.18→18.41	11.12→16.82	9.44→17.96	4.96→7.47
	Ratio CEB/PR	1.84 1.21→2.49	2.41 1.68→3.31	2.11 1.53→3.38	1
Normalized for dose to subject³	(pg*h/mL)	6.33	8.16	6.72	2.61
	μg	3.34→14.85	6.56→10.74	3.95→11.15	1.51→3.75
	Ratio CEB/PR	2.42 1.40→3.96	3.13 2.27→4.74	2.57 2.09→3.50	1
Normalized for emitted dose⁴	(pg*h/mL)	3.39	4.31	3.54	1.18
	μg	1.74→7.27	3.46→6.01	2.30→5.72	0.78→1.42
	Ratio CEB/PR	2.88 1.59→5.19	3.67 2.52→5.24	3.02 2.36→4.03	1

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A determination of dose distribution in subjects (Example 17) was made to determine the efficiency of dose deposition to the lungs. The results are detailed in the table below and in FIG. 22.

	1(A)	2(B)	3(C)	4(D)
	Lung Scintigraphy (mean values)			
Dose in lungs (µg)	90.17	39.56	63.85	94.39
% wt. in lungs	20.61	32.61	31.30	29.61
% wt. in oropharyngeal/stomach region	24.79	21.13	21.68	23.39
% wt. in exhalation filter/mouthpiece	54.61	46.26	47.02	47.00
Mass Balance	100.47	100.97	103.21	103.49

1(A) Budesonide Suspension nebulized to sputter- 100% TTS (time to sputter)

5 2(B) Captisol-Enabled® Budesonide Formulation - 25% TTS

3(C) Captisol-Enabled® Budesonide Formulation - 50% TTS

4(D) Captisol-Enabled® Budesonide Formulation - 75% TTS

The data demonstrate that administration of the solution of the invention resulted in a lower oropharyngeal deposition of the corticosteroid as compared to administration of the control suspension formulation. Moreover, the formulation provided at least a 20% to 85%, or 30% to 80%, or at least 30%, at least 40%, at least 56%, at least 59%, at least 62% wt. pulmonary deposition of corticosteroid based upon the emitted dose. The percentage of pulmonary deposition can be further increased by employing a nebulizer capable of providing a higher respirable fraction and lower MMAD for the nebulized mist. Suitable nebulizers are ultrasonic, vibrating mesh, vibrating cone, vibrating plate nebulizers or those that extrude a liquid formulation through a self-contained nozzle array. For example, the Aradigm AERx pulmonary delivery systems, the AERx Essence and AERx Ultra, is particularly suitable for use according to the invention, as it is recognized in the art as providing controlled dose expression, control of generated aerosol particle size, control of inhaled aerosol particle size, and management of the inhalation and delivery process (Farr et al., Drug Delivery Technology May 2002 Vol. 2, No. 3, 42-44). For

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example, the PARI eFlow vibrating plate nebulizer is particularly suitable for use according to the invention, as it is recognized in the art as providing the above-mentioned desired performance parameters (Keller et al. (ATS 99th International Conference, Seattle, May 16th-21st, 2003; poster 2727).

5 By virtue of the improved systemic bioavailability of a corticosteroid delivered to the lungs by pulmonary administration according to the invention, the composition or formulation of the invention will provide an improved therapeutic benefit or improved clinical benefit over an equivalent dose of corticosteroid administered as an aqueous suspension.

10 Example 30 details a study conducted on dogs wherein a budesonide solution of the invention and a reference formulation (PULMICORT RESPULESsuspension) was administered via nebulization with a Pari LC air-jet nebulizer. The results depicted in FIG. 21 demonstrate an average plasma level of budesonide as a function of time. The $AUC_{0 \rightarrow 8hr}$ of the solution formulation was 1.9 times greater than the reference suspension
15 formulation. The solution formulation also emitted 1.23 times as much budesonide as the reference formulation in the same length of time. Detailed results are also provided in the table below.

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Dog PK Study			
Parameter	Captisol Enabled Budesonide	Pulmicort Respules	Ratio CEB/PR
Loaded Dose (nominal dose)	1000 μg	1000 μg	1
Emitted Dose	769 μg	626 μg	1.23
Average AUC_{0→8hr}	2599 $\text{pg}\times\text{hr}/\text{mL}$	1355 $\text{pg}\times\text{hr}/\text{mL}$	1.91
Average C_{max}	1685 pg/mL	901 pg/mL	1.87
Loaded Dose - normalized AUC_{0→8hr}	2.60 ($\text{pg}\times\text{hr}/\text{mL}$)/ μg	1.36 ($\text{pg}\times\text{hr}/\text{mL}$)/ μg	1.91
Emitted Dose - normalized AUC_{0→8hr}	3.38 ($\text{pg}\times\text{hr}/\text{mL}$)/ μg	2.16 ($\text{pg}\times\text{hr}/\text{mL}$)/ μg	1.56
Loaded Dose - normalized C_{max}	1.69 (pg/mL)/ μg	0.90 (pg/mL)/ μg	1.88
Emitted Dose - normalized C_{max}	2.19 (pg/mL)/ μg	1.44 (pg/mL)/ μg	1.52

According to the table above, the formulation and system of the invention can provide an AUC_{0-8hr} ($\text{pg}\times\text{h}/\text{ml}$) of 2000 to 3000, 2500 to 2700, 2000, 3000, or 2600 on a dose non-normalized basis. The formulation and system of the invention can provide a dose normalized AUC_{inf} ($\text{pg}/\text{ml}/\mu\text{g}$) of: 1) 2 to 3, 2.5 to 2.7, or 2.6 on a loaded dose nominal normalized basis; 2) 3 to 4, 3.4 to 3.5, or 3.4 on an emitted dose normalized basis.

The formulation, system and method of the invention provided a higher AUC_{0-8hr} than did the suspension-based PULMICORT RESPULES formulation. On the basis of the normalized nominal (loaded) dose of corticosteroid, the AUC_{0-8hr} provided by the corticosteroid solution is at least 1.7, 1.8, 1.9, or 2 fold higher than the AUC_{0-8hr} provided by the suspension-based formulation when the dose of corticosteroid in the solution and the suspension is approximately the same. On the basis of normalization to the emitted dose of corticosteroid, the AUC_{0-8hr} provided by the corticosteroid solution is at least 1.5, 1.6, to 2 fold higher than the AUC_{0-8hr} provided by the suspension-based formulation.

According to the table above, the formulation and system of the invention can provide an C_{max} (pg/ml) of 1600 to 1800, 1650 to 1750, or 1700 on a dose non-normalized basis. The formulation and system of the invention can provide a dose normalized C_{max}

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(pg/ml/ μ g) of: 1) 1 to 2, 1.6 to 1.8, or 1.7 on a loaded dose nominal normalized basis; 2) 2 to 2.5, or 2.2 on an emitted dose normalized basis.

The formulation, system and method of the invention provided a higher $C_{\max}$ than did the suspension-based PULMICORT RESPULES formulation. On the basis of the normalized nominal (loaded) dose of corticosteroid, the $C_{\max}$ provided by the corticosteroid solution is at least 1.7, 1.8, 1.9, or 2 fold higher than the $C_{\max}$ provided by the suspension-based formulation when the dose of corticosteroid in the solution and the suspension is approximately the same. On the basis of normalization to the emitted dose of corticosteroid, the $C_{\max}$ provided by the corticosteroid solution is at least 1.5, 1.6, to 2 fold higher than the $C_{\max}$ provided by the suspension-based formulation.

In some aspects, the method and dosage form of the invention thus provides an improved method of administering a corticosteroid suspension-based unit dose, the method comprising the step of adding a sufficient amount of SAE-CD to convert the suspension to a clear solution and then administering the clear solution to a subject. As a result, the method of the invention provides increased total delivery of the corticosteroid as well as increased rate of administration as compared to the initial unit dose suspension formulation.

The data herein can also be used to estimate the percentage of the dose of corticosteroid emitted or delivered that is absorbed into the bloodstream of a patient. In some embodiments, greater than 20%, greater than 25%, greater than 29%, greater than 35% of the emitted dose may be absorbed into the bloodstream of the patient. In some embodiments, greater than 40%, greater than 50%, greater than 55% of the dose to subject may be absorbed into the bloodstream of the patient. In some embodiments, greater than 10%, greater than 12%, greater than 15% of the nominal available dose may be absorbed into the bloodstream of the patient.

The corticosteroids that are useful in the present invention generally include any steroid produced by the adrenocortex, including glucocorticoids and mineralocorticoids, and synthetic analogs and derivatives of naturally occurring corticosteroids having anti-inflammatory activity. Suitable synthetic analogs include prodrugs, ester derivatives. Examples of corticosteroids that can be used in the compositions of the invention include aldosterone, beclomethasone, betamethasone, budesonide, ciclesonide (Altana Pharma AG), cloprednol, cortisone, cortivazol, deoxycortone, desonide, desoximetasone, dexamethasone, difluorocortolone, fluclorolone, flumethasone, flunisolide, fluocinolone,

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fluocinonide, fluocortin butyl, fluorocortisone, fluorocortolone, fluorometholone, flurandrenolone, fluticasone, halcinonide, hydrocortisone, icomethasone, meprednisone, methylprednisolone, mometasone, paramethasone, prednisolone, prednisone, rofleponide, RPR 106541, tixocortol, triamcinolone, and their respective pharmaceutically acceptable
5 derivatives, such as beclomethasone dipropionate (anhydrous or monohydrate), beclomethasone monopropionate, dexamethasone 21-isonicotinate, fluticasone propionate, icomethasone enbutate, tixocortol 21-pivalate, and triamcinolone acetone. Particularly preferred are compounds such as beclomethasone dipropionate, budesonide, flunisolide, fluticasone propionate, mometasone furoate, and triamcinolone acetone. Other
10 corticosteroids not yet commercialized, but that are commercialized subsequent to the filing of this application, are considered useful in the present invention unless it is otherwise established experimentally that they are not suitable.

Corticosteroids can be grouped according to their relative lipophilicity as described by Barnes et al. (*Am. J. Respir. Care Med.* (1998), 157, p. S1-S53), Miller-Larsson et al. (*Am J. Respir. Crit. Care Med.* (2003), 167, A773), D.E. Mager et al. (*J. Pharm. Sci.* (Nov. 2002), 91(11), 2441-2451) or S. Edsbäcker (Uptake, retention, and biotransformation of corticosteroids in the lung and airways. In: Schleimer RP, O'Byrne PMO, Szefer SJ, Brattsand R, editor(s). *Inhaled steroids in asthma: optimizing effects in the airways.* New York: Marcel Dekker, 2002: 213-246). Generally, the less lipophilic a
20 corticosteroid is, the lower the amount of SAE-CD required to dissolve it in an aqueous medium and vice versa. Corticosteroids that are less lipophilic than flunisolide generally require a SAE-CD to corticosteroid molar ratio of less than 10:1 to dissolve the corticosteroid in an aqueous medium. Exemplary corticosteroids of this group include hydrocortisone, prednisolone, prednisone, dexamethasone, betamethasone, methylprednisolone, triamcinolone, and fluocortolone. Some embodiments of the
25 invention exclude corticosteroids that are less lipophilic than flunisolide.

Corticosteroids that are at least as lipophilic as or more lipophilic than flunisolide generally require a SAE-CD to corticosteroid molar ratio of more than 10:1 to dissolve the corticosteroid in an aqueous medium. In some embodiments, the corticosteroid used in the
30 invention is at least as lipophilic as or more lipophilic than flunisolide. Exemplary corticosteroids of this group include beclomethasone, beclomethasone dipropionate, beclomethasone monopropionate, budesonide, ciclesonide, desisobutyryl-ciclesonide,

flunisolide, fluticasone, fluticasone propionate, mometasone, mometasone furoate, triamcinolone acetonide.

The suitability of a corticosteroid for use in the inhalable liquid composition/formulaion can be determined by performing a phase solubility binding study as detailed in Example 23. Phase solubility binding data is used to determine the saturated solubility of a corticosteroid in the presence of varying amounts of SAE-CD in an aqueous liquid carrier. The phase solubility binding curve depicted in FIG. 3 demonstrates the saturated solubility of budesonide in an aqueous liquid carrier comprising γ -CD, HP- β -CD or SBE7- β -CD. A phase solubility curve in the graph defines the boundary for the saturated solubility the corticosteroid in solutions containing various different concentrations of cyclodextrin. A molar phase solubility curve can be used to determine the molar ratio of SAE-CD to corticosteroid or of corticosteroid to SAE-CD at various concentrations of corticosteroid. The area below the phase solubility curve, c.g. of FIG. 3, denotes the region where the corticosteroid is solubilized in an aqueous liquid medium to provide a substantially clear aqueous solution. In this region, the SAE-CD is present in molar excess of the corticosteroid and in an amount sufficient to solubilize the corticosteroid present in the liquid carrier. The boundary defined by the phase solubility curve will vary according to the corticosteroid and SAE-CD within a composition or formulation of the invention. The table below provides a summary of the minimum molar ratio of SAE-CD to corticosteroid required to achieve the saturated solubility of the corticosteroid in the composition or formulation of the invention under the conditions studied.

Corticosteroid	SAE-CD	Approximate Molar Ratio at Saturated Solubility of Corticosteroid* (SAE-CD : corticosteroid)
Beclomethasone dipropionate	SAE- β -CD	358
Beclomethasone dipropionate	SAE- γ -CD	62
Budesonide	SAE- β -CD	16
Budesonide	SAE- γ -CD	13 (SBE6.1), 10.8 (SBE5.2), 10.1 (SPE5.4)
Budesonide	SAE- α -CD	12
Flunisolide	SAE- β -CD	16
Flunisolide	SAE- γ -CD	9
Fluticasone	SAE- β -CD	32
Fluticasone Propionate	SAE- β -CD	797
Fluticasone Propionate	SAE- γ -CD	51

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Corticosteroid	SAE-CD	Approximate Molar Ratio at Saturated Solubility of Corticosteroid* (SAE-CD : corticosteroid)
Fluticasone Propionate	SAE- α -CD	501
Mometasone	SAE- α -CD	73
Mometasone	SAE- β -CD	33
Mometasone furoate	SAE- α -CD	141
Mometasone furoate	SAE- β -CD	274
Mometasone furoate	SAE- γ -CD	101
Triamcinolone acetonide	SAE- β -CD	9

*This value was determined in the presence of SAE-CD under the conditions detailed in Examples 18, 23 accompanying the solubility values presented in the preceding and following text.

- 5 The saturated solubility of a corticosteroid in the presence of a fixed amount of SAE-CD will vary according to the identity of the corticosteroid and the SAE-CD. The table below summarizes some solubility data for the listed corticosteroids in the absence (intrinsic solubility of corticosteroid in the aqueous test medium) and in the presence of two different SAE-CD's as determined herein.

Steroid	[Steroid] x10⁵ M		
	Intrinsic Solubility (in H₂O)	Captisol (0.04 M)	(SBE)_{6.1} γ-CD (0.04 M)
Hydrocortisone	92.4	2656.3	2369.3
Methylprednisolone	43.6	743.1	1215.3
Prednisolone	62.5	1995.3	2095.0
Prednisone	50.5	1832.7	1313.7
Triamcinolone Acetonide	3.56	457.0	1059.5
Flunisolide	11.3	261.5	455.1
Budesonide	6.6	254.8	306.6
Fluticasone Propionate	0.39	5.41	51.8
Beclomethasone Dipropionate	0.41	11.6	46.8
Mometasone Furoate	1.82	16.4	41.5

10

The above data can be used in combination with the phase solubility data to prepare formulations according to the invention having a target concentration of corticosteroid and SAE-CD. Accordingly, some embodiments of the invention comprise a corticosteroid having an intrinsic solubility in water that approximates or is less than the

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intrinsic solubility of flunisolide (less than about 11×10^{-5} M or less than about 11.3×10^{-5} M) in water as determined herein.

Even though a composition or formulation of the invention can comprise the corticosteroid present in an aqueous medium at a concentration up to its saturated solubility in the presence of a particular concentration of SAE-CD, some embodiments of the invention include those wherein the corticosteroid is present at a concentration that is less than its saturated solubility in the presence of that concentration of SAE-CD. The corticosteroid can be present at a concentration that is 95% or less, 90% or less, 85% or less, 80% or less, or 50% or less of its saturated solubility as determined in the presence of SAE-CD. It is generally easier to prepare solutions that comprise the corticosteroid at a concentration that is less than its saturated solubility in the presence of SAE-CD.

Therefore, the molar ratio of SAE-CD to corticosteroid in a formulation or composition of the invention can exceed the molar ratio obtained at the saturated solubility of the corticosteroid in the presence of SAE-CD, such as defined by the phase solubility binding curve for the corticosteroid. In such a case, the molar ratio of SAE-CD to corticosteroid in the composition or formulation will be at least 1%, at least 2%, at least 5%, at least 7.5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 50%, at least 75%, at least 100% , or at least 200% greater than the molar ratio at the saturated solubility of the corticosteroid in the presence of SAE-CD. For example, if the molar ratio at the saturated solubility is about 14:1, then the molar ratio in the composition or formulation can be at least 14.1:1 (for at least 1% higher), at least 14.3:1 (for at least 2% higher), at least 14.7:1 (for at least 5% higher), at least 15.4:1 (for at least 10% higher), at least 16.1:1 (for at least 15% higher), at least 16.8:1 (for at least 20% higher), at least 17.5:1 (for at least 25% higher), at least 21:1 (for at least 50% higher), at least 24.5:1 (for at least 75% higher), at least 28:1 (for at least 100% higher), or at least 42:1 (for at least 100% higher).

Changes in the molar ratio of SAE-CD to corticosteroid can have an impact upon the total output of a nebulizer. A study was conducted using a PARI LC PLUS air jet nebulizer and solutions containing varying amounts of SAE-CD in 2 ml of 250 μ g/ml PULMICORT RESPULES. Each preparation was nebulized for until no further sustained vapor was visibly being emitted. At the end of each run, the amount of budesonide remaining in the reservoir of the nebulizer was determined. The SAE-CD to corticosteroid molar ratios used were 10:1, 14:1, and 20:1. The data indicate that increasing the molar

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ratio resulted in an increase of the amount of budesonide delivered and a decrease in the amount of budesonide remaining in the reservoir.

Changes in the molar ratio of SAE-CD to corticosteroid can also have an impact upon the dissolution rate of corticosteroid in an aqueous medium. A study was conducted on a roller mixer having containers with solutions containing varying amounts of SAE-CD, e.g. CAPTISOL, and a fixed amount of fluticasone propionate or mometasone furoate. The samples were prepared by mixing the corticosteroid with a solution containing the SAE-CD in a vortexer for about 30 seconds and then placing the containers on the roller mixer. Aliquots were taken periodically from each container and the amount of dissolved corticosteroid was determined. The SAE-CD to corticosteroid molar ratios used were 10:1, 14:1, and 20:1. The data indicate that increasing the molar ratio resulted in an increase in the rate of dissolution of the corticosteroid.

The corticosteroid compound is present in the final, diluted corticosteroid composition designed for inhalation in an amount from about 1 µg/ml to about 10 mg/ml, about 10 µg/ml to about 1 mg/ml, or about 20 µg/ml to about 500 µg/ml. For example, the drug concentration can be between about 30 and 1000 µg/ml for triamcinolone acetonide, and between about 50 and 2000 µg/ml for budesonide, depending on the volume to be administered. By following the preferred methods of the present invention, relatively high concentrations of the corticosteroid can be achieved in an aqueous-based composition.

Similarly, the corticosteroid compound is present in the final, diluted corticosteroid composition designed for nasal administration in an amount from about 10 µg/ml to 6 mg/ml, 50 µg/ml to about 10 mg/ml, about 100 µg/ml to about 2 mg/ml, or about 300 µg/ml to about 1 mg/ml. For example, the drug concentration can be between about 250 µg/ml and 1 mg/ml or 250 µg/ml and 6 mg/ml for triamcinolone acetonide, and between about 400 µg/ml and 1.6 mg/ml or 250 µg/ml and 6 mg/ml for budesonide, depending on the volume to be administered.

For the treatment of pulmonary disorders and sinus-related disorders, the diluted corticosteroid composition is prepared as described herein. The corticosteroid for such treatment is preferably, beclomethasone dipropionate, beclomethasone monopropionate, betamethasone, budesonide, ciclesonide, desisobutyryl-ciclesonide, flunisolide, fluticasone, fluticasone propionate, fluticasone furoate, mometasone, mometasone furoate, or triamcinolone acetonide, and is formulated in the concentrations set forth herein. The

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daily dose of the corticosteroid is generally about 0.05 to 10 mg, depending on the drug and the disease, in accordance with the Physician's Desk Reference (PDR). However, in view of the improved bioavailability of a corticosteroid when administered as a solution of the invention, the dose required to achieve a desired clinical endpoint, clinical benefit or therapeutic benefit may be lower than the corresponding dose indicated in the PDR.

A unit dose of budesonide may also be administered once daily, once every two days, once every week, once every month, or even less frequently as set forth in U.S. Patents No. 6,598,603 and No. 6,899,099, wherein a dose comprises 0.05 to 2.0 mg or 0.25 to 1.0 mg of budesonide. Administration can be during the daytime and/or nighttime.

A dose of budesonide, or corticosteroid, can be administered twice, thrice or more times per day or on an as-needed basis.

In some embodiments, a dose comprises 1 µg to 20 mg, 0.01 mg to 10 mg, 0.025 mg to 10 mg, 0.05 mg to 5 mg, 0.1 mg to 5 mg, 0.125 mg to 5 mg, 0.25 mg to 5 mg, 0.5 mg to 5 mg, 0.05 mg to 2 mg, 0.1 mg to 2 mg, 0.125 mg to 2 mg, 0.25 mg to 2 mg, 0.5 mg to 2 mg, 1 µg, 10 µg, 25 µg, 50 µg, 100 µg, 125 µg, 200 µg, 250 µg, 25 to 66 µg, 48 to 81 µg, 73 to 125 µg, 40 µg, 64 µg, 95 µg, 35 to 95 µg, 25 to 125 µg, 60 to 170 µg, 110 µg, 170 µg, 45 to 220 µg, 45 to 85 µg, 48 to 82 µg, 85 to 160 µg, 140 to 220 µg, 120 to 325 µg, 205 µg, 320 µg, 325 µg, 90 to 400 µg, 95 to 170 µg, 165 to 275 µg, or 275 to 400 µg of budesonide, said dose being a nominal dose, nominal available dose, emitted dose, delivered dose, dose to subject, or dose to lung.

The corticosteroid can be present in its neutral, ionic, salt, basic, acidic, natural, synthetic, diastereomeric, isomeric, isomeric, enantiomerically pure, racemic, solvate, anhydrous, hydrate, chelate, derivative, analog, esterified, non-esterified, or other common form. Whenever an active agent is named herein, all such forms available are included.

For example, all known forms of budesonide are considered within the scope of the invention.

The formulation of the invention can be used to deliver two or more different active agents (active ingredients, therapeutic agents, etc.). Particular combinations of active agents can be provided by the present formulation. Some combinations of active agents include: 1) a first drug from a first therapeutic class and a different second drug from the same therapeutic class; 2) a first drug from a first therapeutic class and a different second drug from a different therapeutic class; 3) a first drug having a first type of biological activity and a different second drug having about the same biological activity;

4) a first drug having a first type of biological activity and a different second drug having a different second type of biological activity. Exemplary combinations of active agents are described herein.

A corticosteroid, such as budesonide, can be administered in combination with one or more other drugs (active ingredients, therapeutic agents, active agents, etc., the terms being used interchangeably herein unless otherwise specified). Such other drugs include: B₂ adrenoreceptor agonist, topical anesthetic, D₂ receptor agonist, anticholinergic agent.

B₂-Adrenoreceptor agonists for use in combination with the compositions provided herein include, but are not limited to, Albuterol (α^1 -(((1,1-dimethylethyl)amino)methyl)-4-hydroxy-1,3-benzenedimethanol); Bambuterol (dimethylcarbamic acid 5-(2-(((1,1-dimethylethyl)amino)-1-hydroxyethyl)-1,3-phenylene ester); Bitolterol (4-methylbenzoic acid 4-(2-(((1,1-dimethylethyl)amino)-1-hydroxyethyl)-1,2-phenyleneester); Broxaterol (3-bromo- α -(((1,1-dimethylethyl)amino)methyl)-5-isoxazolemethanol); Isoproterenol (4-(1-hydroxy-2-((1-methylethyl)amino)ethyl)-1,2-benzene-diol); Trimetoquinol (1,2,3,4-tetrahydro-1-((3,4-, 5-trimethoxyphenyl)-methyl)-6,7-isoquinolinediol); Clenbuterol (4-amino-3,5-dichloro- α -(((1,1-dimethylethyl)amino)methyl)benzenemethanol); Fenoterol (5-(1-hydroxy-2-((2-(4-hydroxyphenyl)-1-methylethyl)amino)ethyl)-1,3-benzenediol); Formoterol (2-hydroxy-5-((1RS)-1-hydroxy-2-(((1RS)-2-(p-methoxyphenyl)-1-methylethyl)amino)ethyl)formanilide); (R,R)-Formoterol; Desformoterol ((R,R) or (S,S)-3-amino-4-hydroxy- α -(((2-(4-methoxyphenyl)-1-methyl-ethyl)amino)methyl)benzenemethanol); Hexoprenaline (4,4'-(1,6-hexane-diyl)-bis(imino(1-hydroxy-2,1-ethanediyl)))bis-1,2-benzenediol); Isoetharine (4-(1-hydroxy-2-((1-methylethyl)amino)butyl)-1,2-benzenediol); Isoprenaline (4-(1-hydroxy-2-((1-methylethyl)amino)ethyl)-1,2-benzenediol); Meta-proteranol (5-(1-hydroxy-2-((1-methylethyl)amino)ethyl)-1,3-benzenediol); Picumeterol (4-amino-3,5-dichloro- α -(((6-(2-(2-pyridinyl)ethoxy)hexyl)-amino)methyl)benzenemethanol); Pirbuterol (α^6 -(((1,1-dimethylethyl)-amino)methyl)-3-hydroxy-2,6-pyridinemethanol); Procaterol (((R*,S*)-(+,-)-8-hydroxy-5-(1-hydroxy-2-((1-methylethyl)amino)butyl)-2(1H)-quinolin-one); Reproterol ((7-(3-((2-(3,5-dihydroxyphenyl)-2-hydroxyethyl)amino)-propyl)-3,7-dihydro-1,3-dimethyl-1H-purine-2,6-dione); Rimiterol (4-(hydroxy-2-piperidinylmethyl)-1,2-benzenediol); Salbutamol ((+,-)- α^1 -(((1,1-dimethylethyl)amino)methyl)-4-hydroxy-1,3-benzenedimethanol); (R)-Salbutamol; Salmeterol ((+,-)-4-hydroxy- α^1 -(((6-(4-phenylbutoxy)hexyl)-

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amino)methyl)-1,3-benzenedimethanol); (R)-Salmeterol; Terbutaline (5-(2-((1,1-dimethylethyl)amino)-1-hydroxyethyl)-1,3-benzenediol); Tulobuterol (2-chloro-.alpha.-(((1,1 -dimethylethyl)amino)methyl)benzenemethanol); and TA-2005 (8-hydroxy-5-((1R)-1-hydroxy-2-(N-((1R)-2-(4-methoxyphenyl)-1-methylethyl)amino)ethyl)carbostyryl
 5 hydrochloride).

Dopamine (D₂) receptor agonists include, but are not limited to, Apomorphine ((r)-5,6,6a,7-tetrahydro-6-methyl-4H-dibenzo[de,gl]quinoline-10,11-diol); Bromocriptine ((5'.alpha.)-2-bromo-12'-hydroxy-2'-(1-methylethyl)-5'-(2-methylpropyl)ergotaman-3',6',18-trione); Cabergoline ((8.beta.)-N-(3-(dimethylamino)propyl)-N-((ethylamino)carbonyl)-6-(2-propenyl)ergoline-8-carboxamide); Lisuride (N'-((8.alpha.)-9,10-didehydro-6-methylergolin-8-yl)-N,N-diethylurea); Pergolide ((8-beta)-8-((methylthio)methyl)-6-propylergoline); Levodopa (3-hydroxy-L-tyrosine); Pramipexole ((s)-4,5,6,7-tetrahydro-N⁶-prop-yl-2,6-benzothiazolediamine); Quinpirole hydrochloride (trans-(-)-4aR-4,4a,5,6,7,8,8a,9-octahydro-5-propyl-1H-pyrazolo[3,4-g]quinoline hydrochloride);
 10 Ropinirole (4-(2-(dipropylamino)ethyl)-1,3-dihydro-2H-indol-2-one); and Talipexole (5,6,7,8-tetrahydro-6-(2-propenyl)-4H-thia-zolo[4,5-d]azepin-2-amine). Other dopamine D₂ receptor agonists for use herein are disclosed in International Patent Application Publication No. WO 99/36095, the relevant disclosure of which is hereby incorporated by
 15 reference.

Anticholinergic agents for use herein include, but are not limited to, ipratropium bromide, oxitropium bromide, atropine methyl nitrate, atropine sulfate, ipratropium, belladonna extract, scopolamine, scopolamine methobromide, homatropine methobromide, hyoscyamine, isopriopramide, orphenadrine, benzalkonium chloride, tiotropium bromide and glycopyrronium bromide. In certain embodiments, the
 20 compositions contain an anticholinergic agent, such as ipratropium bromide or tiotropium bromide, at a concentration of about 5 µg/mL to about 5 mg/mL, or about 50 µg/mL to about 200 µg/mL. In other embodiments, the compositions for use in the methods herein contain an anticholinergic agent, including ipratropium bromide and tiotropium bromide, at a concentration of about 83 µg/mL or about 167 µg/mL.

Other active ingredients for use herein in combination therapy, include, but are not limited to, IL-5 inhibitors such as those disclosed in U.S. Patents No. 5,668,110, No. 5,683,983, No. 5,677,280, No. 6,071,910 and No. 5,654,276, the relevant disclosures of which are hereby incorporated by reference; antisense modulators of IL-5 such as those

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disclosed in U.S. Pat. No. 6,136,603, the relevant disclosure of which is hereby incorporated by reference; milrinone (1,6-dihydro-2-methyl-6-oxo-[3,4'-bipyridine]-5-carbonitrile); milrinone lactate; tryptase inhibitors such as those disclosed in U.S. Pat. No. 5,525,623, the relevant disclosure of which is hereby incorporated by reference;

5 tachykinin receptor antagonists such as those disclosed in U.S. Patents No. 5,691,336, No. 5,877,191, No. 5,929,094, No. 5,750,549 and No. 5,780,467, the relevant disclosures of which are hereby incorporated by reference; leukotriene receptor antagonists such as montelukast sodium (Singular™, R-(E)-1-[[[1-[3-[2-(7-chloro-2-quinolinyl)ethenyl]-phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]-propyl]thio]methyl] cyclopropaneacetic acid, monosodium salt), 5-lipoxygenase inhibitors such as zileuton (Zyflo™, Abbott Laboratories, Abbott Park, Ill.), and anti-IgE antibodies such as Xolair™ (recombinant humanized anti-IgE monoclonal antibody (CGP 51901; IGE 025A; rhuMAb-E25), Genentech, Inc., South San Francisco, Calif.), and topical anesthetics such as lidocaine, N-arylamide, aminoalkylbenzoate, prilocaine, etidocaine (U.S. Patents No. 5,510,339, No.

10 5,631,267, and No. 5,837,713, the relevant disclosures of which are hereby incorporated by reference).

Exemplary combination formulations of the invention comprise the following components.

FORM.	Corticosteroid (A)	Other Active Ingredient (B)
I	Budesonide	Formoterol
II	Budesonide	Salmeterol
III	Budesonide	Albuterol
IV	Fluticasone propionate	Salmeterol

20 A formulation comprising a corticosteroid and another active ingredient can be prepared according to the examples below. In one embodiment, the SAE-CD is present in an amount sufficient to solubilize the corticosteroid and the other active ingredient. In another embodiment, the SAE-CD is present in an amount sufficient to solubilize the corticosteroid or the other active ingredient.

25 Depending upon the other active ingredient used, it may or may not bind competitively against the corticosteroid with the SAE-CD. In some embodiments, the SAE-CD has a higher equilibrium binding constant for the other active ingredient than it has for the corticosteroid. In some embodiments, the SAE-CD has a higher equilibrium binding constant for the corticosteroid than it has for the other active ingredient. In some

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embodiments, the SAE-CD has approximately the same equilibrium binding constant for the other active ingredient as it has for the corticosteroid. Alternatively, the other active ingredient does not bind with the SAE-CD even though the corticosteroid does. Accordingly, the invention provides embodiments wherein, the SAE-CD solubilizes the corticosteroid, the other active ingredient, or a combination thereof. The invention also provides embodiments wherein, the SAE-CD solubilizes at least a major portion of the corticosteroid, the other active ingredient, or of each. The invention also provides embodiments wherein, the SAE-CD does not solubilize the other active ingredient.

The molar ratio of SAE-CD to corticosteroid and SAE-CD to other active ingredient can vary as needed to provide a combination formulation as described herein. The SAE-CD is generally present in molar excess over the corticosteroid, the other active ingredient, or both.

The phase solubility binding curve for Salmeterol xinafoate and budesonide was determined as described herein, and the approximate equilibrium binding constant (K_i) of each with CAPTISOL was determined. The approximate K_i for Salmeterol xinafoate was approximately 3,500, and the approximate K_i for budesonide was 600 under the test conditions used. The molar ratio of CAPTISOL to Salmeterol xinafoate at saturated solubility was about 3.2 under the test conditions used. There may be a reduction in the amount of budesonide solubilized by CAPTISOL in the presence of Salmeterol xinafoate; however, clear aqueous liquid solution formulations comprising therapeutically effective amounts of budesonide and Salmeterol xinafoate were prepared.

A formulation comprising budesonide, albuterol and SAE-CD was prepared according to Example 25. The formulation was clear after preparation. The combination of budesonide and albuterol has been shown to be physically and chemically stable and would be expected to provide the same improved aerosol performance and AUC per μg budesonide dosed as the solution of budesonide alone. Furthermore, the patient would benefit from the simultaneous administration of the two drugs.

The invention includes methods for the treatment, prevention, or amelioration of one or more symptoms of a corticosteroid-responsive disorder, a disease or disorder of the air passageways e.g. respiratory or pulmonary disorders such as bronchoconstrictive disorders; sinus disorders such as sinusitis. The method further includes administering one or more of (a), (b), (c) or (d) as follows: (a) a β_2 -adrenoreceptor agonist; (b) a dopamine (D_2) receptor agonist; (c) a prophylactic therapeutic, such as a steroid; or (d) an

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anticholinergic agent; simultaneously with, prior to or subsequent to the composition provided herein.

Embodiments of the present invention allow for combinations to be prepared in a variety of ways:

5 1) Mixing ready to use solutions of a β 2-agonist such as levalbuterol or anticholinergic such as ipatropium bromide with a ready to use solution of a corticosteroid in SAE-CD;

 2) Mixing ready to use solutions of a β 2-agonist or anticholinergic with a concentrated solution of a corticosteroid dissolved using SAE-CD;

10 3) Mixing a ready to use solution of a β 2-agonist or anticholinergic with substantially dry SAE-CD and a substantially dry corticosteroid;

 4) Mixing a ready to use solution of a β 2-agonist or anticholinergic with a substantially dry mixture of SAE-CD and a corticosteroid or more conveniently a pre-measured amount of the mixture in a unit container such as a capsule (empty a capsule
15 into ready to use solution);

 5) Mixing a ready to use solution of a corticosteroid such as budesonide with a substantially dry long acting or short acting β 2-agonist and/or with a substantially dry anticholinergic such as ipatropium bromide or tiotropium bromide; or

 6) Dissolving a substantially dry β 2-agonist, and/or a substantially dry
20 anticholinergic and a substantially dry SAE-CD plus a substantially dry corticosteroid.

The materials used herein can be used in micronized or non-micronized form and crystalline, polymorphic or amorphous form. This is particularly true of the corticosteroids and other active ingredients.

It is well understood by those of ordinary skill in the art that the above solutions or
25 powders may optionally contain other ingredients such as buffers and/or tonicity adjusters and/or antimicrobials and/or additives or other such excipients as set forth herein or as presently used in inhalable liquid formulations to improve the output of the nebulizer.

Dosing, use and administration of the therapeutic agents disclosed herein is generally intended to follow the guidelines set forth in the Physician's Desk Reference,
30 55th Edition (Thompson Healthcare, Montvale, NJ, 2005) the relevant disclosure of which is hereby incorporated by reference.

The bronchoconstrictive disorder to be treated, prevented, or whose one or more symptoms are to be ameliorated is associated with asthma, including, but not limited to,

bronchial asthma, allergic asthma and intrinsic asthma, e.g., late asthma and airway hyper-responsiveness; and, particularly in embodiments where an anticholinergic agent is used, other chronic obstructive pulmonary diseases (COPDs), including, but not limited to, chronic bronchitis, emphysema, and associated cor pulmonale (heart disease secondary to
5 disease of the lungs and respiratory system) with pulmonary hypertension, right ventricular hypertrophy and right heart failure. COPD is frequently associated with cigarette smoking, infections, environmental pollution and occupational dust exposure.

A formulation according to the invention will generally have a storage shelf life of no less than 6 months. In this case, shelf life is determined only as regards the increase in
10 the amount of corticosteroid degradation by-products or a reduction in the amount of corticosteroid remaining in the formulation. For example, for a formulation having a shelf life of at least six months, the formulation will not demonstrate an unacceptable and substantial increase in the amount of degradants during the storage period of at least six months. The criteria for acceptable shelf-life are set as needed according to a given
15 product and its storage stability requirements. In other words, the amount of degradants in a formulation having an acceptable shelf-life will not increase beyond a predetermined value during the intended period of storage. On the other hand, the amount of degradants of a formulation having an unacceptable shelf-life will increase beyond the predetermined value during the intended period of storage.

The method of Example 3 was followed to determine the stability of budesonide in
20 solution. The shelf-life was defined as the time to loss of 10% potency. Under the conditions tested, the loss of potency was first order. The shelf life of a *Captisol-Enabled*® Budesonide Inhalation Solution (a solution comprising budesonide and SBE7- β -CD) is greater than about 3 years at a pH between 4 and 5, i.e. about 90 months at pH
25 4.0 and about 108 months at pH 5.0 without the need to add any other stabilizers, such as EDTA, in water in the presence of about 5% wt./vol. SAE-CD. This shelf-life is greater than that reported by Otterbeck (U.S. Patent 5,914,122; up to six weeks at pH 4.0-6.0 in water in the presence of EDTA, HP- β -CD and other additives.)

The inventors have also discovered that SAE-CD is capable of stabilizing the
30 isomers of budesonide to different extents. A study to determine if SBE7- β -CD stabilized budesonide solutions and if it preferentially stabilized one isomer was conducted according to Example 13. Figure 11 is a semi-log plot of the % of initial concentration at each time point for the samples stored at 60 °C. Loss of budesonide was first order at each

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temperature. The table below shows the pseudo-first order rate constants calculated for each isomer at 60 °C and 80 °C.

Pseudo 1st Order Rate constant (hours⁻¹)					
Temperature 60 °C					
Ph	Rate constant R-isomer	With/without CAPTISOL ratio for R-isomers	Rate constant S-isomer	With/without CAPTISOL ratio for S-isomers	R/S rate constant ratio
4 w/ CAPTISOL	0.000597	0.547	0.00012	0.323	5.06
4 no CAPTISOL	0.00109		0.0037		2.99
6 w/ CAPTISOL	0.001661	0.385	0.000361	0.193	4.60
6 no CAPTISOL	0.00432		0.001872		2.31

Pseudo 1st Order Rate constant (hours⁻¹)					
Temperature 80 °C					
pH	Rate Constant R-isomer	With/without CAPTISOL ratio for R-isomers	Rate constant S-isomer	With/without CAPTISOL ratio for S-isomers	R/S rate constant ratio
4 w/ CAPTISOL	0.002250	0.607	0.000644	0.491	3.49
4 no CAPTISOL	0.003704		0.00131		2.83
6 w/ CAPTISOL	0.00732	0.529	0.00254	0.384	2.88
6 no CAPTISOL	0.0138		0.00661		2.09

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SBE7- β -CD stabilized both R- and S-isomers of budesonide in solutions at both pH 4 and 6. The with/without CAPTISOL ratio of rate constants was much less than 1 at all temperatures. SBE7- β -CD had a greater effect on the stability of both the R and S-isomer at pH 6 than at pH 4. At a given temperature the ratio of rate constants with/without SBE7- β -CD was less at pH 6 than at pH 4. Although SBE7- β -CD stabilized both isomers, the S-isomer appears to be stabilized to an even greater extent than the R. At all temperatures and pHs tested, the ratio of rate constants with/without SBE7- β -CD was lower for the S isomer. The degree of stabilization affected by SBE7- β -CD at 60°C is

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greater than at 80°C. An even greater degree of stabilization would be expected at 40°C and/or room temperature (20-30 C).

Samples of the above solutions were also placed in a chamber under a bank of fluorescent lights. Vials were periodically removed and assayed for budesonide. Figure 12 shows the semi-log plot of the % of initial value remaining as a function of light exposure (light intensity * time). As noted in the table below, SBE7- β -CD significantly reduced the photodecomposition of budesonide. The loss of budesonide was first order and independent of pH.

Light Stability of Budesonide Pseudo 1st Order Rate constant (hour⁻¹)		
	pH 4	pH 6
Captisol	0.0585	0.0562
No Captisol	0.0812	0.0822

The formulation of the invention can be provided as a kit adapted to form an inhalable solution for nebulization. The kit can comprise a corticosteroid, SAE-CD, an aqueous carrier, and optionally one or more other components. The corticosteroid and SAE-CD can be provided together or separately in solid, suspended or dissolved form. After mixing SAE-CD with corticosteroid in the presence of an aqueous carrier, the solids will dissolve to form an inhalable solution rather than suspension for nebulization. Each component can be provided in an individual container or together with another component. For example, SAE-CD can be provided in an aqueous solution while budesonide is provided in dry solid form or wet suspended form. Alternatively, SAE-CD is provided in dry form and budesonide is provided as an aqueous suspension, e.g., PULMICORT RESPULES™. The kit can instead comprise an admixture of a solid derivatized cyclodextrin and solid corticosteroid and, optionally, at least one solid pharmaceutical excipient, such that a major portion of the active agent is not complexed with the derivatized cyclodextrin prior to reconstitution of the admixture with an aqueous carrier. Alternatively, the composition can comprise a solid mixture comprising the inclusion complex of a derivatized cyclodextrin and an active agent, wherein a major portion of the active agent is complexed with the derivatized cyclodextrin prior to reconstitution of the solid mixture with an aqueous carrier. Depending upon the storage temperature of the kit,

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the aqueous carrier may be a liquid or frozen solid. In one embodiment, the kit excludes the aqueous carrier during storage, but the aqueous carrier is added to the SAE-CD and corticosteroid prior to use to form the nebulization solution. The corticosteroid and SAE-CD can be complexed and present in aqueous concentrated form prior to addition of the aqueous carrier, which is later added to bring the solution to volume and proper viscosity and concentration for nebulization. A reconstitutable formulation can be prepared according to any of the following processes. A liquid formulation of the invention is first prepared, then a solid is formed by lyophilization (freeze-drying), spray-drying, spray freeze-drying, antisolvent precipitation, various processes utilizing supercritical or near supercritical fluids, or other methods known to those of ordinary skill in the art to make a solid for reconstitution. Example 29 details a method for the preparation of a lyophilized solid composition comprising corticosteroid and SAE-CD by lyophilization of a liquid composition or formulation of the invention. The lyophilized solid can be dissolved in an aqueous liquid carrier prior to administration via nebulization. The dried powder would provide a stable form for long-term storage and would also be useful to rapidly prepare inhalation compositions on a larger scale, or as an additive to another inhalation solution medication to prepare combination products.

While the liquid composition or formulation of the invention can be administered to the lung, it would also be suitable for nasal, oral, ophthalmic, otic or topical administration. The liquid composition or formulation may also be administered via inhalation using a device such as a pump spray, metered dose inhaler, or pressurized metered dose inhaler. Accordingly, the invention provides a method of treating a corticosteroid-responsive disease or disorder by administration of the liquid to a subject in need of such treatment.

A liquid vehicle (carrier) included in a formulation of the invention comprises an aqueous liquid carrier, such as water, aqueous alcohol, propylene glycol, or aqueous organic solvent. Example 30 details the preparation of a liquid formulation comprising 20% w/v SAE-CD, corticosteroid, water and ethanol (0-5%). Increasing the concentration of the ethanol in the liquid resulted in a decrease in the maximum saturated solubility of the corticosteroid.

Although not necessary, the formulation of the present invention may include a conventional preservative, antioxidant, buffering agent, acidifying agent, alkalizing agent, colorant, solubility-enhancing agent, complexation-enhancing agent, electrolyte, glucose,

stabilizer, tonicity modifier, bulking agent, antifoaming agent, oil, emulsifying agent, cryoprotectant, plasticizer, flavors, sweeteners, a tonicity modifier, surface tension modifier, viscosity modifier, density modifier, volatility modifier, other excipients known by those of ordinary skill in the art for use in preserved formulations, or a combination thereof.

As used herein, the term "alkalizing agent" is intended to mean a compound used to provide alkaline medium, such as for product stability. Such compounds include, by way of example and without limitation, ammonia solution, ammonium carbonate, diethanolamine, monoethanolamine, potassium hydroxide, sodium borate, sodium carbonate, sodium bicarbonate, sodium hydroxide, triethanolamine, diethanolamine, organic amine base, alkaline amino acids and triethanolamine and others known to those of ordinary skill in the art.

As used herein, the term "acidifying agent" is intended to mean a compound used to provide an acidic medium for product stability. Such compounds include, by way of example and without limitation, acetic acid, acidic amino acids, citric acid, fumaric acid and other alpha hydroxy acids, hydrochloric acid, ascorbic acid, phosphoric acid, sulfuric acid, tartaric acid and nitric acid and others known to those of ordinary skill in the art.

Inclusion of a conventional preservative in the inhalable solution formulation is optional, since the formulation is self-preserved by SAE-CD depending upon its concentration in solution. Nonetheless, a conventional preservative can be further included in the formulation if desired. Preservatives can be used to inhibit microbial growth in the compositions. The amount of preservative is generally that which is necessary to prevent microbial growth in the composition for a storage period of at least six months. As used herein, a conventional preservative is a compound used to at least reduce the rate at which bioburden increases, but preferably maintains bioburden steady or reduces bioburden after contamination has occurred. Such compounds include, by way of example and without limitation, benzalkonium chloride, benzethonium chloride, benzoic acid, benzyl alcohol, cetylpyridinium chloride, chlorobutanol, phenol, phenylethyl alcohol, phenylmercuric nitrate, phenylmercuric acetate, thimerosal, metacresol, myristylgamma picolinium chloride, potassium benzoate, potassium sorbate, sodium benzoate, sodium propionate, sorbic acid, thymol, and methyl, ethyl, propyl or butyl parabens and others known to those of ordinary skill in the art.

As used herein, the term “antioxidant” is intended to mean an agent that inhibits oxidation and thus is used to prevent the deterioration of preparations by the oxidative process. Such compounds include, by way of example and without limitation, acetone, potassium metabisulfite, potassium sulfite, ascorbic acid, ascorbyl palmitate, citric acid, butylated hydroxyanisole, butylated hydroxytoluene, hypophosphorous acid, monothioglycerol, propyl gallate, sodium ascorbate, sodium citrate, sodium sulfide, sodium sulfite, sodium bisulfite, sodium formaldehyde sulfoxylate, thioglycolic acid, EDTA, pentetate, and sodium metabisulfite and others known to those of ordinary skill in the art.

As used herein, the term “buffering agent” is intended to mean a compound used to resist change in pH upon dilution or addition of acid or alkali. Buffers are used in the present compositions to adjust the pH to a range of between about 2 and about 8, about 3 to about 7, or about 4 to about 5. Such compounds include, by way of example and without limitation, acetic acid, sodium acetate, adipic acid, benzoic acid, sodium benzoate, boric acid, sodium borate, citric acid, glycine, maleic acid, monobasic sodium phosphate, dibasic sodium phosphate, HEPES, lactic acid, tartaric acid, potassium metaphosphate, potassium phosphate, monobasic sodium acetate, sodium bicarbonate, tris, sodium tartrate and sodium citrate anhydrous and dihydrate and others known to those of ordinary skill in the art. Other buffers include citric acid/phosphate mixture, acetate, barbitol, borate, Britton-Robinson, cacodylate, citrate, collidine, formate, maleate, McIlvaine, phosphate, Prideaux-Ward, succinate, citrate-phosphate-borate (Teorell-Stanhagen), veronal acetate, MES (2-(N-morpholino)ethanesulfonic acid), BIS-TRIS (bis(2-hydroxyethyl)imino-tris(hydroxymethyl)methane), ADA (N-(2-acetamido)-2-iminodiacetic acid), ACES (N-(carbamoylmethyl)-2-aminoethanesulfonic acid), PIPES (piperazine-N,N'-bis(2-ethanesulfonic acid)), MOPSO (3-(N-morpholino)-2-hydroxypropanesulfonic acid), BIS-TRIS PROPANE (1,3-bis(tris(hydroxymethyl)methylamino)propane), BES (N,N-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid), MOPS (3-(N-morpholino)propanesulfonic acid), TES (N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid), HEPES (N-(2-hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid), DIPSO (3-(N,N-bis(2-hydroxyethyl)amino)-2-hydroxypropanesulfonic acid), MOBS (4-(N-morpholino)-butanesulfonic acid), TAPSO (3-(N-tris(hydroxymethyl)methylamino)-2-hydroxypropanesulfonic acid), TRIZMA™ (tris(hydroxymethylaminomethane), HEPPSO (N-(2-hydroxyethyl)piperazine-N'-(2-hydroxypropanesulfonic acid), POPSO (piperazine-

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N,N'-bis(2-hydroxypropanesulfonic acid)), TEA (triethanolamine), EPPS (N-(2-hydroxyethyl)piperazine-N'-(3-propanesulfonic acid), TRICINE (N-tris(hydroxymethyl)methylglycine), GLY-GLY (glycylglycine), BICINE (N,N-bis(2-hydroxyethyl)glycine), HEPBS (N-(2-hydroxyethyl)piperazine-N'-(4-butanesulfonic acid)), TAPS (N-tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid), AMPD (2-amino-2-methyl-1,3-propanediol), and/or any other buffers known to those of skill in the art.

A complexation-enhancing agent can be added to a formulation of the invention. When such an agent is present, the ratio of cyclodextrin /active agent can be changed. A complexation-enhancing agent is a compound, or compounds, that enhance(s) the complexation of the active agent with the cyclodextrin. Suitable complexation enhancing agents include one or more pharmacologically inert water soluble polymers, hydroxy acids, and other organic compounds typically used in liquid formulations to enhance the complexation of a particular agent with cyclodextrins.

Hydrophilic polymers can be used as complexation-enhancing, solubility-enhancing and/or water activity reducing agents to improve the performance of formulations containing a cyclodextrin. Loftsson has disclosed a number of polymers suitable for combined use with a cyclodextrin (underivatized or derivatized) to enhance the performance and/or properties of the cyclodextrin. Suitable polymers are disclosed in *Pharmazie* (2001), 56(9), 746-747; *International Journal of Pharmaceutics* (2001), 212(1), 29-40; Cyclodextrin: From Basic Research to Market, International Cyclodextrin Symposium, 10th, Ann Arbor, MI, United States, May 21-24, 2000 (2000), 10-15 (Wacker Biochem Corp.: Adrian, Mich.); PCT International Publication No. WO 9942111; *Pharmazie*, 53(11), 733-740 (1998); *Pharm. Technol. Eur.*, 9(5), 26-34 (1997); *J. Pharm. Sci.* 85(10), 1017-1025 (1996); European Patent Application EP0579435; Proceedings of the International Symposium on Cyclodextrins, 9th, Santiago de Comostela, Spain, May 31-June 3, 1998 (1999), 261-264 (Editor(s): Labandeira, J. J. Torres; Vila-Jato, J. L. Kluwer Academic Publishers, Dordrecht, Neth); *S.T.P. Pharma Sciences* (1999), 9(3), 237-242; ACS Symposium Series (1999), 737(Polysaccharide Applications), 24-45; *Pharmaceutical Research* (1998), 15(11), 1696-1701; *Drug Development and Industrial Pharmacy* (1998), 24(4), 365-370; *International Journal of Pharmaceutics* (1998), 163(1-2), 115-121; Book of Abstracts, 216th ACS National Meeting, Boston, August 23-27 (1998), CELL-016, American Chemical Society; *Journal of Controlled Release*, (1997),

44/1 (95-99); *Pharm.Res.* (1997) 14(11), S203; *Investigative Ophthalmology & Visual Science*, (1996), 37(6), 1199-1203; Proceedings of the International Symposium on Controlled Release of Bioactive Materials (1996), 23rd, 453-454; *Drug Development and Industrial Pharmacy* (1996), 22(5), 401-405; Proceedings of the International Symposium on Cyclodextrins, 8th, Budapest, Mar. 31-Apr. 2, (1996), 373-376. (Editor(s): Szejtli, J.; Szente, L. Kluwer: Dordrecht, Neth.); *Pharmaceutical Sciences* (1996), 2(6), 277-279; *European Journal of Pharmaceutical Sciences*, (1996) 4(SUPPL.), S144; Third European Congress of Pharmaceutical Sciences Edinburgh, Scotland, UK September 15-17, 1996; *Pharmazie*, (1996), 51(1), 39-42; *Eur. J. Pharm. Sci.* (1996), 4(Suppl.), S143; U.S. Patents No. 5,472,954 and No. 5,324,718; *International Journal of Pharmaceutics* (Netherlands), (Dec. 29, 1995) 126, 73-78; Abstracts of Papers of the American Chemical Society, (02 APR 1995) 209(1), 33-CELL; *European Journal of Pharmaceutical Sciences*, (1994) 2, 297-301; *Pharmaceutical Research* (New York), (1994) 11(10), S225; *International Journal of Pharmaceutics* (Netherlands), (Apr 11, 1994) 104, 181-184; and *International Journal of Pharmaceutics* (1994), 110(2), 169-77, the entire disclosures of which are hereby incorporated by reference.

Other suitable polymers are well-known excipients commonly used in the field of pharmaceutical formulations and are included in, for example, *Remington's Pharmaceutical Sciences, 18th Edition*, Alfonso R. Gennaro (editor), Mack Publishing Company, Easton, PA, 1990, pp. 291-294; Alfred Martin, James Swarbrick and Arthur Commarata, *Physical Pharmacy. Physical Chemical Principles in Pharmaceutical Sciences, 3rd edition* (Lea & Febinger, Philadelphia, PA, 1983, pp. 592-638); A.T. Florence and D. Altwood, (*Physicochemical Principles of Pharmacy, 2nd Edition*, MacMillan Press, London, 1988, pp. 281-334. The entire disclosures of the references cited herein are hereby incorporated by references. Still other suitable polymers include water-soluble natural polymers, water-soluble semi-synthetic polymers (such as the water-soluble derivatives of cellulose) and water-soluble synthetic polymers. The natural polymers include polysaccharides such as inulin, pectin, algin derivatives (e.g. sodium alginate) and agar, and polypeptides such as casein and gelatin. The semi-synthetic polymers include cellulose derivatives such as methylcellulose, hydroxyethylcellulose, hydroxypropyl cellulose, their mixed ethers such as hydroxypropyl methylcellulose and other mixed ethers such as hydroxyethyl ethylcellulose and hydroxypropyl ethylcellulose, hydroxypropyl methylcellulose phthalate and carboxymethylcellulose and its salts,

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especially sodium carboxymethylcellulose. The synthetic polymers include polyoxyethylene derivatives (polyethylene glycols) and polyvinyl derivatives (polyvinyl alcohol, polyvinylpyrrolidone and polystyrene sulfonate) and various copolymers of acrylic acid (e.g. carbomer). Other natural, semi-synthetic and synthetic polymers not
5 named here which meet the criteria of water solubility, pharmaceutical acceptability and pharmacological inactivity are likewise considered to be within the ambit of the present invention.

An emulsifying agent is intended to mean a compound that aids the formation of an emulsion. An emulsifier can be used to wet the corticosteroid and make it more
10 amenable to dissolution. Emulsifiers for use herein include, but are not limited to, polyoxyethylene sorbitan fatty esters or polysorbates, including, but not limited to, polyethylene sorbitan monooleate (Polysorbate 80), polysorbate 20 (polyoxyethylene (20) sorbitan monolaurate), polysorbate 65 (polyoxyethylene (20) sorbitan tristearate), polyoxyethylene (20) sorbitan mono-oleate, polyoxyethylene (20) sorbitan monopalmitate,
15 polyoxyethylene (20) sorbitan monostearate; lecithins; alginic acid; sodium alginate; potassium alginate; ammonium alginate; calcium alginate; propane-1,2-diol alginate; agar; carrageenan; locust bean gum; guar gum; tragacanth; acacia; xanthan gum; karaya gum; pectin; amidated pectin; ammonium phosphatides; microcrystalline cellulose; methylcellulose; hydroxypropylcellulose; hydroxypropylmethylcellulose;
20 ethylmethylcellulose; carboxymethylcellulose; sodium, potassium and calcium salts of fatty acids; mono- and di-glycerides of fatty acids; acetic acid esters of mono- and di-glycerides of fatty acids; lactic acid esters of mono- and di-glycerides of fatty acids; citric acid esters of mono- and di-glycerides of fatty acids; tartaric acid esters of mono- and di-glycerides of fatty acids; mono- and diacetyltartaric acid esters of mono- and di-glycerides
25 of fatty acids; mixed acetic and tartaric acid esters of mono- and di-glycerides of fatty acids; sucrose esters of fatty acids; sucroglycerides; polyglycerol esters of fatty acids; polyglycerol esters of polycondensed fatty acids of castor oil; propane-1,2-diol esters of fatty acids; sodium stearyl-2-lactylate; calcium stearyl-2-lactylate; stearyl tartrate; sorbitan monostearate; sorbitan tristearate; sorbitan monolaurate; sorbitan monooleate;
30 sorbitan monopalmitate; extract of quillaia; polyglycerol esters of dimerised fatty acids of soya bean oil; oxidatively polymerised soya bean oil; and pectin extract.

As used herein, the term "stabilizer" is intended to mean a compound used to stabilize the therapeutic agent against physical, chemical, or biochemical process that

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would reduce the therapeutic activity of the agent. Suitable stabilizers include, by way of example and without limitation, albumin, sialic acid, creatinine, glycine and other amino acids, niacinamide, sodium acetyltryptophonate, zinc oxide, sucrose, glucose, lactose, sorbitol, mannitol, glycerol, polyethylene glycols, sodium caprylate and sodium saccharin and other known to those of ordinary skill in the art.

As used herein, the term "tonicity modifier" is intended to mean a compound or compounds that can be used to adjust the tonicity of the liquid formulation. Suitable tonicity modifiers include glycerin, lactose, mannitol, dextrose, sodium chloride, sodium sulfate, sorbitol, trehalose and others known to those of ordinary skill in the art. Other tonicity modifiers include both inorganic and organic tonicity adjusting agents. Tonicity modifiers include, but are not limited to, ammonium carbonate, ammonium chloride, ammonium lactate, ammonium nitrate, ammonium phosphate, ammonium sulfate, ascorbic acid, bismuth sodium tartrate, boric acid, calcium chloride, calcium disodium edetate, calcium gluconate, calcium lactate, citric acid, dextrose, diethanolamine, dimethylsulfoxide, edetate disodium, edetate trisodium monohydrate, fluorescein sodium, fructose, galactose, glycerin, lactic acid, lactose, magnesium chloride, magnesium sulfate, mannitol, polyethylene glycol, potassium acetate, potassium chlorate, potassium chloride, potassium iodide, potassium nitrate, potassium phosphate, potassium sulfate, propylene glycol, silver nitrate, sodium acetate, sodium bicarbonate, sodium biphosphate, sodium bisulfite, sodium borate, sodium bromide, sodium cacodylate, sodium carbonate, sodium chloride, sodium citrate, sodium iodide, sodium lactate, sodium metabisulfite, sodium nitrate, sodium nitrite, sodium phosphate, sodium propionate, sodium succinate, sodium sulfate, sodium sulfite, sodium tartrate, sodium thiosulfate, sorbitol, sucrose, tartaric acid, triethanolamine, urea, urethan, uridine and zinc sulfate. In one embodiment, the tonicity of the liquid formulation approximates the tonicity of the tissues in the respiratory tract.

An osmotic agent can be used in the compositions to enhance the overall comfort to the patient upon delivery of the corticosteroid composition. Osmotic agents can be added to adjust the tonicity of SAE-CD containing solutions. Osmolality is related to concentration of SAE-CD in water. At SBE7- β -CD concentrations below about 11-13% w/v, the solutions are hypotonic or hypoosmotic with respect to blood and at SBE7- β -CD concentrations above about 11-13% w/v the SBE7- β -CD containing solutions are hypertonic or hyperosmotic with respect to blood. When red blood cells are exposed to solutions that are hypo- or hypertonic, they can shrink or swell in size, which can lead to

hemolysis. As noted above and in Figure 1, SBE-CD is less prone to induce hemolysis than other derivatized cyclodextrins. Suitable osmotic agents include any low molecular weight water-soluble species pharmaceutically approved for pulmonary and nasal delivery such as sodium chloride, lactose and glucose. The formulation of the invention can also include biological salt(s), potassium chloride, or other electrolyte(s).

As used herein, the term "antifoaming agent" is intended to mean a compound or compounds that prevents or reduces the amount of foaming that forms on the surface of the liquid formulation. Suitable antifoaming agents include dimethicone, simethicone, octoxynol, ethanol and others known to those of ordinary skill in the art.

As used herein, the term "bulking agent" is intended to mean a compound used to add bulk to the lyophilized product and/or assist in the control of the properties of the formulation during lyophilization. Such compounds include, by way of example and without limitation, dextran, trehalose, sucrose, polyvinylpyrrolidone, lactose, inositol, sorbitol, dimethylsulfoxide, glycerol, albumin, calcium lactobionate, and others known to those of ordinary skill in the art.

As used herein, the term "cryoprotectant" is intended to mean a compound used to protect an active therapeutic agent from physical or chemical degradation during lyophilization. Such compounds include, by way of example and without limitation, dimethyl sulfoxide, glycerol, trehalose, propylene glycol, polyethylene glycol, and others known to those of ordinary skill in the art.

A solubility-enhancing agent or solubility enhancer can be added to the formulation of the invention. A solubility-enhancing agent is a compound, or compounds, that enhance(s) the solubility of the corticosteroid when in an aqueous liquid carrier. When another solubility enhancing agent is present, the ratio of SAE-CD to corticosteroid can be changed, thereby reducing the amount of SAE-CD required to dissolve the corticosteroid. Suitable solubility enhancing agents include one or more cyclodextrins, cyclodextrin derivatives, SAE-CD, organic solvents, detergents, soaps, surfactant and other organic compounds typically used in parenteral formulations to enhance the solubility of a particular agent. Exemplary solubility enhancers are disclosed in U.S. Patent No. 6,451,339; however, other surfactants used in the pharmaceutical industry can be used in the formulation of the invention. Some suitable cyclodextrin include underivatized cyclodextrins and cyclodextrin derivatives, such as SAE-CD, SAE-CD derivatives, hydroxyalkyl ether cyclodextrin and derivatives, alkyl ether cyclodextrin and

derivatives, sulfated cyclodextrin and derivatives, hydroxypropyl- β -cyclodextrin, 2-HP- β -CD, methyl- β -cyclodextrin, carboxyalkyl thioether derivatives, succinyl cyclodextrin and derivatives, and other cyclodextrin suitable for pharmaceutical use. SAE-CD cyclodextrins are particularly advantageous.

5 Suitable organic solvents that can be used in the formulation include, for example, ethanol, glycerin, poly(ethylene glycol), propylene glycol, poloxamer, aqueous forms thereof and others known to those of ordinary skill in the art.

 It should be understood that compounds used in the art of pharmaceutical formulations generally serve a variety of functions or purposes. Thus, if a compound
10 named herein is mentioned only once or is used to define more than one term herein, its purpose or function should not be construed as being limited solely to that named purpose(s) or function(s).

 An active agent contained within the present formulation can be present as its pharmaceutically acceptable salt. As used herein, "pharmaceutically acceptable salt"
15 refers to derivatives of the disclosed compounds wherein the active agent is modified by reacting it with an acid or base as needed to form an ionically bound pair. Examples of pharmaceutically acceptable salts include conventional non-toxic salts or the quaternary ammonium salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. Suitable non-toxic salts include those derived from inorganic acids such
20 as hydrochloric, hydrobromic, sulfuric, sulfonic, sulfamic, phosphoric, nitric and others known to those of ordinary skill in the art. The salts prepared from organic acids such as amino acids, acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pantoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic,
25 oxalic, isethionic, and others known to those of ordinary skill in the art. The pharmaceutically acceptable salts of the present invention can be synthesized from the parent active agent which contains a basic or acidic moiety by conventional chemical methods. Lists of other suitable salts are found in *Remington's Pharmaceutical Sciences*, 17th. ed., Mack Publishing Company, Easton, PA, 1985, the relevant disclosure of which is
30 hereby incorporated by reference.

 The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and

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animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

As used herein, the term "patient" or "subject" are taken to mean warm blooded animals such as mammals, for example, cats, dogs, mice, guinea pigs, horses, bovine
5 cows, sheep and humans.

A formulation of the invention will comprise an active agent present in an effective amount, effective dose or therapeutically effective dose. By the term "effective amount" or "effective dose" or "therapeutically effective dose", is meant the amount or quantity of active agent that is sufficient to elicit the required or desired response, or in other words,
10 the amount that is sufficient to elicit an appreciable biological response when administered to a subject.

In view of the above description and the examples below, one of ordinary skill in the art will be able to practice the invention as claimed without undue experimentation. The foregoing will be better understood with reference to the following examples that
15 detail certain procedures for the preparation of formulations according to the present invention. All references made to these examples are for the purposes of illustration. The following examples should not be considered exhaustive, but merely illustrative of only a few of the many embodiments contemplated by the present invention.

20 **EXAMPLE 1**

Exemplary formulations according to the invention were made according to the following general procedures.

Method A

Cyclodextrin is dissolved in water (or buffer) to form a solution containing a
25 known concentration of cyclodextrin. This solution is mixed with an active agent in solid, suspension, gel, liquid, paste, powder or other form while mixing, optionally while heating to form an inhalable solution.

Method B

A known amount of substantially dry cyclodextrin is mixed with a known amount
30 of substantially dry active agent. A liquid is added to the mixture to form a suspension,

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gel, solution, syrup or paste while mixing, optionally while heating and optionally in the presence of one or more other excipients, to form an inhalable solution.

Method C

5 A known amount of substantially dry cyclodextrin is added to a suspension, gel, solution, syrup or paste comprising a known amount of active agent while mixing, optionally while heating and optionally in the presence of one or more other excipients, to form an inhalable solution.

10 The methods of this example may be modified by the inclusion of a wetting agent in the composition in order to facilitate dissolution and subsequent inclusion complexation of the corticosteroid. A surfactant, soap, detergent or emulsifying agent can be used as a wetting agent.

Method D

15 To a solution comprising a known concentration or amount of SAE-CD, aqueous liquid carrier, and optionally one or more other excipients, is added a molar excess of the corticosteroid based upon the molar ratio of SAE-CD to corticosteroid at the point of saturated solubility of the corticosteroid, in the presence of the SAE-CD, as determined herein. For example, corticosteroid would be added at a 5%, 10%, 15%, 20%, 25%, 30% or greater molar excess. The components are mixed until equilibration, the point at which there is only a minor change in the concentration of budesonide over a one-hour period of
20 time. Then, the excess corticosteroid is removed leaving behind the target solution of the invention.

The budesonide is added to the SAE-CD-containing solution as either a solid or suspension in an aqueous liquid carrier, which can be water, buffer, aqueous alcohol, aqueous organic solvent or a combination thereof. The alcohol and organic solvent are of
25 a pharmaceutically acceptable grade, such as ethanol, propylene glycol, and others as described herein.

Method E

The SAE-CD and corticosteroid are triturated to form a mixture. Then, an aqueous liquid carrier is added to the mixture form the target solution of the invention.

30 The trituration can be conducted dry or in the presence of moisture, water, buffer, alcohol, surfactant, organic solvent, glycerin, poly(ethylene glycol), poloxamer, or a combination thereof.

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Method F

Any of the methods herein are conducted in the presence of heat, e.g. at a temperature of least 40 °C.

Method G

5 Any of the methods herein are conducted with cooling, e.g. at a temperature of less than 20 °C or less than 10 °C or less than 5 °C.

Method H

Any of the methods herein are conducted in the presence of high shear mixing such as with a sonicator, narrow gauge syringe(s), mixer/homogenizer (POLYTRON from
10 KINEMATICA, Europe; FLUKO, Shanghai, China; ULTIMAGRAL from GEA Niro, Inc., Columbia, MD), rotor-stator mixer, or saw tooth mixer.

Method I

Any of the methods herein are conducted under reduced pressure.

EXAMPLE 2

15 The MMD of nebulized solutions containing SBE7-β-CD and budesonide was determined as follows.

Placebo solutions of three different cyclodextrins were prepared at different concentrations. Two ml of the solutions were added to the cup of a Pari LC Plus nebulizer supplied with air from a Pari Proneb Ultra compressor. The particle size of the emitted
20 droplets was determined using a Malvern Mastersizer S laser light scattering instrument.

EXAMPLE 3

The stability of liquid formulations containing SAE-CD was determined by HPLC chromatography of aliquots periodically drawn from the liquid in storage.

Citrate-phosphate (McIlvaines) buffer solutions at a pH of 4, 5, 6, 7, or 8 were
25 prepared by mixing various portions of 0.01M citric acid with 0.02 M Na₂HPO₄. These stock solutions contained 5% w/w Captisol. Approximately 250 µg /mL of budesonide was dissolved in each buffer solution. Aliquots of the solutions were stored at 40 °C, 50 °C and 60 °C. Control samples were stored at 5 °C but are not reported here. HPLC analysis of the samples was performed initially and after 1, 2, and 3 months storage.

30 The HPLC conditions included:

Instrument:	PE Series 200
Column:	Phenomenex Luna C18(2) 4.6x150 mm 3 μ m
Mobile Phase:	58% Phosphate Buffer pH 3.4/ 39.5% ACN / 2.5% MeOH
Mobile Phase Program:	100% A (isocratic)
Wavelength	240
Flow Rate:	0.6 mL/min
Standard Range:	Seven standards - 1 to 500 μ g/mL

EXAMPLE 4

The viscosity of aqueous solutions containing SAE-CD were measured using a cone and plate viscometer.

A Brookfield Programmable DV-III+ Rheometer, CPE-40 cone and CPE 40Y plate (Brookfield Engineering Laboratories, Middleboro, MA) was used to make measurements on 0.5ml samples at 1, 2, 3, 5 and 10 rpm. Samples were sheered for approximately 5 revolutions prior to each measurement. This allowed accurate rheological characterization of the samples. The temperature of all samples was equilibrated to 25 $\pm$ 1 degree centigrade using a double wall viscometer cone supplied with water from an electronically controlled thermostatic circulating water bath (Model, 8001, Fisher Scientific, Pittsburgh, PA). The viscometer was calibrated using 5 and 50 centipoise using silicon oil calibration standards. Viscosity measurements were made at 5 or more rotation speeds to look for shear thinning behavior (viscosities that decrease as the rate of shear increases). Higher rotation speeds result in increased rates of shear.

EXAMPLE 5

Nebulizer output rate as a function of SAE-CD concentration was measured according to the following general procedure.

Nebulizer Output was tested using Pari LC Plus Nebulizer with a Pari ProNeb Ultra Air Compressor (Minimum Nebulizer Volume = 2ml, Maximum Nebulizer Volume = 8ml) for solutions containing 43%, 21.5%, 10.75% and 5.15%w/w SBE7- β -CD. Percentage of sample emitted was estimated gravimetrically. The nebulizer cup was weighed before and after nebulization was complete. Nebulization Time was defined as the duration of time when nebulizer run was started until the time of first sputter. Nebulizer Output Rate was calculated by dividing % Emitted with Nebulization Time.

EXAMPLE 6

Preparation of an inhalable solution containing budesonide.

A buffer solution containing 3mM Citrate Buffer and 82mM NaCl at pH 4.45 is prepared. ~12.5 grams of CAPTISOL was placed into a 250 ml volumetric flask. ~62.5
5 mg of budesonide was placed into the same flask. Flask was made to volume with the 3mM citrate buffer/82mM NaCl solution. The flask was well-mixed on a vortexer for 10 minutes and sonicated for 10 minutes. The flask was stirred over weekend with magnetic stirrer. Stirring was stopped after ~62 hours and flask was revortexed and resonicated again for 10 minutes each. The solution was filtered through a 0.22 μ m Durapore Millex-
10 GV Millipore syringe filter unit. The first few drops were discarded before filter rest of solution into an amber glass jar with a Teflon-lined screw cap. Sample concentration was ~237 μ g/ml.

EXAMPLE 7

Preparation of an inhalable solution containing budesonide.

15 Approximately 5 grams of CAPTISOL was placed into a 100 mL volumetric flask. ~26.3 mg of budesonide was placed into the same flask. The flask was made to volume with the 3mM citrate buffer/82mM NaCl solution. The mixture was well-mixed on a vortexer for 10 minutes and sonicated for 10 minutes. The mixture was stirred overnight with a magnetic stirrer. Stirring was stopped after ~16 hours and flask was revortexed and
20 resonicated again for 10 minutes each. The solution was filtered through 0.22 μ m Durapore Millex-GV Millipore syringe filter unit. The first 5 drops were discarded before filter rest of solution into an amber glass jar with a Teflon-lined screw cap. Sample was analyzed to be 233 μ g budesonide/ml.

EXAMPLE 8

25 Preparation of an inhalable solution containing budesonide.

The procedure of Example 7 was followed except that 12.5 g of CAPTISOL, 62.5 mg of budesonide and about 250 ml of buffer were used. Sufficient disodium EDTA was added to prepare a solution having an EDTA concentration of about 0.01 or 0.05 % wt/v EDTA.

EXAMPLE 9

Preparation of a solution containing SAE-CD and budesonide as prepared from a PULMICORT RESPULES suspension.

Method A.

5 To the contents of one or more containers of the Pulmicort Respules (nominally 2 mL of the suspension), about 50 mg (corrected for water content) of CAPTISOL was added per mL of Respule and mixed or shaken well for several minutes. After standing from about 30 minutes to several hours, the solution was used as is for in vitro characterization. In addition to budesonide and water, the PULMICORT RESPULE
10 (suspension) also contains the following inactive ingredients per the label: citric acid, sodium citrate, sodium chloride, disodium EDTA and polysorbate 80.

Method B.

Weigh approximately 200 mg amounts of CAPTISOL (corrected for water content) into 2-dram amber vials. Into each vial containing the weighed amount of
15 CAPTISOL empty the contents of two Pulmicort Respules containers (0.5 mg/2 mL, Lot # 308016 Feb05) by gently squeezing the deformable plastic container to the last possible drop. The Respules were previously swirled to re-suspend the budesonide particles. The vials are screw capped, mixed vigorously by vortex and then foil wrapped. The material can be kept refrigerated until use.

20 The inhalable liquid composition prepared according to any of these methods can be used in any known nebulizer. By converting the suspension to a liquid, an improvement in delivery of budesonide (a corticosteroid) is observed.

EXAMPLE 10

Other solutions according to the invention can be prepared as detailed below.

Component	Mg per ml (as prepared)		Mg per ml (per target)
	Concentrate A	Concentrate B	Final Solution
Budesonide EP	1	~1.6 (sat'd)	0.250
CAPTISOL	200	200	50
Sodium Citrate tribasic dihydrate	0	0	0.44
Citric Acid	0	0	0.32
Sodium Chloride	0	0	4.8

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Disodium EDTA	0	0	0-0.5
Polysorbate 80 (Tween 80)	0	0	0-1
Water	Qs	Qs	QS with buffer containing CAPTISOL or budesonide

- 5 Dilute Concentrate A at a ratio of 1 to 4 with pH 4.5 salinated citrate buffer (4 mM containing 109 mM sodium chloride) to contain 5% w/v CAPTISOL on an anhydrous basis. Filter the diluted concentrate through a 0.22 μ m Millipore Durapore Millex-GV syringe filter unit. Assay the filtered solution by HPLC then add supplemental budesonide as needed to give a solution final concentration of about 250 μ g/mL ($\pm < 5\%$).
- 10 Dilute Concentrate B at a ratio of 1 to 4 with pH 4.5 salinated citrate buffer (4 mM containing 109 mM sodium chloride) to contain 5% w/v CAPTISOL on an anhydrous basis. Filter the diluted concentrate through a 0.22 μ m Millipore Durapore Millex-GV syringe filter unit. Assay the filtered solution by HPLC then dilute further with pH 4.5 salinated citrate buffer (3 mM containing 82 mM sodium chloride containing 5% w/v CAPTISOL) as required to give a final

15 solution concentration of about 250 μ g/mL ($\pm < 5\%$). This technique takes advantage of the excess solid budesonide used to saturate the solution.

EXAMPLE 11

Clarity of solutions was determined by visual inspection or instrumentally. A clear solution is at least clear by visual inspection with the unaided eye.

20 EXAMPLE 12

The following method was used to determine the performance of nebulization compositions emitted from a nebulizer according to FIGS. 10a-10b.

Two ml of the test CD solution or Pulmicort suspension was accurately pipetted by volumetric pipettes into a clean nebulizer cup prior to starting each experiment. The test

25 nebulizer was assembled and charged with the test inhalation solution or suspension according to the manufacturer instructions. The end of the mouthpiece was placed at a height of approximately 18 cm from the platform of the MALVERN MASTERSIZER to the middle point of tip of the nebulizer mouthpiece. A vacuum source was positioned

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opposite the mouthpiece approximately 6 cm away to scavenge aerosol after sizing. The distance between the mouthpiece and the detector was approximately 8 cm. The center of the mouthpiece was level with the laser beam (or adjusted as appropriate, depending on the individual design of each nebulizer). The laser passed through the center of the emitted cloud when the nebulizer was running. Measurements were manually started 15 seconds into nebulization. Data collection started when beam obscuration reached 10% and was averaged over 15,000 sweeps (30 seconds). Scattered light intensity data on the detector rings was modeled using the "Standard-Wet" model. Channels 1 and 2 were killed due to low relative humidity during measurement to prevent beam steering. The volume diameter of droplets defining 10, 50 (volume median), and 90% of the cumulative volume undersize was determined. (Dv10 is the size below which 10% of the volume of material exists, Dv50 is the size below which 50% of the volume of material exists and Dv90 is the size below which 90% of the volume of material exists.

The procedure above may be practiced with slight modification on a MALVERN SPRAYTEC to determine the particle size of droplets emitted by a nebulizer.

EXAMPLE 13

Solutions of budesonide with and without SBE7- β -CD were prepared at two different pHs (4 and 6) and stored at 2 different temperatures (60 °C and 80 °C). Citrate buffers (50mM) at each pH value were prepared by mixing differing portions of 50mM citric acid and 50mM sodium citrate (tribasic, dihydrate) solutions. To achieve a concentration of budesonide in the buffers without SBE7- β -CD sufficient for accurate measurement, the budesonide was dissolved first in 100% ethyl alcohol. An aliquot of the ethanol/budesonide solution was then added drop-wise with stirring to each buffer solution. The theoretical budesonide concentration was 100 μ g/mL with a final ethanolic content of 5% in each buffer. All solution preps and procedures involving budesonide were done in a darkened room under red light. After shaking solutions for 24 hours, both buffer solutions were filtered through Millipore Millex-GV 0.22 μ m syringe filters to remove any solid that had precipitated (no significant amounts observed) from the solutions. The final budesonide concentration was about 50 μ g/mL. Both the pH 4 and 6 solutions were split in two, and solid SBE7- β -CD was added to one of the portions to create solutions with and without 1% w/v SBE7- β -CD at each pH. Each solution was aliquoted into individual amber vials. They were then placed in ovens at 60 °C and 80 °C.

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Sample vials were removed from the ovens and analyzed by HPLC at 0, 96, 164, and 288 hours. The HPLC assay conditions are summarized below.

Chromatographic Conditions

- 5 (Adapted from Hou, S., Hindle, M., and Byron, P. R. A. **Stability-Indicating HPLC Assay Method for Budesonide**. *Journal of Pharmaceutical and Biomedical Analysis*, 2001; 24: 371-380.)

Instrument:	PE Series 200
Column:	Phenomenex Luna C18(2) 4.6x150 mm 3um
Mobile Phase:	58% Phosphate Buffer pH 3.4/ 39.5% ACN / 2.5% MeOH
Mobile Phase Program:	100% A (isocratic)
Wavelength	240 nm
Flow Rate:	0.6 mL/min
Standard Range:	Seven standards - 1 to 500 µg/mL

EXAMPLE 14

- 10 Preparation and use of a combination solution containing SAE-CD, budesonide, and albuterol sulfate.

A budesonide solution is prepared per EXAMPLE 9 (mixing SAE-CD with the PULMICORT RESPULES suspension) and added to 3 ml of a solution containing 2.5 mg albuterol (The World Health Organization recommended name for albuterol base is
15 salbutamol) provided as albuterol sulfate. The albuterol solution is commercially available prediluted and sold under the name PROVENTIL[®] Inhalation Solution, 0.083%, or prepared from a commercially available concentrate 0.5% (sold under the names: PROVENTIL[®] Solution for inhalation and VENTOLIN[®] Inhalation Solution).

To provide the required dose for children 2 to 12 years of age, the initial dosing
20 should be based upon body weight (0.1 to 0.15 mg/kg per dose), with subsequent dosing titrated to achieve the desired clinical response. Dosing should not exceed 2.5 mg three to four times daily by nebulization. The appropriate volume of the 0.5% inhalation solution should be diluted in sterile normal saline solution to a total volume of 3 mL prior to administration via nebulization. To provide 2.5 mg, 0.5 mL of the concentrate is diluted to
25 3 mL with sterile normal saline. The albuterol aqueous solutions also contain benzalkonium chloride; and sulfuric acid is used to adjust the pH to between 3 and 5. Alternatively, an aqueous solution of an appropriate strength of albuterol may be prepared from albuterol sulfate, USP with or without the added preservative benzalkonium chloride and pH adjustment using sulfuric acid may also be unnecessary when combining with the

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corticosteroid solution. Furthermore the volume containing the appropriate dose of corticosteroid may be decreased four-fold as described in the following example allowing the total volume to be less and the time of administration to diminish accordingly.

EXAMPLE 15

5 Preparation and use of a combination solution containing SAE-CD, budesonide, and albuterol sulfate or levalbuterol HCl (XOPENEX).

A citrate buffer (3 mM pH 4.5) was prepared as follows. Approximately 62.5 mg of citric acid was dissolved in and brought to volume with water in one 100 ml volumetric flask. Approximately 87.7 mg of sodium citrate was dissolved in and brought to volume
10 with water in another 100 mL volumetric flask. In a beaker the sodium citrate solution was added to the citric acid solution until the pH was approximately 4.5.

Approximately 10.4 mg of budesonide and 1247.4 mg of Captisol were ground together with a mortar and pestle and transferred to a 10 mL flask. Buffer solution was added, and the mixture was vortexed, sonicated and an additional 1.4 mg budesonide
15 added. After shaking overnight, the solution was filtered through a 0.22 μ m Durapore Millex-GV Millipore syringe filter unit. The resulting budesonide concentration was ~1 mg/ml. Approximately 0.5 ml of the budesonide solution was added to a unit dose of either Proventil (2.5 mg/3 mL) or Xopenex (1.25 mg/3 mL) thereby forming the combination clear liquid dosage form of the invention. The resulting mixture remained
20 essentially clear for a period of at least 17 days at ambient room conditions protected from light.

EXAMPLE 16

Preparation and use of a combination solution containing SAE-CD, budesonide, and formoterol (FORADIL® (formoterol fumarate inhalation powder)).

25 The contents of one capsule containing 12 mcg of formoterol fumarate blended with 25 mg of lactose was emptied into a vial to which was added 3-mL of 3 mM citrate buffer (pH 4.5) prepared as described in the previous example. The contents of the vial were vortexed to dissolve the solids present. The budesonide concentrate was prepared as described in the previous example to provide a concentration of ~1 mg/mL.
30 Approximately 1 ml of the budesonide solution was added to the formoterol fumarate

buffered solution. The resulting solution remained essentially clear for a period of at least one month at room ambient conditions protected from light.

EXAMPLE 17

Clinical evaluation of a dosage form according to the invention was conducted by performing gamma scintigraphy analyses on subjects before and after administration of the dosage form by nebulization.

A single centre, four-way crossover gamma scintigraphy study to compare pulmonary delivery of budesonide via Pulmicort Respules®, and Captisol-Enabled® budesonide formulations using a Pari LC air-jet nebulizer was conducted. The purpose of the study was to determine, by gamma scintigraphy, the intra-pulmonary deposition of radiolabeled budesonide following nebulization of a budesonide suspension (Pulmicort Respules®, Astra Zeneca, reference formulation) and a Captisol®-Enabled budesonide solution (test formulation) in healthy male volunteers. During the course of the study, blood was collected periodically up to 24 hours, and the plasma was isolated. The concentration of budesonide in the plasma was determined using a validated HPLC MS-MS method in order to determine pharmacokinetic parameters. Dosing was conducted using a Pari LC Plus air-jet nebulizer. The use of gamma scintigraphy in conjunction with radiolabeled study drug and/or vehicle is the standard technique for the quantitative assessment of pulmonary deposition and clearance of inhaled drugs and/or vehicle.

The study dosage forms consisted of: 1) 1mg Budesonide as 2mL x 0.5mg/mL Pulmicort Respules®; or 2) 1 mg Budesonide as 2mL x 0.5mg/mL Pulmicort Respules to which 7.5% w/v Captisol® has been added.

Each subject received each of four study administrations of radiolabeled Budesonide in a non-randomized manner. A non-randomized design was utilized for this study since the reference formulation (Pulmicort Respule®) must be administered first to all subjects in order to determine the time to sputter (TTS). The TTS differed between subjects. For subsequent administrations the dose administered was controlled by the length of administration, expressed as a fraction of the time to sputter determined following administration of the reference formulation (i.e. 25% TTS, 50% TTS and 75% TTS). It was expected that even though the same concentration of budesonide would be nebulized for a shorter time, the amount of drug reaching the volunteers lungs would be essentially the same as the reference suspension for one of the legs of the study.

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Scintigraphic images were acquired using a gamma camera immediately after completion of dosing the volunteers.

Comparison of the image from the reference product and the 25% TTS leg indicated that a greater percentage of the budesonide from the Respule was in the stomach and throat immediately after administration. Thus a greater percentage of the budesonide reached the target lung tissue when Captisol was used to dissolve the budesonide. This could reduce undesirable side effects caused by the drug. One aspect of the method and dosage form of the invention thus provides an improved method of administering a corticosteroid suspension-based unit dose, the method comprising the step of adding a sufficient amount of SAE-CD to convert the suspension to a clear solution and then administering the clear solution to a subject. As a result, the method of the invention provides increased rate of administration as well as increased total pulmonary delivery of the corticosteroid as compared to the initial unit dose suspension formulation.

EXAMPLE 18

Comparative evaluation of various forms of SAE-CD in the solubilization of corticosteroid derivatives.

The solubility of beclomethasone dipropionate (BDP), beclomethasone 17-monopropionate (B17P), beclomethasone 21-monopropionate (B21P) and beclomethasone (unesterified) in solutions containing CAPTISOL and various SBE_nγ-CD was evaluated. BDP, B17P and B21P were obtained from Hovione. Beclomethasone was obtained from Spectrum Chemicals. CAPTISOL, SBE(3.4) γ-CD, SBE(5.23) γ-CD and SBE(6.1) γ-CD were provided by CyDex, Inc. (Lenexa, KS). γ-CD was obtained from Wacker Chemical Co. SBE(5.24) γ-CD and SBE(7.5) γ-CD were provided by the University of Kansas.

A 0.04M solution of each selected CD was prepared. Each form of beclomethasone required 2ml of CD solution, therefore the 0.04M solutions were prepared in 20 or 25 mL volumetric flasks in duplicate (N=2). The following table indicates the amount of each CD used after accounting for the content of water in each CD.

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CD	MW (g/mole)	mg of CD (volume)
SBE(6.7) β -CD	2194.6	2297.0 (25ml)
γ -CD	1297	1433.0 (25ml)
SBE(3.4) γ -CD	1834.9	1891.6 (25ml)
SBE(5.24) γ -CD	2119.5	1745.7 (20ml)
SBE(6.1) γ -CD	2261.9	1866.8 (20ml)
SBE(7.5) γ -CD	2483.3	2560.0 (25ml)

Beclomethasone forms were weighed in amounts in excess of the anticipated solubilities directly into 2-dram Teflon-lined screw-capped vials. These amounts typically provided approximately 6 mg/mL of solids. Each vial then received 2 ml of the appropriate CD solution. The vials were vortexed and sonicated for about 10 minutes to aid in wetting the solids with the fluid. The vials were then wrapped in aluminum foil to protect from light and placed on a lab quake for equilibration. The vials were visually inspected periodically to assure that the solids were adequately being wetted and in contact with the fluid. The time points for sampling were at 24 hrs for all samples and 72 hours for BDP only.

Solutions of SBE(6.1) γ -CD were prepared at 0.04, 0.08, and 0.1M and solutions of SBE (5.23) γ -CD were prepared at only 0.04 and 0.08M. Beclomethasone dipropionate was weighed in amounts in excess of the anticipated solubilities directly into 2-dram teflon-lined screw-capped vials. These amounts typically provided approximately 2 mg/mL of solids. Each vial then received 2 mL of the appropriate CD solution (N = 1). The vials were vortexed and sonicated for about 10 minutes to aid in wetting the solids with the fluid. The vials were then wrapped in aluminum foil to protect from light and placed on a lab quake for a five-day equilibration.

Solutions of γ -CD were prepared at 0.01 and 0.02M. Beclomethasone dipropionate was weighed in amounts in excess of the anticipated solubilities directly into 2-dram teflon-lined screw-capped vials. These amounts typically provided approximately 2 mg/mL of solids. Each vial then received 2 mLs of the γ -CD solution (N = 2). A solution was also prepared to measure the intrinsic solubility of BDP using HPLC grade water in place of the CD. The samples were wrapped in foil and placed on a lab quake for five days.

At the end of the equilibration time for each stage, the vials were centrifuged and 1 ml of the supernatant removed. The removed supernatant was then filtered using the

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Durapore PVDF 0.22 μ m syringe filter (discarded first few drops), and diluted with the mobile phase to an appropriate concentration within the standard curve. The samples were then analyzed by HPLC to determine concentration of solubilized corticosteroid.

EXAMPLE 19

5 Preparation and use of a combination solution containing SAE-CD, budesonide, and formoterol fumarate.

Formoterol fumarate dry powder is blended with Captisol dry powder which are both sized appropriately to provide for content uniformity at a weight ratio of 12 mcg formoterol fumarate/100 mg Captisol. An amount of powder blend corresponding to a
10 unit dose of formoterol fumarate is placed in a suitable unit dose container such as a HPMC capsule for later use or is added directly to a unit dose of Pulmicort Respules budesonide inhalation suspension (500 mcg / 2 mL), then mixed to achieve dissolution of all solids (a clear solution) and placed in the nebulizer reservoir for administration.

EXAMPLE 20

15 Preparation and use of a combination solution containing SAE-CD, budesonide, and ipratropium bromide.

A budesonide solution is prepared as per EXAMPLE 9 and added to a ipratropium bromide solution that is commercially available and sold under the name ATROVENT® Inhalation Solution Unit Dose. ATROVENT® (ipratropium bromide) Inhalation Solution
20 is 500 mcg (1 unit dose Vial) administered three to four times a day by oral nebulization, with doses 6 to 8 hours apart. ATROVENT® inhalation solution unit dose Vials contain 500 mcg ipratropium bromide anhydrous in 2.5 ml sterile, preservative-free, isotonic saline solution, pH-adjusted to 3.4 (3 to 4) with hydrochloric acid. Furthermore the volume containing the appropriate dose of corticosteroid may be decreased four-fold as
25 described in the above example (budesonide concentrate ~ 1 mg/mL) allowing the total volume to be less and the time of administration to diminish accordingly.

EXAMPLE 21

Evaluation of the AERONEB GO nebulizer versus a RAINDROP nebulizer with a solution comprising budesonide, aqueous liquid carrier and SAE-CD.

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The AERONEB GO nebulizer (AEROGEN Inc., Mountainview, California) is detailed in U.S. Pregrant Publication No. 2005-011514 to Power et al. (Application USSN 10/833,932 filed April 27, 2004), PCT International Publication No. WO 2005/009323 to Aerogen, Inc. et al. (PCT Application No. PCT/US2004/021268 filed July 6, 2004), and European Application No. EP 16426276, the entire disclosures of which are hereby incorporated by reference.

The RAINDROP nebulizer is available from Nellcor (Tyco Healthcare).

The solution of the invention used for this study was prepared according to Example 28.

Characterization of droplet size distribution of an aerosolized solution using a cascade impactor was determined according to Example 26.

Determination of total drug output and drug output rate from a nebulizer containing a liquid of the invention was determined according to Example 27.

EXAMPLE 22

Evaluation of the pulsating membrane nebulizer of U.S. Patent No. 6,962,151 with a solution comprising budesonide, aqueous liquid carrier and SAE-CD. Inertial Impaction Characterization of Tc^{99m} -DTPA Labelled *Captisol-Enabled*[®] Budesonide Aerosols Generated via a Pari Electronic Nebulizer

The nebulizer detailed in U.S. Patent No. 6,962,151, is also described in PCT International Application No. PCT/US00/29541 filed October 27, 2000, and U.S. Application Serial No. 11/269,783 filed November 7, 2005.

Aerosol characterization was conducted by standard *in vitro* inertial impaction tests using an Andersen Cascade Impactor (ACI). Technetium-99m (^{99m}Tc), in the form of diethylenetriaminepenta-acetic acid (DTPA, GE Healthcare), was added to the *Captisol-Enabled*[®] Budesonide Inhalation Solution (CEBUD). The suitability of ^{99m}Tc -DTPA to function as a surrogate for budesonide in CEBUD preparations was validated in the course of an earlier clinical trial (see Example 17). Preparation of the budesonide solution formulation for testing was conducted as per the description below. It was calculated that approximately 10MBq of ^{99m}Tc should be added to the budesonide formulation (11.05g) on the day of testing, in order to provide sufficient activity for *in vitro* imaging.

Two Pari electronic vibrating membrane nebulizers were used. At the core of this electronic nebulizer is a stainless steel membrane with thousands of laser drilled holes.

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Laser drilling allows flexibility to customize particle size, ensure reproducibility, and maintain a high output rate with smaller particles. The perforated membrane is vibrated at high frequencies in a resonant "bending" mode which yields high particle output rates. The nebulizer provides rapid drug delivery, efficiency, ideal particle sizing, low residual volume, and optimal performance matched to the inhaled drug formulation (See Rajiv Dhand, *Respiratory Care* 2002;(12): 1406 – 1416). Approximately 0.5 mL of drug solution was loaded and subsequently delivered via each nebulizer on 3 separate occasions. Runs 1, 3 and 5 were conducted with Device 1 and runs 2, 4 and 6 were conducted with Device 2.

10 *Pre-dose*

On each occasion, prior to dose delivery, the filled nebulizer was imaged for 60 seconds on Head I of the dual head gamma camera (Axis, Philips Medical Systems). Also, the nebulizers were weighed before and after addition of the budesonide formulation.

15 *Inertial impaction testing*

The nebulizer was positioned at the USP (United States Pharmacopoeia) inlet of the ACI and a flow rate of 28.3 L/min was drawn through the impactor using a vacuum pump. Flow through the impactor was started prior to activation of the electronic nebulizer. A stopwatch was used in order to measure the duration of dose delivery.

20 The ACI test conditions were the same as those used for Pari LC Plus air-jet nebulizer evaluation in the course of the earlier clinical study.

Following deposition the USP throat was removed from the ACI and imaged for 120 seconds. The collection plates were removed from the impactor and placed on Head I of the gamma camera and imaged for 120 seconds. The plates were subsequently washed and dried before conduct of further impaction tests.

25 *Post-dose*

On each occasion, the nebulizer weight was recorded after delivery of the dose. The nebulizer was imaged as described below.

30 *Image Processing*

A rectangular ROI was applied to image to the nebulizer pre-dose. This ROI was then re-applied to image the nebulizer after dose delivery.

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A rectangular ROI was also applied to the USP Inlet image.

A circular ROI was drawn around collection plate 0, copied and placed around plate 1. This was repeated for plates 2-7 and the filter. A rectangular ROI was also drawn to assess the background counts. Raw counts were corrected for background activity and
5 adjusted to counts per minute (cpm).

Aerosol performance is characterized in the table, in terms of the fine particle fraction (FPF) i.e. % emitted dose with a particle size < 5.8µm, mass median aerodynamic diameter (MMAD), geometric standard deviation (GSD) and the nebulization delivery time.

10 *CEBUD Preparation*

The expelled contents of five Pulmicort Respules (1mg/mL) were combined together. Captisol® (165 mg) on a dried basis was added per Respule used to the combined contents of the commercial suspension to provide a Captisol concentration of about 7.5 %w/v.

15 The mixture was vortexed briefly to disperse and dissolve the Captisol®. Then placed on a roller-bed mixer and allowed to mix for two-four or several hours. Aliquots of the equilibrated mixture were used to recover any budesonide retained in the original Respule container, and the recombined together. The mixture was the further equilibrated overnight (~20 hours) on the roller-bed mixer. After visually checking that all the
20 suspended solids had dissolved, the required volume of 99m Tc-D5PA / saline solution (provided by Medical Physical Department, UHW) was added. So about 180µl of the Radiolabel solution was added to the *Captisol-Enabled*® Budesonide Inhalation Solution and vortexed briefly.

EXAMPLE 23

25 Determination of the phase solubility curve for corticosteroid dissolution with SAE-CD.

The solubility of corticosteroid solutions containing SAECD was determined by HPLC chromatography of aliquots from equilibrated filtered or centrifuged corticosteroid solutions as follows.

30 SAE-CD/steroid solutions were prepared by weighing dry solids of SAE-CD (to provide 0.04 molar) and excess steroid drug (6 mg/mL) together into a screw-capped vial. A volume of water was aliquoted to each vial (separate vial for each steroid). Intrinsic

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solubility was determined by weighing excess steroid (6 mg/mL) and adding a volume of water in the absence of CD. Vials were capped, initially vortexed and sonicated. Vials were then placed on a roller-mixer (model: SRT2; Manufacturer: Stuart Scientific; Serial number: R000100052) or rocker/mixer (Model: LabQuake; Manufacturer: Barnstead/Thermolyne; Serial number: 1104010438202). Higher excesses of solid steroid (up to 10 mg/mL) were then added to any vial where the liquid contents clarified overnight (e.g. prednisolone, hydrocortisone, and prednisone). Samples were rolled and mixed on the roller or rocker for 72 hours. At various times during the equilibration, samples were additionally vortexed or sonicated briefly (up to 30 minutes). After the designated equilibration time, samples were filtered (0.22 μ m, 25 mm, Duropore – PVDF, manufacturer: Millipore) into clean vials except for the intrinsic solubility sample for Beclomethasone Dipropionate which was centrifuged and the supernatant transferred to a clean vial. Samples were analyzed by conventional HPLC methods.

EXAMPLE 24

Preparation of clear liquid formulations containing SAE-CD, budesonide and Salmeterol xinafoate. A formulation of this kind can be prepared according to other examples herein containing a combination of corticosteroid, SAE-CD and a second therapeutic agent.

EXAMPLE 25

Preparation of clear liquid formulations containing SAE-CD, budesonide and albuterol sulfate.

A stock solution containing CAPTISOL (40.0 g), water (1 L), citric acid (387.6 mg), sodium citrate (519.6 mg), EDTA (120 mg), and NaCl (6.36 mg) was prepared by mixing the components together.

Budesonide (37.5 mg) and albuterol sulfate (150 mg) were added to an aliquot (150 ml) of the stock solution and mixed on a roller mixer at ambient temperature until dissolution of all components. The final solution was clear, however, it could be filtered if needed. The final concentration of components in the formulation was as follows: CAPTISOL (0.018 M); budesonide (5.9 mM or 254 μ g/ml); and albuterol sulfate (1 mg/ml).

EXAMPLE 26

Characterization of droplet size distribution of an aerosolized solution using a cascade impactor.

The droplet size distribution of an aerosolized solution of the invention was characterized using an NGI cascade impactor. Approximately 0.5 mL of the 1000 µg/mL was placed into a nebulizer, such as the AERONEB GO. The vacuum pump associated with the impactor was turned on. The nebulizer mouthpiece was positioned into the center of the USP induction port and sealed to it with parafilm. The nebulizer was turned on until no more vapors were visible. Collection continued for an additional 45 seconds to ensure complete collection of the sample. Vacuum was turned off and the impactor disassembled. The collection cups at each stage were extracted with known volumes of HPLC mobile phase and assayed for budesonide. The cumulative amount of budesonide on each stage was quantified. The percent of drug exiting the nebulizer was 81%, and the percent of drug in the fine particle fraction (<5 µm) was 66%.

EXAMPLE 27

Determination of total drug output and drug output rate from a nebulizer containing a liquid of the invention.

A dose collection apparatus was constructed with a 300 ml glass filter unit of the type used to filter HPLC mobile phases. A specially fabricated Plexiglas lid covered the reservoir and had an opening to admit aerosol into the reservoir. The reservoir tapered towards a glass fiber filter supported by a metal mesh. The mesh was contained in a conical glass housing that terminated in a glass tube, which was attached to a flow controller and vacuum pump. The reservoir and filter support housing were clamped together. Two filters were sandwiched together in the apparatus to collect the emitted aerosol. This was necessary to avoid saturation of the filter, which can result in it tearing with subsequent drug loss into the vacuum, and changes in airflow rate over the course of aerosol collection. The end of nebulization was judged by intermittent aerosol output and/or a change in pitch of the nebulizer sound. To prevent loss of nebulized budesonide to the atmosphere, air was drawn into the filter faster than it was ejected from the nebulizers. By visually confirming that no aerosol escaped from the back of any nebulizer, a flow rate of $15 \pm 5\%$ l/min was deemed satisfactory. This was regulated by a TPK flow

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controller (Copley Instruments, 4312) and vacuum pump, and confirmed before each experiment using a calibrated flow meter.

EXAMPLE 28

Preparation of a liquid formulation comprising SAE-CD and budesonide,
5 optionally containing Tween.

A 3mM citrate buffer at pH 4.5 was added to 2 grams of CAPTISOL and 25 mg of budesonide in a serum vial to make the final volume 10 mL. The suspension was well mixed by vortexing and sonication. A 20% stock solution of CAPTISOL without budesonide was also prepared in 3mM citrate buffer. These mixtures, along with the
10 buffer were sealed in separate vials and autoclaved using the 20-minute hold at 121 °C cycle. HPLC analysis of the clear budesonide solution showed the concentration was 2100 µg/mL. The 20% CAPTISOL stock solution was used to dilute the sample to 2000 µg/mL. A portion of the above resulting solution was optionally diluted with an equal volume of the 3mM citrate buffer. HPLC analysis showed the final concentration was 990
15 µg of budesonide/mL.

The Tween could be added to the above solution as follows. A solution of 0.02% Tween was prepared with the autoclaved buffer only solution to form a Tween stock solution for use as a diluent for the above solutions. The dilutions for the 10% captisol/1 mg/mL budesonide were done by weight. Approximately 9 grams of the 20%
20 captisol/2000 µg/mL was mixed with ~9 grams of either the autoclaved buffer only solution or the autoclaved buffer/0.02% Tween solution. These solutions were well-mixed, filtered and reassayed by HPLC.

The budesonide concentrations of the above formulations were found to be 986 µg/mL for the solution without Tween and 962 µg/mL for the solution with Tween.

25 The solutions can be nebulized with any nebulizer; however, with an AERx nebulizer, an initial sample volume of 50 µl can be used. Administration of this solution with the nebulizer makes it feasible for a therapeutic dose to be administered to a subject in a single puff (a single full inhalation by a subject) via nebulization.

EXAMPLE 29

30 Preparation and dissolution of a lyophilized formulation comprising SAE-CD and budesonide.

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An excess of budesonide, 3.5 mg/mL, was added to 3L of 30% Captisol in 3 mM citrate buffer containing 0.1 mg/mL EDTA. After mixing for 2 days, an additional 1 mg/mL budesonide was added and equilibrated an additional 4 days. The preparation was filtered through a 0.22 μ Durapore filter and placed in three stainless steel trays in a freeze
5 dryer. The solution was frozen at -30 °C for one hour and lyophilized over 30 hours to remove essentially all the water. The lyophile was powdered, screened and the powder transferred to a plastic bottle. The final composition contained 8.2 mg budesonide per gram of powder.

When approximately 65 mg of powder was added to 2 mL of water, an essentially
10 clear solution containing the same amount of budesonide as in the reference suspension product was rapidly obtained.

EXAMPLE 30

Preparation of an aqueous liquid formulation comprising SAE-CD, ethanol and budesonide.

15 Captisol/Ethanol solutions were prepared by making a stock captisol solution at 22.2% (~0.1 M) w/v which was diluted with either ethanol or water in varying amounts to create four solutions of 0, 1, 2, 5 % ethanol and about 20% w/v Captisol. Captisol/Ethanol/Budesonide solutions were prepared by adding dry Budesonide (2.5 mg/mL) to a volume of the prepared Captisol/ethanol solutions and then these were
20 equilibrated on a Labquake for 72 hours. These solutions were filtered (Duropore syringe filters) and analyzed by HPLC to determine the concentration (μ g/ml) of budesonide dissolved in the formulation.

EXAMPLE 31

An in-vivo evaluation of a dosage form according to the invention was conducted
25 by performing a pharmacokinetic analysis on budesonide after administration of the dosage form by nebulization.

A four dog crossover study to compare pulmonary delivery of budesonide via Pulmicort Respules®, and a Captisol-Enabled® budesonide formulation using a Pari LC air-jet nebulizer was conducted. The purpose of the study was to determine, by HPLC-
30 MS/MS analysis, the pharmacokinetics of budesonide the plasma, following nebulization of a budesonide suspension (Pulmicort Respules®, AstraZeneca, reference formulation)

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and a Captisol-Enabled® budesonide inhalation solution (test formulation) in healthy dogs. Dosing was conducted using a Pari LC Star air-jet nebulizer and anesthesia masks.

The study dosage forms consisted of: 1) 1 mg Budesonide as two 2 mL x 0.25 mg/mL Pulmicort Respules®; or 2) 1 mg Budesonide as two 2 mL x 0.25 mg/mL Pulmicort Respules to which 5% w/v Captisol® was added.

Prior to dosing, two capsules containing activated charcoal followed with approximately 30 mL of water were administered to each dog to block oral absorption of the budesonide. Each dog received each of the study administrations one week apart in a randomized manner. Administration time was the same for each formulation (23.9 min. for test formulation and 23.4 min. for reference). Blood was collected periodically up to 8 hours, the plasma was isolated and stored at -70 °C until analysis. After each administration, the nebulizer and test solution container were assayed for residual budesonide to determine the amount of drug delivered.

EXAMPLE 32

To investigate how the incorporation of Captisol at 5% w/v into Pulmicort Respules impact performance of different types of nebulizers.

The emitted dose of budesonide from four different nebulizers (PARI LC PLUS (air jet), OMRON MICROAIR NE-U22, AIRSEP MYSTIQUE (ultrasonic), AEROGEN AERONEB) was determined. The package insert-approved Pari air jet system was used as the benchmark to judge performance of the other nebulizers. The emitted dose was from 1.25 to 3.7 times higher when Captisol was added to the budesonide suspension. The Emitted dose (ED) was determined by:

- 1) Drawing the nebulized formulations through a 300 mL glass filter apparatus at 15 l/min, and collecting drug on double or triple layers of glass fiber depth filter and the interior walls. Collection was stopped every two minutes, the budesonide quantitatively recovered, and filters were changed to prevent filter saturation or alterations in airflow. Budesonide recovery was quantified by HPLC; and/or
- 2) Summing the amount of budesonide on the cascade impactor stages after nebulization.

The results are detailed below. (ND means “not determined.”)

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Formulation	Total Delivered (ED) (μg , mean & SD), Filter ¹	Total Delivered (ED) (μg , mean & SD), Impactor ²
Pari LC Plus (Air Jet) Listed in the Pulmicort package insert		
Pulmicort	171.5 $\pm$ 6.3	137.8 $\pm$ 14.9
Pulmicort + 5% Captisol	247.4 $\pm$ 11.3	172.4 $\pm$ 6.6
Omron MicroAir NE-U22		
Pulmicort	179.9 $\pm$ 17.2	168.8 $\pm$ 30.1
Pulmicort + 5% Captisol	380.1 $\pm$ 8.5	349.6 $\pm$ 10.0
AirSep Mystique (Ultrasonic)		
Pulmicort	32.9 $\pm$ 6.4	ND*
Pulmicort + 5% Captisol	120.8 $\pm$ 19.6	ND*
Aerogen AeroNeb		
Pulmicort	90.7 $\pm$ 4.5	ND*
Pulmicort + 5% Captisol	301.2 $\pm$ 19.5	ND*

All documents cited herein are each incorporated by reference herein in its entirety. The above is a detailed description of particular embodiments of the invention. It will be appreciated that, although specific embodiments of the invention have been described

5 herein for purposes of illustration, various modifications may be made without departing from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims. All of the embodiments disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure.

CLAIMS

1. An aqueous liquid formulation comprising a therapeutically effective amount of corticosteroid dissolved therein, SAE-CD, and an aqueous liquid carrier, wherein the molar ratio of SAE-CD to corticosteroid is greater than 10:1.
- 5 2. The formulation of claim 1, wherein the molar ratio of SAE-CD to corticosteroid required by the corticosteroid at the saturated solubility of the corticosteroid in the presence of SAE-CD is greater than 10:1.
3. The formulation of claim 2, wherein the corticosteroid is at least as lipophilic as or more lipophilic than flunisolide.
- 10 4. The formulation of claim 3, wherein the corticosteroid is selected from the group consisting of beclomethasone, beclomethasone dipropionate, beclomethasone monopropionate, budesonide, ciclesonide, desisobutyryl-ciclesonide, flunisolide, fluticasone, fluticasone propionate, fluticasone furoate, mometasone, mometasone furoate, icomethasone enbutate, tixocortol 21-pivalate, and triamcinolone acetonide.
- 15 5. The formulation of claim 3, wherein the corticosteroid is selected from the group consisting of beclomethasone dipropionate, beclomethasone monopropionate, budesonide, ciclesonide, desisobutyryl-ciclesonide, flunisolide, fluticasone propionate, fluticasone furoate, mometasone furoate, icomethasone enbutate, tixocortol 21-pivalate, and triamcinolone acetonide.
- 20 6. The formulation of claim 3, wherein the corticosteroid is present at a concentration that is less than its saturated solubility as determined in the presence of SAE-CD.
7. The formulation of claim 6, wherein the molar ratio of SAE-CD to corticosteroid in the composition or formulation is at least 1% greater than the molar ratio at the saturated solubility of the corticosteroid as determined in the presence of SAE-CD.
- 25 8. The formulation of claim 7, wherein the molar ratio of SAE-CD to corticosteroid is in the range of greater than 10:1 to about 10,000:1 or in the range of greater than 10:1 to about 1000:1.
9. The formulation of claim 7, wherein the molar ratio of SAE-CD to corticosteroid at the saturated solubility of the corticosteroid in the presence of SAE-CD is at least 14:1.

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10. The formulation of claim 9, wherein the formulation is a substantially clear solution comprising less than 5% wt. undissolved corticosteroid.
11. The formulation of claim 10 comprising $21.5 \pm 2\%$ wt./wt. or less of SAE-CD.
12. The formulation of claim 2, wherein the corticosteroid is present at a concentration
5 that is less than its saturated solubility as determined in the presence of SAE-CD.
13. The formulation of claim 12, wherein the corticosteroid is present at a concentration that is 95% or less of its saturated solubility as determined in the presence of SAE-CD.
14. The formulation of claim 2, wherein the molar ratio of SAE-CD to corticosteroid in the composition or formulation is at least 1% greater than the molar ratio at the saturated
10 solubility of the corticosteroid as determined in the presence of SAE-CD.
15. The formulation of claim 14, wherein the molar ratio of SAE-CD to corticosteroid in the composition or formulation is at least 5% greater than the molar ratio at the saturated solubility of the corticosteroid as determined in the presence of SAE-CD.
16. The formulation of claim 1, wherein the formulation is a substantially clear solution
15 comprising less than 5% wt. undissolved corticosteroid.
17. The formulation of claim 1, wherein the molar ratio of SAE-CD to corticosteroid at the saturated solubility of the corticosteroid in the presence of SAE-CD is at least 14:1.
18. The formulation of claim 1, wherein the aqueous liquid carrier comprises water, buffer, alcohol, organic solvent, glycerin, poly(ethylene glycol), poloxamer, surfactant
20 or a combination thereof.
19. The formulation of claim 1 comprising $21.5 \pm 2\%$ wt./wt. or less of SAE-CD.
20. The formulation of claim 1, wherein the molar ratio of SAE-CD to corticosteroid is in the range of greater than 10:1 to about 10,000:1 or in the range of greater than 10:1 to about 1,000:1.
- 25 21. The formulation of claim 20, wherein the molar ratio of SAE-CD to corticosteroid is in the range of greater than 10:1 to about 333:1, from $> 10:1$ to about 1000:1, about from $> 10:1$ to about 100:1, from $> 10:1$ to about 50:1, from $> 10:1$ to about 30:1, or from $> 10:1$ to about 500:1.

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22. The formulation of claim 1, wherein the corticosteroid excludes any corticosteroid having a lipophilicity less than that of flunisolide.
23. The formulation of claim 22 excluding hydrocortisone, prednisolone, prednisone, dexamethasone, betamethasone, methylprednisolone, triamcinolone, and
5 fluocortolone.
24. The formulation of claim 1, wherein the corticosteroid is selected from the group consisting of aldosterone, beclomethasone, betamethasone, budesonide, ciclesonide, cloprednol, cortivazol, deoxycortone, desonide, desoximetasone, dexamethasone, difluorocortolone, fluclorolone, flumethasone, flunisolide, fluocinolone, fluocinonide,
10 fluocortin butyl, fluorocortisone, fluorocortolone, fluorometholone, flurandrenolone, fluticasone, halcinonide, icomethasone, mometasone, paramethasone, rofleponide, RPR 106541, tixocortol, triamcinolone, and their respective pharmaceutically acceptable derivatives.
25. The formulation of claim 1, wherein the corticosteroid possesses an intrinsic solubility
15 in water that approximates or is less than the intrinsic solubility of flunisolide in water.
26. The formulation of claim 1, wherein the SAE-CD is present in an amount sufficient to solubilize at least 90% of the corticosteroid.
27. The formulation of claim 1, wherein the SAE-CD is present in an amount sufficient to solubilize at least 95% of the corticosteroid.
- 20 28. The formulation of claim 1, wherein the SAE-CD is present at a concentration of about 10 mg to about 500 mg of SAE-CD per ml of formulation.
29. The formulation of claim 1, wherein the formulation has a shelf-life of at least 6 months.
30. The formulation of claim 1 further comprising a conventional preservative, an
25 antioxidant, a buffering agent, an acidifying agent, a solubilizing agent, a colorant, a complexation enhancing agent, saline, an electrolyte, another therapeutic agent, an alkalizing agent, a tonicity modifier, surface tension modifier, viscosity modifier, density modifier, volatility modifier, antifoaming agent, flavor, sweetener, hydrophilic polymer, surfactant or a combination thereof.

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31. The formulation of claim 1 further comprising one or more therapeutic agents independently selected at each occurrence from the group consisting of a β_2 -adrenoreceptor agonist, a dopamine (D_2) receptor agonist, a topical anesthetic, an anticholinergic agent, IL-5 inhibitor, antisense modulator of IL-5, milrinone (1,6-dihydro-2-methyl-6-oxo-[3,4'-bipyridine]-5-carbonitrile); milrinone lactate; tryptase inhibitor, tachykinin receptor antagonist, leukotriene receptor antagonist, 5-lipoxygenase inhibitor, and anti-IgE antibody.
32. The formulation of claim 31, wherein the β_2 -adrenoreceptor agonist is selected from the group consisting of Albuterol (α^1 -(((1,1-dimethylethyl)amino)methyl)-4-hydroxy-1,3-benzenedimethanol); Bambuterol (dimethylcarbamic acid 5-(2-((1,1-dimethylethyl)amino)-1-hydroxyethyl)-1,3-phenylene ester); Bitolterol (4-methylbenzoic acid 4-(2-((1,1-dimethylethyl)amino)-1-hydroxyethyl)-1,2-phenyleneester); Broxaterol (3-bromo- α -(((1,1-dimethylethyl)amino)methyl)-5-isoxazolemethanol); Isoproterenol (4-(1-hydroxy-2-((1-methylethyl)amino)ethyl)-1,2-benzene-diol); Trimetoquinol (1,2,3,4-tetrahydro-1-((3,4,5-trimethoxyphenyl)-methyl)-6,7-isoquinolinediol); Clenbuterol (4-amino-3,5-dichloro- α -(((1,1-dimethylethyl)amino)methyl)benzenemethanol); Fenoterol (5-(1-hydroxy-2-((2-(4-hydroxyphenyl)-1-methylethyl)amino)ethyl)-1,3-benzenediol); Formoterol (2-hydroxy-5-((1R)-1-hydroxy-2-(((1R)-2-(p-methoxyphenyl)-1-methylethyl)amino)ethyl) formanilide); (R,R)-Formoterol; Desformoterol ((R,R) or (S,S)-3-amino-4-hydroxy- α -(((2-(4-methoxyphenyl)-1-methylethyl)amino)methyl)benzenemethanol); Hexoprenaline (4,4'-(1,6-hexane-diyl)-bis(imino(1-hydroxy-2,1-ethanediyl)))bis-1,2-benzenediol); Isoetharine (4-(1-hydroxy-2-((1-methylethyl)amino)butyl)-1,2-benzenediol); Isoprenaline (4-(1-hydroxy-2-((1-methylethyl)amino)ethyl)-1,2-benzenediol); Meta-proterenol (5-(1-hydroxy-2-((1-methylethyl)amino)ethyl)-1,3-benzenediol); Picumeterol (4-amino-3,5-dichloro- α -(((6-(2-(2-pyridinyl)ethoxy)hexyl)-amino)methyl)benzenemethanol); Pirbuterol (α^6 -(((1,1-dimethylethyl)-amino)methyl)-3-hydroxy-2,6-pyridinemethanol); Procaterol (((R*,S*)-(+,-)-8-hydroxy-5-(1-hydroxy-2-((1-methylethyl)amino)butyl)-2(1H)-quinolin-one); Reproterol ((7-(3-((2-(3,5-dihydroxyphenyl)-2-hydroxyethyl)amino)-propyl)-3,7-dihydro-1,3-dimethyl-1H-purine-2,6-dione); Rimiterol (4-(hydroxy-2-piperidinylmethyl)-1,2-benzenediol); Salbutamol ((+,-)-

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- alpha¹-(((1,1-dimethylethyl)amino)methyl)-4-hydroxy-1,3-benzenedimethanol); (R)-Salbutamol; Salmeterol ((+/-)-4-hydroxy-.alpha.¹-(((6-(4-phenylbutoxy)hexyl)-amino)methyl)-1,3-benzenedimethanol); (R)-Salmeterol; Terbutaline (5-(2-((1,1-dimethylethyl)amino)-1-hydroxyethyl)-1,3-benzenediol); Tulobuterol (2-chloro-.alpha.-(((1,1-dimethylethyl)amino)methyl)benzenemethanol); and TA-2005 (8-hydroxy-5-((1R)-1-hydroxy-2-(N-((1R)-2-(4-methoxyphenyl)-1-methylethyl)amino)ethyl)carbostyryl hydrochloride).
33. The formulation of claim 31, wherein the dopamine (D2) receptor agonist is selected from the group consisting of Apomorphine ((r)-5,6,6a,7-tetrahydro-6-methyl-4H-dibenzo[de,gl]quinoline-10,11-diol); Bromocriptine ((5'.alpha.)-2-bromo-12'-hydroxy-2'-(1-methylethyl)-5'-(2-methylpropyl)ergotaman-3',6',18-trione); Cabergoline ((8.beta.)-N-(3-(dimethylamino)propyl)-N-((ethylamino)carbonyl)-6-(2-propenyl)ergoline-8-carboxamide); Lisuride (N'-((8-alpha)-9,10-di-dehydro-6-methylergolin-8-yl)-N,N-diethylurea); Pergolide ((8-beta)-8-((methylthio)methyl)-6-propylergoline); Levodopa (3-hydroxy-L-tryrosine); Pramipexole ((s)-4,5,6,7-tetrahydro-N.sup.6-propyl-2,6-benzothiazolediamine); Quinpirole hydrochloride (trans-(-)-4aR-4,4a,5,6,7,8,8a,9-octahydro-5-propyl-1H-pyrazolo[3,4-g]quinoline hydrochloride); Ropinirole (4-(2-(dipropylamino)ethyl)-1,3-dihydro-2H-indol-2-one); and Talipexole (5,6,7,8-tetrahydro-6-(2-propenyl)-4H-thiazolo[4,5-d]azepin-2-amine).
34. The formulation of claim 31, wherein the anticholinergic agent is selected from the group consisting of ipratropium bromide, oxitropium bromide, atropine methyl nitrate, atropine sulfate, ipratropium, belladonna extract, scopolamine, scopolamine methobromide, homatropine methobromide, hyoscyamine, isopripramide, orphenadrine, benzalkonium chloride, tiotropium bromide and glycopyrronium bromide.
35. The formulation of claim 31 wherein the topical anesthetic is selected from the group consisting of lidocaine, an N-arylamide, an aminoalkylbenzoate, prilocaine, and etidocaine.
36. The formulation of claim 31 comprising:

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a corticosteroid selected from the group consisting of beclomethasone dipropionate, beclomethasone monopropionate, budesonide, ciclesonide, desisobutryl-ciclesonide, flunisolide, fluticasone propionate, fluticasone furoate, mometasone furoate, icomethasone enbutate, tixocortol 21-pivalate, and triamcinolone acetonide; and

a therapeutic agent selected from the group consisting of salmeterol, formoterol, albuterol, ipatropium, a pharmaceutically acceptable salt thereof, and an isomer thereof.

37. The formulation of claim 31, wherein the corticosteroid is present in a molar excess over the other therapeutic agent.

38. The formulation of claim 31, wherein the other therapeutic agent is present in a molar excess over the corticosteroid.

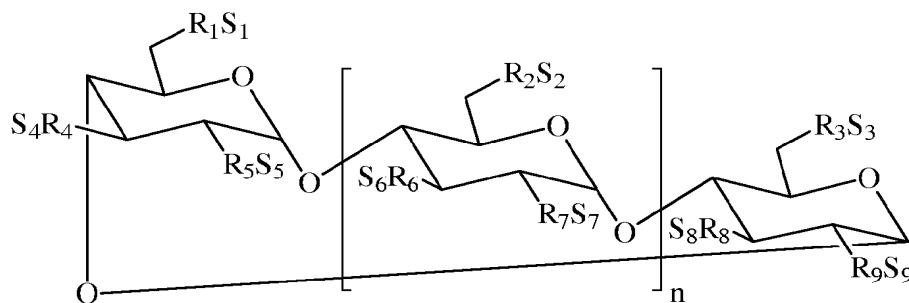
39. The formulation of claim 31, wherein the SAE-CD is present in a molar excess over the other therapeutic agent.

40. The formulation of claim 31, wherein the SAE-CD has a higher equilibrium binding constant for the corticosteroid than for the other therapeutic agent.

41. The formulation of claim 31, wherein the SAE-CD has a higher equilibrium binding constant for the other therapeutic agent than for the corticosteroid.

42. The formulation of claim 31, wherein the SAE-CD has an equilibrium binding constant approximating that for the other therapeutic agent.

43. The invention according to any one of claims 1-42, 46-110 or 111, wherein the cyclodextrin is a compound of the Formula 1:



Formula 1

wherein:

n is 4, 5 or 6;

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R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈ and R₉ are each, independently, -O- or a-O-(C₂ - C₆ alkylene)-SO₃⁻ group, wherein at least one of R₁ - R₉ is independently a -O-(C₂ - C₆ alkylene)-SO₃⁻ group, a -O-(CH₂)_mSO₃⁻ group wherein m is 2 to 6, -OCH₂CH₂CH₂SO₃⁻, or -OCH₂CH₂CH₂CH₂SO₃⁻; and

5 S₁, S₂, S₃, S₄, S₅, S₆, S₇, S₈ and S₉ are each, independently, a pharmaceutically acceptable cation.

44. The invention according to any one of claims 1-42, 46-110 or 111, wherein the cyclodextrin is a compound of the Formula II (SAEx-α-CD), wherein "x" ranges from 1 to 18; of the Formula III (SAEy-β-CD), wherein "y" ranges from 1 to 21; or of the
10 Formula IV (SAEz-γ-CD), wherein "z" ranges from 1 to 24, and wherein "SAE" represents a sulfoalkyl ether substituent, and the values "x", "y" and "z" represent the average degree of substitution in terms of the number of sulfoalkyl ether groups per CD molecule.

45. The invention according to claim 44, wherein the cyclodextrin is selected from the
15 group consisting of:

SAEx-α-CD	SAEy-β-CD	SAEz-γ-CD
SEEx-α-CD	SEEy-β-CD	SEEz-γ-CD
SPEx-α-CD	SPEy-β-CD	SPEz-γ-CD
SBEx-α-CD	SBEy-β-CD	SBEz-γ-CD
SPtEx-α-CD	SPtEy-β-CD	SPtEz-γ-CD
SHEx-α-CD	SHEy-β-CD	SHEz-γ-CD.

46. A solid composition prepared by drying a formulation according to claim 1.

47. A liquid formulation according to claim 1, wherein the formulation is adapted for nasal, oral, ophthalmic, otic or topical administration.

20 48. A method of treating a disease or disorder of the airways, in a subject in need thereof, comprising administering via inhalation a liquid formulation comprising an aqueous liquid carrier, a sulfoalkyl ether cyclodextrin, and a therapeutically effective amount of corticosteroid dissolved therein, the corticosteroid being present in an amount sufficient to provide a mean plasma AUC_t of 160-1600 pg*h/ml, wherein the molar
25 ratio of SAE-CD to corticosteroid is greater than 10:1.

49. The method of claim 48, wherein the therapeutically effective amount comprises 45-1000 μg of corticosteroid.

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50. The method of claim 49, wherein the molar ratio of SAE-CD to corticosteroid required by the corticosteroid at the saturated solubility of the corticosteroid in the presence of SAE-CD is greater than 10:1.
51. The method of claim 50, wherein the liquid formulation is administered via nebulization.
52. The method of claim 51, wherein the formulation comprises less than or about 5 ml of aqueous liquid carrier.
53. The method of claim 52, wherein the formulation comprises 0.05 to 2 ml of aqueous liquid carrier.
54. The method of claim 51, wherein the liquid formulation is administered with an ultrasonic nebulizer, air jet nebulizer, electronic nebulizer, vibrating membrane nebulizer, vibrating mesh nebulizer, forced carrier/perforated disc combination nebulizer, vibrating plate nebulizer, a nebulizer comprising a vibration generator and an aqueous chamber, and a nebulizer comprising a nozzle array.
55. The method of claim 50, wherein the corticosteroid is selected from the group consisting of beclomethasone dipropionate, beclomethasone monopropionate, budesonide, ciclesonide, desisobutyl-ciclesonide, flunisolide, fluticasone propionate, fluticasone furoate, mometasone furoate, icomethasone enbutate, tixocortol 21-pivalate, and triamcinolone acetonide.
56. A method of providing a mean plasma AUC_t of 150-1600 pg*h/ml for the corticosteroid in an individual subject comprising: administering to the subject, via nebulization, 48-220 µg, as dose to subject, of a corticosteroid dissolved in an aqueous liquid carrier comprising sulfoalkyl ether cyclodextrin.
57. The method of claim 56, wherein nebulization is conducted with a nebulizer selected from the group consisting of an air jet nebulizer, ultrasonic nebulizer, electronic nebulizer, vibrating membrane nebulizer, vibrating mesh nebulizer, vibrating plate nebulizer, a nebulizer comprising a vibration generator and an aqueous chamber, and a nebulizer comprising a nozzle array.
58. The method of claim 57, wherein the corticosteroid is budesonide.

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59. A method of providing in a subject a mean plasma AUC_t, normalized for dose of corticosteroid to subject, of at least 6 (pg*h/ml)/μg of corticosteroid delivered, as dose to subject, comprising: administering to the subject, via nebulization, at least 45 μg of corticosteroid dissolved in an aqueous liquid carrier comprising sulfoalkyl ether cyclodextrin.
60. The method of claim 59, wherein nebulization is conducted with a nebulizer selected from the group consisting of an air jet nebulizer, ultrasonic nebulizer, electronic nebulizer, vibrating membrane nebulizer, vibrating mesh nebulizer and vibrating plate nebulizer.
61. The method of claim 60, wherein the unit dose is from 45 – 1000 μg.
62. The method of claim 60, wherein the corticosteroid is budesonide.
63. A method of providing in a subject a mean AUC_i, normalized for dose of corticosteroid to subject, of at least 8 (pg*h/ml)/μg of corticosteroid delivered, as dose to subject, comprising: administering to the subject, via nebulization, at least 45 μg of corticosteroid dissolved in an aqueous liquid carrier comprising sulfoalkyl ether cyclodextrin.
64. The method of claim 63, wherein nebulization is conducted with a nebulizer selected from the group consisting of an air jet nebulizer, ultrasonic nebulizer, electronic nebulizer, vibrating membrane nebulizer, vibrating mesh nebulizer and vibrating plate nebulizer.
65. The method of claim 64, wherein the corticosteroid is budesonide.
66. The method of claim 64, wherein the unit dose is from 45-1000 μg.
67. The method of claim 59, wherein the method provides a two- to less than four-fold increase in the mean plasma AUC_t per μg of corticosteroid delivered to a subject as compared to administration via nebulization of a reference unit dose aqueous suspension-based liquid formulation.
68. The method of claim 59, wherein the method provides a 1.5 to 5-fold increase in the individual plasma AUC_t per μg of corticosteroid delivered to a subject as compared to administration via nebulization of a reference unit dose aqueous suspension-based liquid formulation.

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69. The method of claim 63, wherein the method provides a two- to less than four-fold increase in the mean plasma AUCi per μg of corticosteroid delivered to a subject as compared to administration via nebulization of a reference unit dose aqueous suspension-based liquid formulation.

5 70. The method of claim 63, wherein the method provides a 1.5 to 5-fold increase in the individual plasma AUCi per μg of corticosteroid delivered to a subject as compared to administration via nebulization of a reference unit dose aqueous suspension-based liquid formulation.

10 71. A method of treating a disease or disorder of the airways, in a subject in need thereof, comprising administering or delivering, via inhalation, to the subject an aqueous liquid formulation comprising an aqueous liquid carrier, a sulfoalkyl ether cyclodextrin, and a dose of corticosteroid dissolved therein, wherein the formulation provides an enhanced pharmacokinetic profile over a suspension based formulation administered under similar conditions.

15 72. A method of administering or delivering a dose of corticosteroid to the air passageways of a subject in need thereof, the method comprising administering or delivering the corticosteroid with a nebulizer comprising a charge of an aqueous liquid formulation, the formulation comprising an aqueous liquid carrier, sulfoalkyl ether cyclodextrin, and the corticosteroid dissolved therein, wherein, during operation, the
20 system provides enhanced drug delivery, increased rate of drug administration, reduced treatment time, reduced toxicity, improved stability, enhanced bioabsorption, increased output rate, increased total output, enhanced pharmacokinetic profile, reduced corticosteroid-related side effects, enhanced pulmonary deposition, reduced oropharyngeal deposition, and/or improved nebulization performance over another
25 system comprising the nebulizer and a suspension based formulation of the corticosteroid.

30 73. The method of claim 71, wherein the formulation provides enhanced drug delivery, increased rate of drug administration, reduced treatment time, reduced toxicity, improved stability, enhanced bioabsorption, increased output rate, increased total output, enhanced pharmacokinetic profile, reduced corticosteroid-related side effects, enhanced pulmonary deposition, reduced oropharyngeal deposition, and/or improved

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nebulization performance over a suspension based formulation administered under similar conditions.

74. The method of claim 71 or 72, wherein the dose of corticosteroid is at least 25 μ g, 40 μ g, at least 45 μ g, at least 48 μ g, 45-1000 μ g, about 1 μ g to 20 mg, or 1 μ g to 10 mg, 0.01 mg to 10 mg, 0.025 mg to 10 mg, 0.05 mg to 5 mg, 0.1 mg to 5 mg, 0.125 mg to 5 mg, 0.25 mg to 5 mg, 0.5 mg to 5 mg, 0.05 mg to 2 mg, 0.1 mg to 2 mg, 0.125 mg to 2 mg, 0.25 mg to 2 mg, 0.5 mg to 2 mg, 1 μ g, 10 μ g, 25 μ g, 50 μ g, 100 μ g, 125 μ g, 200 μ g, 250 μ g, 25 to 66 μ g, 48 to 81 μ g, 73 to 125 μ g, 40 μ g, 64 μ g, 95 μ g, 35 to 95 μ g, 25 to 125 μ g, 60 to 170 μ g, 110 μ g, 170 μ g, 45 to 220 μ g, 45 to 85 μ g, 48 to 82 μ g, 85 to 160 μ g, 140 to 220 μ g, 120 to 325 μ g, 205 μ g, 320 μ g, 325 μ g, 90 to 400 μ g, 95 to 170 μ g, 165 to 275 μ g, or 275 to 400 μ g.
75. The method of claim 71 or 72, wherein the liquid has a volume of 10 μ l to 100 ml, 50 μ l to 50 ml, 50 μ l to 10 ml, 0.1 to 10 ml, 0.1 ml to less than 10 ml, 0.1 ml to 7.5 ml, 0.1 ml to 5 ml, 0.1 ml to 3 ml, 0.1 ml to 2 ml, 0.1 ml to 1 ml, 0.05 ml to 7.5 ml, 0.05 ml to 5 ml, 0.05 ml to 3 ml, 0.05 ml to 2 ml, or 0.05 ml to 1 ml.
76. The method of claim 71 or 72, wherein the step of delivering or administering is conducted over a period of less than 30 min, less than 20 min, less than 10 min, less than 7 min, less than 5 min, less than 3 min, or less than 2 min, or the time is about 0.05 to 10 min, about 0.1 to 5 min, about 0.1 to 3 min, about 0.1 to 2 min, about 0.1 to 1.5 min, about 0.5 min to about 1.5 min, or about 1 min, or the time is about the time it takes for a subject to take a single breath (about 1 to 3 or 1 to 5 sec).
77. The method of claim 71 or 72, wherein the enhanced pharmacokinetic profile is an enhancement of 1.5 to 8 fold higher, 1.5 to 6 fold higher, 1.5 to 4 fold higher, 1.5 to 2 fold higher, 1.64 to 3.55 fold higher for C_{max} , 1.48 to 6.25 fold higher for C_{max} , 1.59 to 3.55 or 1.19 to 6.11 fold higher for AUC_{inf} , 1.69-3.67 or 1.21 to 7.66 fold higher for AUC_{last} as compared to administration of the suspension formulation.
78. The method of claim 71 or 72, wherein 20% to 85%, or 30% to 80%, or at least 30%, at least 40%, at least 56%, at least 59%, at least 62% of the dose of corticosteroid is delivered to the lungs of the subject.

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79. The method of claim 71 or 72, wherein the method provides in the subject a $C_{\max}$ (pg of corticosteroid/ml) of 90 to 900, 200 to 600, 200 to 550, 200 to 250, 400 to 450, 500 to 600, 225, 437, or 545 on a dose non-normalized basis.
80. The method of claim 71 or 72, wherein the method provides in the subject a dose normalized $C_{\max}$ (pg/ml/ μ g) of: 1) 0.3 to 2, 0.35 to 2, 0.6 to 1.5, 0.5 to 1.2, 0.8 to 1, 0.8 to 0.9, 0.7 to 0.8, 0.4, 1.9, 0.6, 1.5, 0.5, 1.2, 0.35, 2, 0.7, 0.8, or 0.9 on a nominal available dose normalized basis; 2) 3.4 to 9.2, 3.5 to 8.5, 5.5 to 9.2, 4.5 to 7.5, 5.8 to 7, 3.4, 3.5, 4.5, 5.5, 9.2, 8.5, 7.5, 5.8, 5.9, 6, or 7 on a dose to lung normalized basis; 3) 1.7 to 7.5, 3.2 to 4.1, 1.9 to 6, 3.2 to 7.5, 1.7 to 5.2, 3.6, 4.1, 3.2, 1.9, 6, 3.2, 7.4, 7.5, 1.7, 5.2 or 5.3 on a dose to subject normalized basis; 4) 0.9 to 3.3, 1.7 to 2.2, 0.9 to 3, 1 to 3, 1.7 to 3.3, 1 to 2.7, 1.9, 2.1, 2.2, 1.7, 0.9, 1, 2, 3, 2.9, 3.2, 3.3, or 2.7 on an emitted dose normalized basis.
81. The method of claim 71 or 72, wherein the, on the basis of the non-normalized dose of corticosteroid, the $C_{\max}$ provided by the formulation is 1.6 to 2, 1.5 to 3, 1.5 to 2.5, 1.5 to 2, 1.5, 1.6, 2, 2.5, or 3 fold higher than the $C_{\max}$ provided by the suspension-based formulation when the dose of corticosteroid in the formulation and the suspension is approximately the same amount loaded.
82. The method of claim 71 or 72, wherein, on the basis of normalization to the nominal available dose of corticosteroid, the $C_{\max}$ provided by the corticosteroid formulation is 1.8 to 6.2, 1.5 to 6.5, 2 to 6.5, 1.5 to 5.5, 2 to 4, 1.5 to 4, 1.5 to 3, 2.7, 3.3, 3.4, 1.5, 6.5, 2, 5.5, 4, or 3 fold higher than the $C_{\max}$ provided by the suspension-based formulation.
83. The method of claim 71 or 72, wherein, on the basis of normalization to the dose of corticosteroid to lung, the $C_{\max}$ provided by the corticosteroid formulation is 1.4 to 4.3, 1.4 to 4.5, 1.5 to 4.5, 1.5 to 3.5, 1.5 to 3, 1.4 to 3, 1.5 to 2.5, 1.5 to 2, 2, 2.3, 1.4, 4.5, 3.5, 3, 1.5 or 2.5 fold higher than the $C_{\max}$ provided by the suspension-based formulation.
84. The method of claim 71 or 72, wherein, on the basis of normalization to the dose of corticosteroid to subject, the $C_{\max}$ provided by the corticosteroid formulation is 2 to 3.5, 2 to 5, 1.7 to 3.8, 1.7 to 5, 2.7, 3, 2.4, 2, 3.5, 5, 1.7, or 3.8 fold higher than the $C_{\max}$ provided by the suspension-based formulation.

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85. The method of claim 71 or 72, wherein, on the basis of normalization to the emitted dose of corticosteroid, the C_{max} provided by the corticosteroid formulation is 1.9 to 6.3, 1.75 to 6.5, 2.2 to 4.2, 2.2 to 6.3, 1.9 to 4.2, 3.2, 3.5, 3.6, 2.8, 1.75, 6.5, 2.2, 4.2, or 6.3 fold higher than the C_{max} provided by the suspension-based formulation.
- 5 86. The method of claim 71 or 72, wherein the C_{max} provided by the corticosteroid formulation is at least 1.5, 1.6, 2, 2.6, and 3 fold higher than the C_{max} provided by the suspension-based formulation when the dose of corticosteroid in the formulation is about 2 fold lower than the dose in the suspension.
87. The method of claim 71 or 72, wherein the method provides a C_{max} (pg/ml) of 1600 to 10 1800, 1650 to 1750, or 1700 on a dose non-normalized basis.
88. The method of claim 71 or 72, wherein the method provides a dose normalized C_{max} (pg/ml/ μ g) of: 1) 1 to 2, 1.6 to 1.8, or 1.7 on a loaded dose nominal normalized basis; 2) 2 to 2.5, or 2.2 on an emitted dose normalized basis.
89. The method of claim 71 or 72, wherein, on the basis of the normalized nominal dose of 15 corticosteroid, the C_{max} provided by the corticosteroid formulation is at least 1.7, 1.8, 1.9, or 2 fold higher than the C_{max} provided by the suspension-based formulation when the dose of corticosteroid in the formulation and the suspension is approximately the same.
90. The method of claim 71 or 72, wherein, on the basis of normalization to the emitted 20 dose of corticosteroid, the C_{max} provided by the corticosteroid formulation is at least 1.5, 1.6, to 2 fold higher than the C_{max} provided by the suspension-based formulation.
91. The method of claim 71 or 72, wherein the method provides in the subject an AUC_{inf} (pg*h/ml) of 500 to 1700, 530 to 1650, 250 to 2500, 280 to 1300, 780 to 1300, 980 to 2450, 275, 775, 980, 2400, 2500, 1300, 1290, 530, 1650, 250, 280 or 780 on a dose 25 non-normalized basis.
92. The method of claim 71 or 72, wherein the method provides in the subject a dose normalized AUC_{inf} (pg/ml/ μ g) of: 1) 1 to 5.5, 2 to 2.2, 1 to 5.3, 1.1 to 5.2, 1.5 to 2.6, 1.3 to 3.3, 2, 2.1, 2.2, 1, 5.5, 5.3, 5.2, 1.5, 2.6, 1.3 or 3.3 on a nominal available dose normalized basis; 2) 10 to 25, 14 to 18, 10.2 to 20, 13.6 to 18.8, 11.2 to 24.7, 10.2, 20, 30 13.6, 14, 19, 18.8, 11, 11.2, 25, 24.7, 14.2, 16.2, 17.3 on a dose to lung normalized basis; 3) 4 to 16, 4.2 to 16.1, 8 to 12.2, 5.4 to 16, 5.4 to 17, 8.5 to 9.6, 8.5, 9.5, 9.6, 4.2,

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16.1, 8, 12.2, 12, 5.4, 16, 17, or 16.5 on a dose to subject normalized basis; 4) 2.5 to 9, 2.6 to 8.5, 4.5 to 5.1, 2.5 to 8, 2.6 to 7.9, 4.2 to 6.7, 3.1 to 8.5, 3.2 to 8.5, 4.5, 4.6, 5, 5.1, 2.5, 2.6, 4.2, 3.1, 9, 8.5, 5.1, 8, 7.9, or 6.7 to on an emitted dose normalized basis.

- 5 93. The method of claim 71 or 72, wherein, on the basis of the non-normalized dose of corticosteroid, the AUC_{inf} provided by the corticosteroid formulation is 1.6 to 2.5, 1.6 to 3.1, 1.5 to 3.5, 1.5 to 3.3, 2.5 to 3.3, 3.1, 1.5, 3.3, 1.6, or 2.5 fold higher than the AUC_{inf} provided by the suspension-based formulation when the dose of corticosteroid in the formulation and the suspension is approximately the same.
- 10 94. The method of claim 71 or 72, wherein, on the basis of normalization to the nominal available dose of corticosteroid, the AUC_{inf} provided by the corticosteroid formulation is 1.75 to 6.5, 1.75 to 6.1, 2 to 6.5, 2 to 6.1, 2 to 4.5, 2 to 4.4, 3.3, 3.2, 3.5, 3.4, 1.75, 6.5, 6.1, 2, 4.5, or 4.4 fold higher than the AUC_{inf} provided by the suspension-based formulation.
- 15 95. The method of claim 71 or 72, wherein, on the basis of normalization to the dose of corticosteroid to lung, the AUC_{inf} provided by the corticosteroid formulation is 1.2 to 3.5, 1.2 to 4, 1.2 to 3, 1.2 to 2.85, 1.5 to 3.5, 1.4 to 3.5, 2, 2.2, 2.3, 2.4, 1.2, 3, 4, 2.85, 1.5, 3.5, or 1.4 fold higher than the AUC_{inf} provided by the suspension-based formulation.
- 20 96. The method of claim 71 or 72, wherein, on the basis of normalization to the dose of corticosteroid to subject, the AUC_{inf} provided by the corticosteroid formulation is 1.6 to 4.9, 1.5 to 5, 1.6 to 5, 1.6 to 3.7, 1.6 to 3.6, 2 to 4.9, 1.9 to 4, 2.6, 1.5, 5, 1.6, 3.7, 3.6, 2, 4.9, 1.9, or 4 fold higher than the AUC_{inf} provided by the suspension-based formulation.
- 25 97. The method of claim 71 or 72, wherein, on the basis of normalization to the emitted dose of corticosteroid, the AUC_{inf} provided by the corticosteroid formulation is 1.5 to 6, 1.7 to 6, 1.9 to 6, 1.9 to 5.4, 2.3 to 5.8, 1.9 to 5.5, 1.9 to 5.8, 1.5, 6, 1.7, 1.9, 5.4, 2.3, 5.8, 5.8, 3.2, 3.5, or 3.6 fold higher than the AUC_{inf} provided by the suspension-based formulation.
- 30 98. The method of claim 71 or 72, wherein the AUC_{inf} provided by the corticosteroid formulation is at least 1.5, 1.6, 2, 2.5, 3 and 3.1 fold higher than the AUC_{inf} provided

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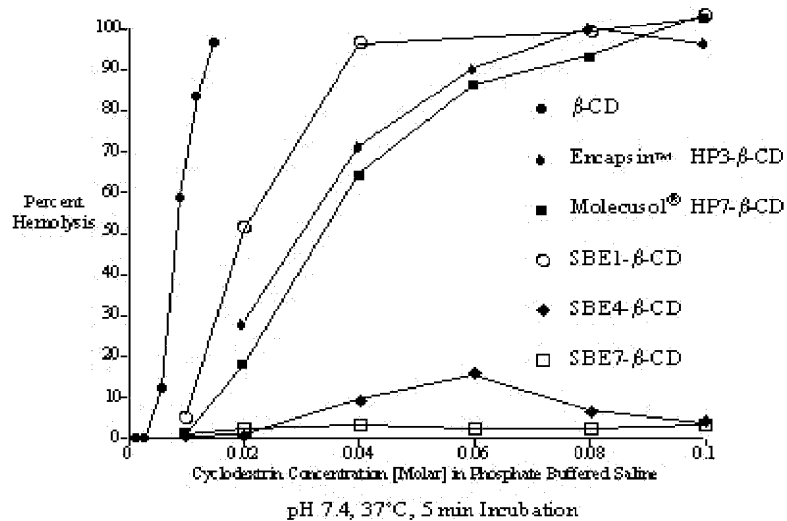
by the suspension-based formulation when the dose of corticosteroid in the formulation is about 2 fold lower than the dose in the suspension.

99. The method of claim 71 or 72, wherein the method provides in the subject an AUC_{0-8hr} (pg*h/ml) of 2000 to 3000, 2500 to 2700, 2000, 3000, or 2600 on a dose non-normalized basis for the corticosteroid.
100. The method of claim 71 or 72, wherein the method provides in the subject a dose normalized AUC_{inf} (pg/ml/ μ g) of: 1) 2 to 3, 2.5 to 2.7, or 2.6 on a loaded dose nominal normalized basis; and/or 2) 3 to 4, 3.4 to 3.5, or 3.4 on an emitted dose normalized basis.
101. A system comprising: a nebulizer equipped with a reservoir; and an aqueous liquid formulation comprising an aqueous liquid carrier, solubility enhancer, and a dose of corticosteroid, wherein, during nebulization of the formulation, the nebulizer provides a percentage decrease in the rate of increasing concentration of corticosteroid in the formulation in the reservoir as compared to the rate of increasing concentration of a suspension-based formulation nebulized with the nebulizer under similar conditions.
102. The system of claim 101, wherein the percentage decrease in the rate of increasing concentration is 10% to 60%, 15% to 60%, 20% to 60%, 30% to 60%, or 40% to 60%.
103. The system of claim 101, wherein the system provide a rate of increasing concentration of 0 to 40, 1 to 40, 5 to 30, or 10 to 30 mcg of corticosteroid/mL of formulation volume per min of nebulization.
104. A method of treating a disease or disorder of the airways, in a subject in need thereof, comprising administering via inhalation to the subject a liquid formulation comprising an aqueous liquid carrier, a sulfoalkyl ether cyclodextrin, and a dose of corticosteroid dissolved therein, wherein the dose of corticosteroid is present in an amount sufficient to provide a mean plasma AUC_t of 160-1600 pg*h/ml, and wherein the molar ratio of SAE-CD to corticosteroid is greater than 10:1.
105. A method of providing a corticosteroid to a subject, the method comprising: administering to the subject, via nebulization, 25-400 μ g of a corticosteroid dissolved in an aqueous liquid carrier comprising sulfoalkyl ether cyclodextrin to provide a plasma AUC_t of 150-1600 pg*h/ml for the corticosteroid.

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106. A method of providing in a subject a plasma AUC_t , normalized for dose of corticosteroid, of at least 6 (pg*h/ml)/ μ g of corticosteroid, comprising: administering to the subject, via nebulization, a dose of at least 25 μ g of corticosteroid dissolved in an aqueous liquid carrier comprising sulfoalkyl ether cyclodextrin.
- 5 107. A method of providing in a subject an AUC_i , normalized for dose of corticosteroid, of at least 8 (pg*h/ml)/ μ g of corticosteroid, comprising: administering to the subject, via nebulization, a dose of at least 25 μ g of corticosteroid dissolved in an aqueous liquid carrier comprising sulfoalkyl ether cyclodextrin.
- 10 108. The invention according to any one of claims 71-107, wherein the corticosteroid is budesonide.
109. The invention according to any one of claims 71-107, wherein the corticosteroid has a lipophilicity approximating or exceeding that of flunisolide.
- 15 110. The invention according to claim 109, wherein the corticosteroid is selected from the group consisting of beclomethasone dipropionate, beclomethasone monopropionate, budesonide, ciclesonide, desisobutyryl-ciclesonide, flunisolide, fluticasone propionate, fluticasone furoate, mometasone furoate, icomethasone enbutate, tixocortol 21-pivalate, and triamcinolone acetonide.
- 20 111. The invention according to any one of claims 71-107, wherein the step of administering or delivering is conducted with a nebulizer selected from the group consisting of an air jet nebulizer, ultrasonic nebulizer, electronic nebulizer, vibrating membrane nebulizer, vibrating mesh nebulizer, vibrating plate nebulizer, a nebulizer comprising a vibration generator and an aqueous chamber, and a nebulizer comprising a nozzle array.

FIG. 1



PRIOR ART

FIG. 2

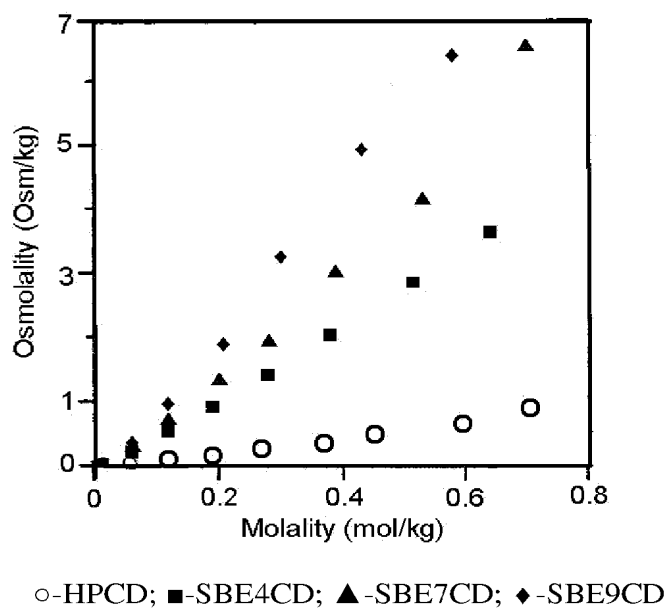


FIG. 3

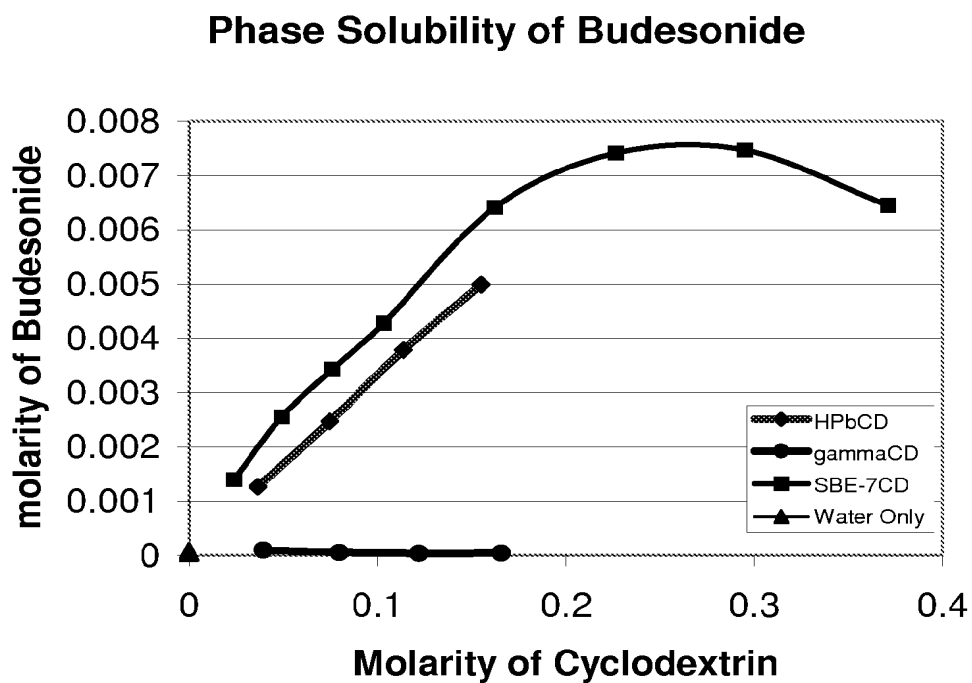


FIG. 4

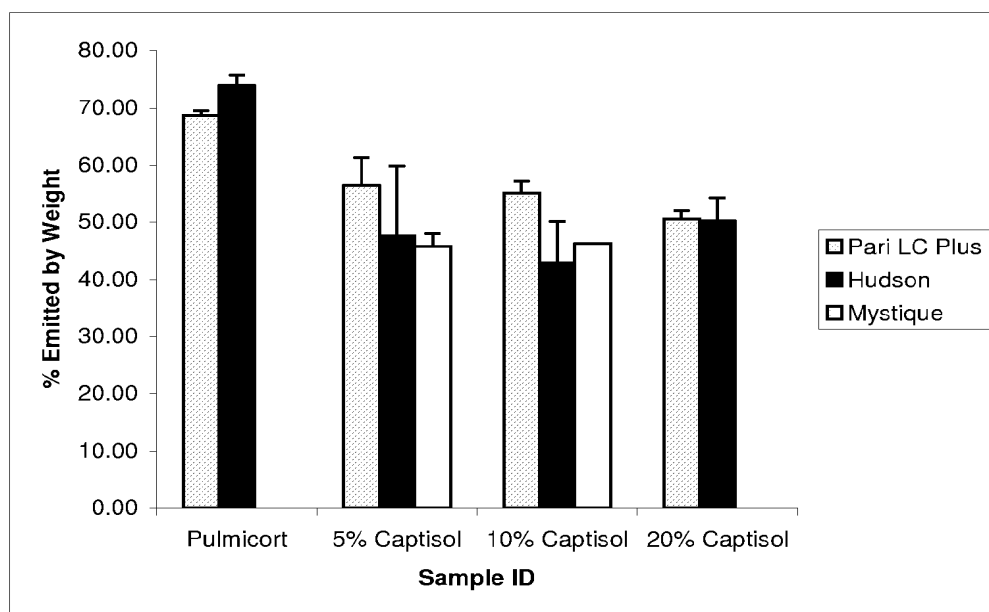


FIG. 5a

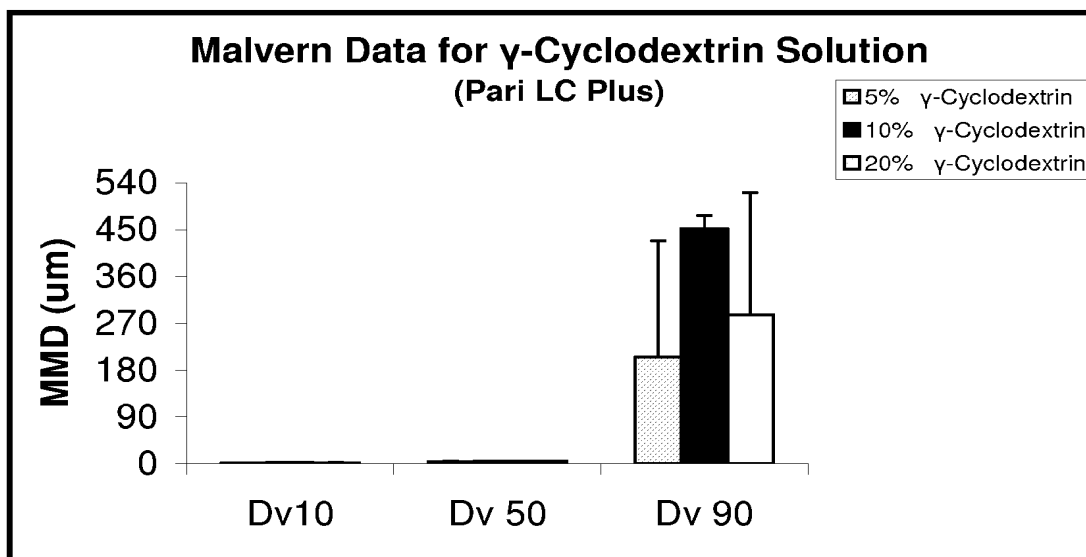


FIG. 5b

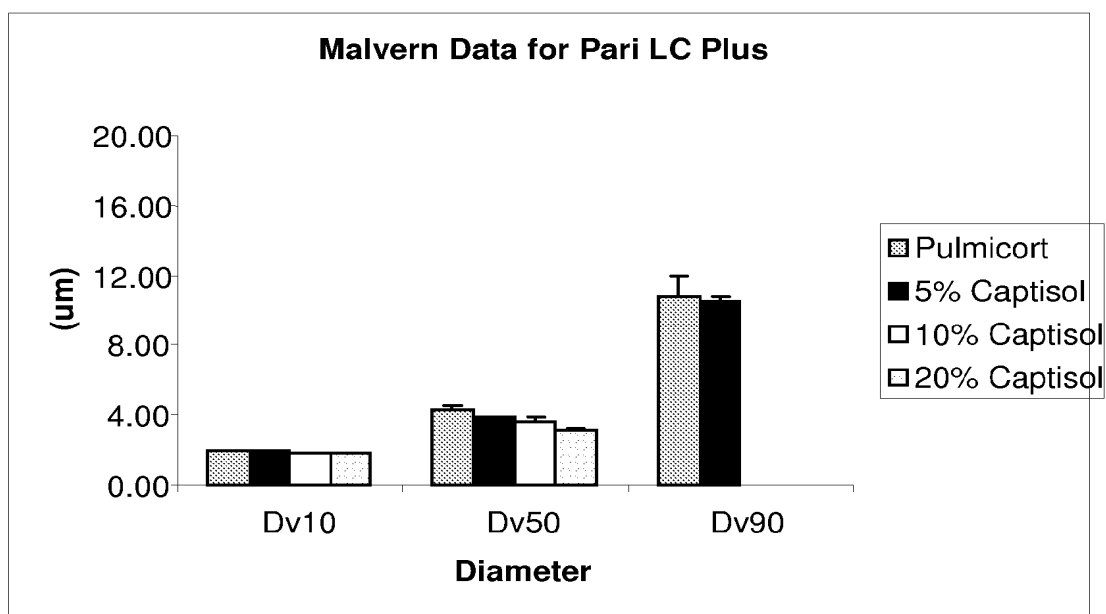


FIG. 6

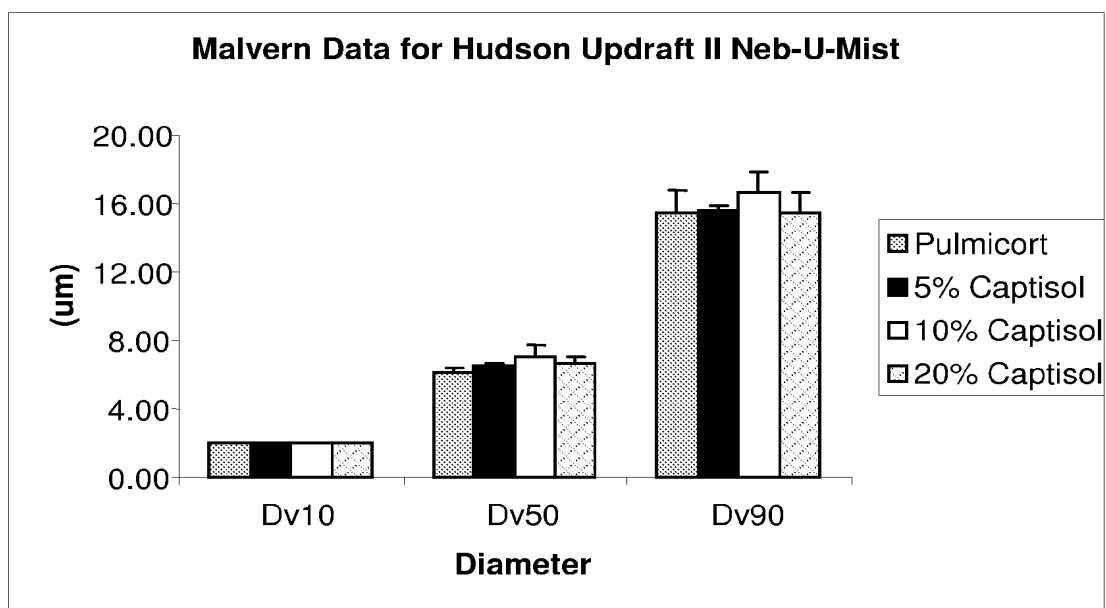


FIG. 7

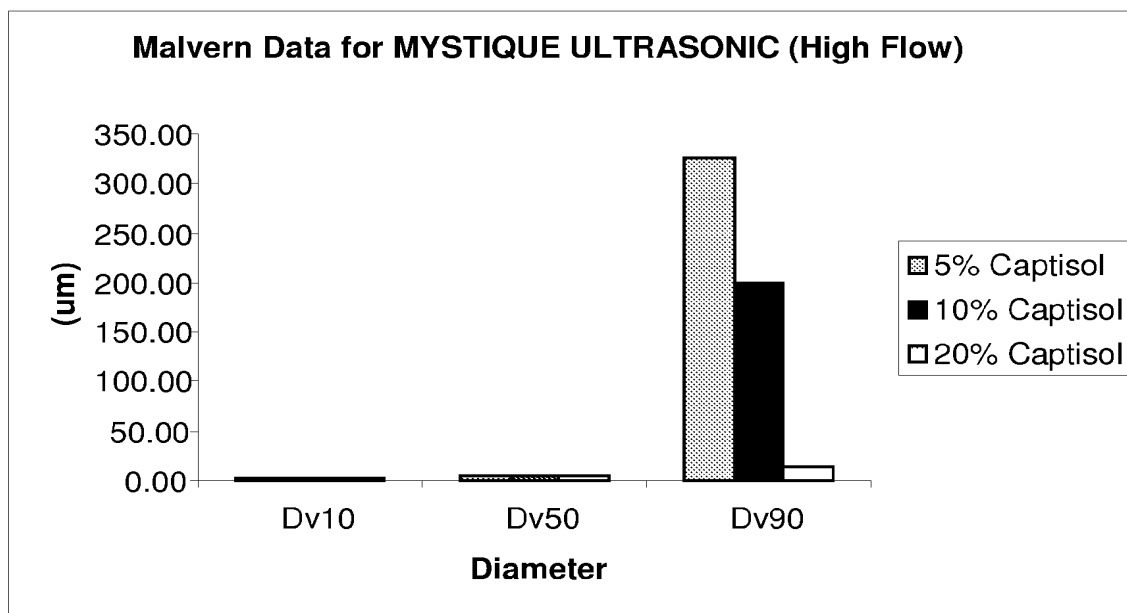


FIG. 8

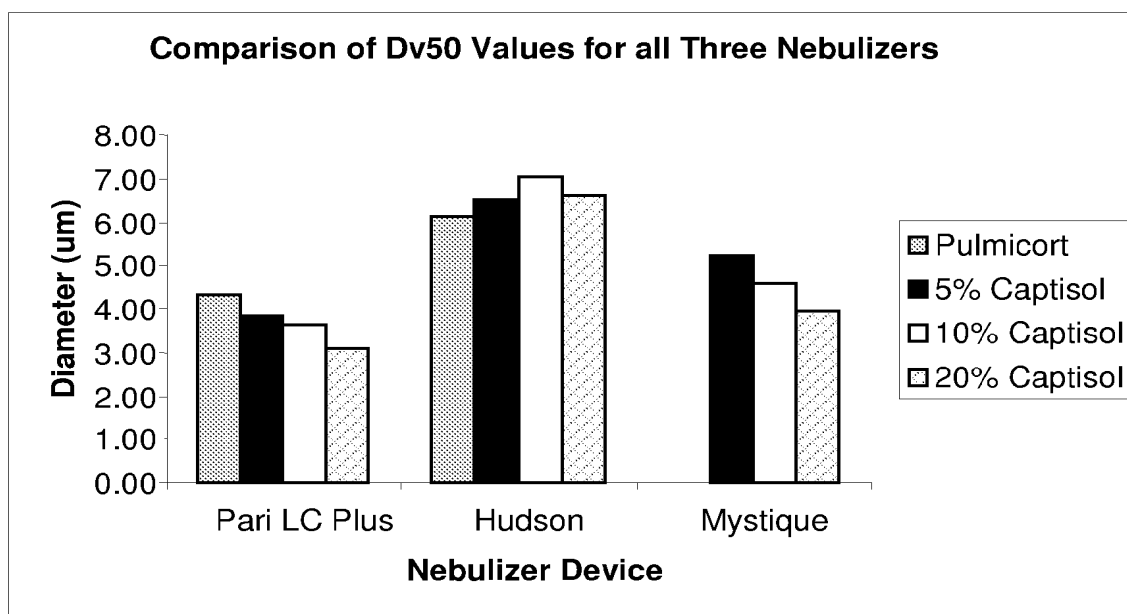


FIG. 9

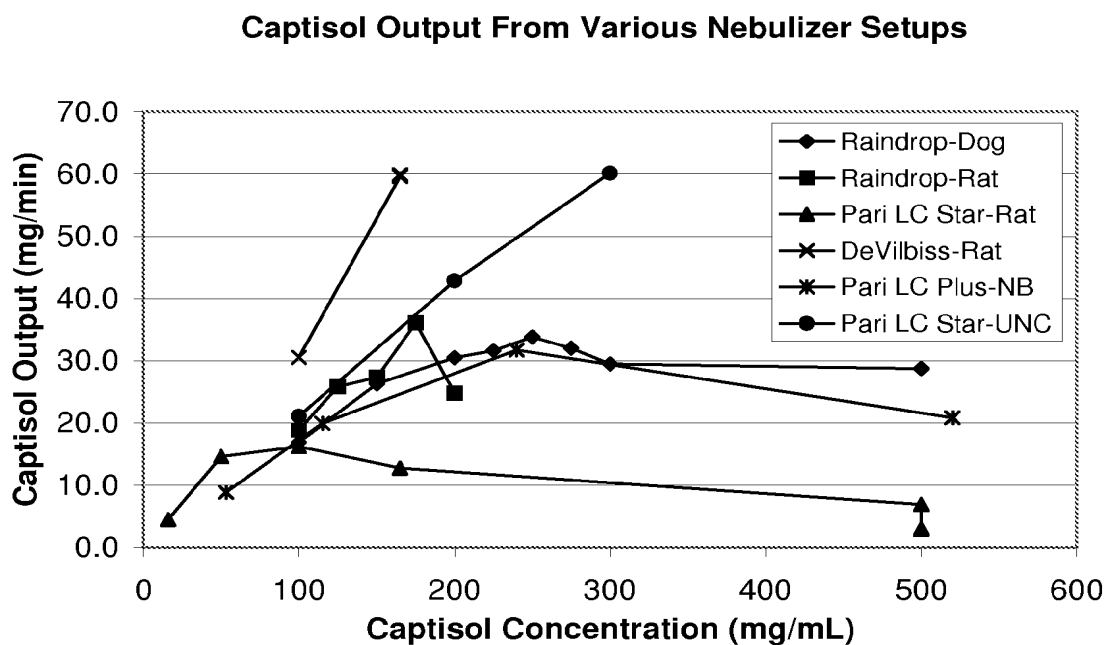


FIG. 10a

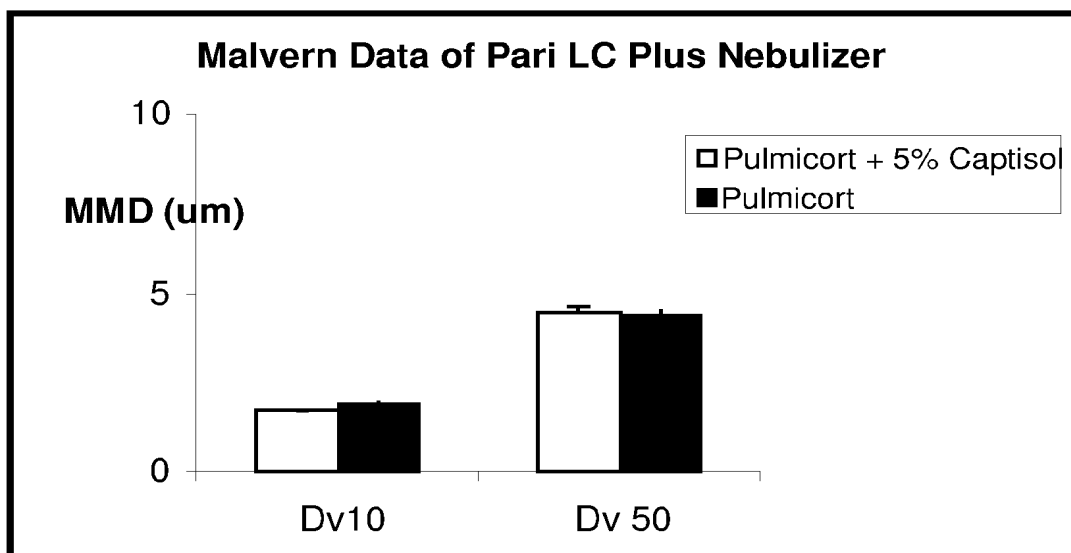


FIG. 10b

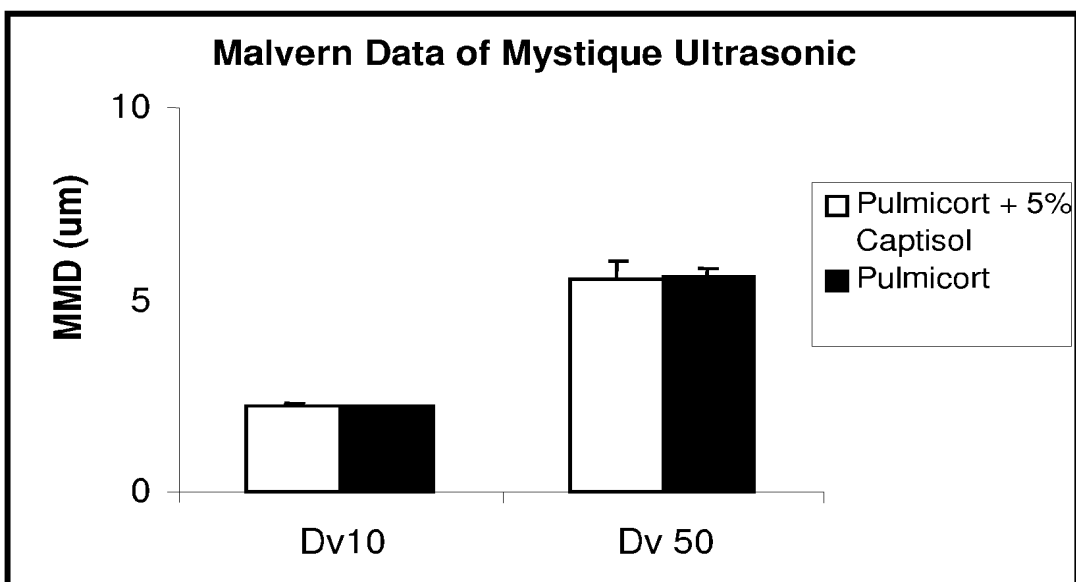


FIG. 11

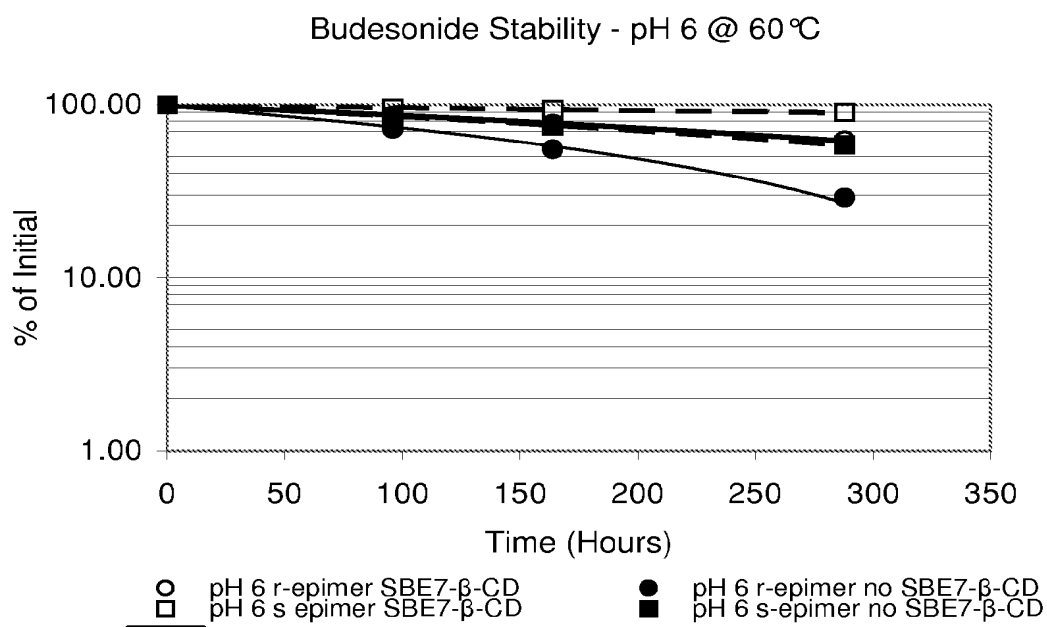


FIG. 12

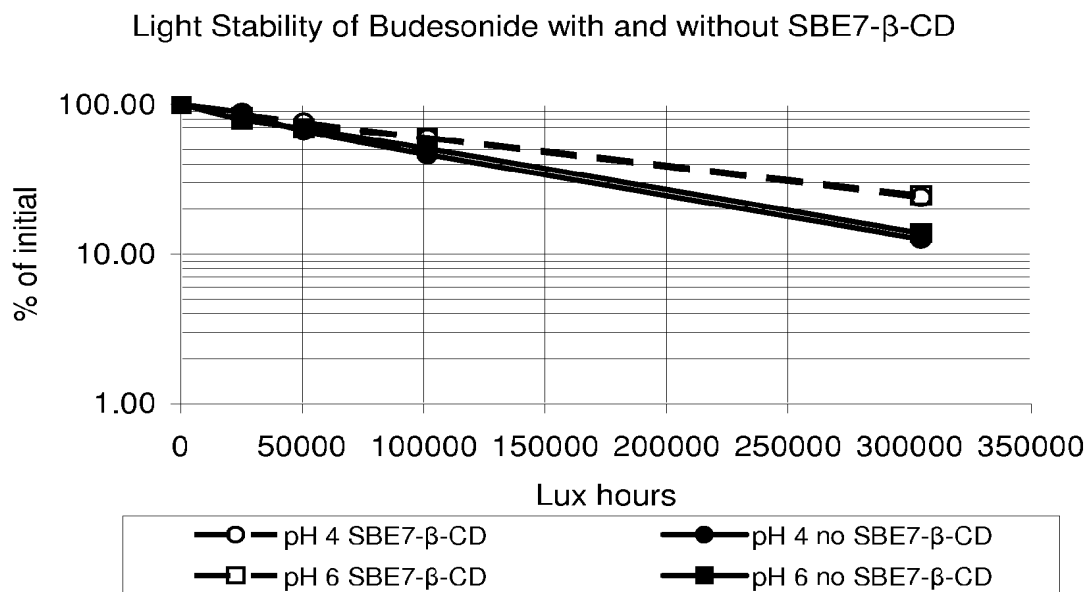
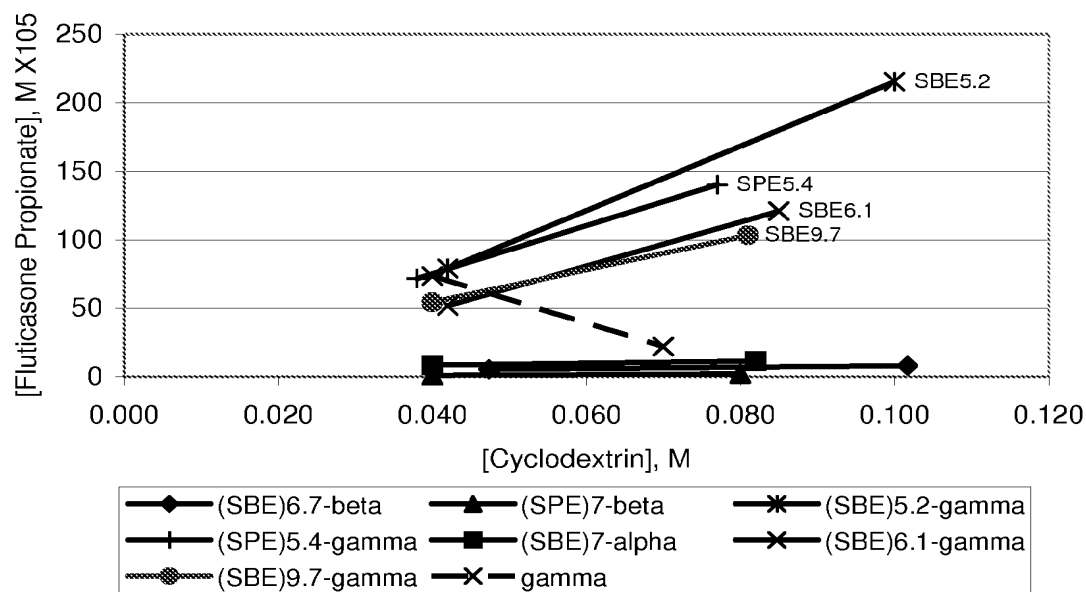


FIG. 13

Effect of CD Ring Size and DS on Solubility of Fluticasone Propionate

**FIG. 14**

Effect of CD Ring Size and DS on Solubility of Mometasone Furoate

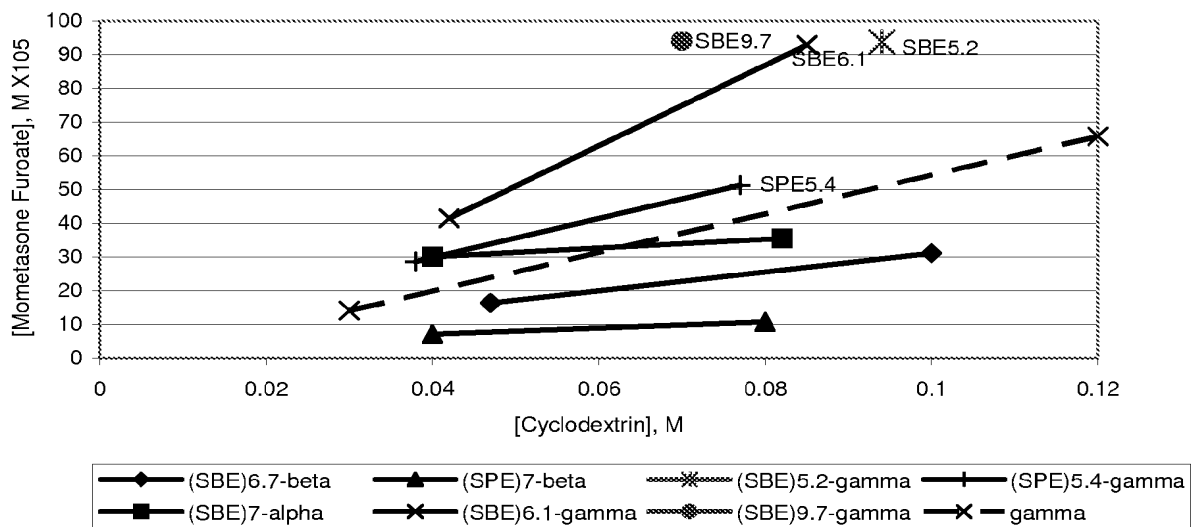


FIG. 15

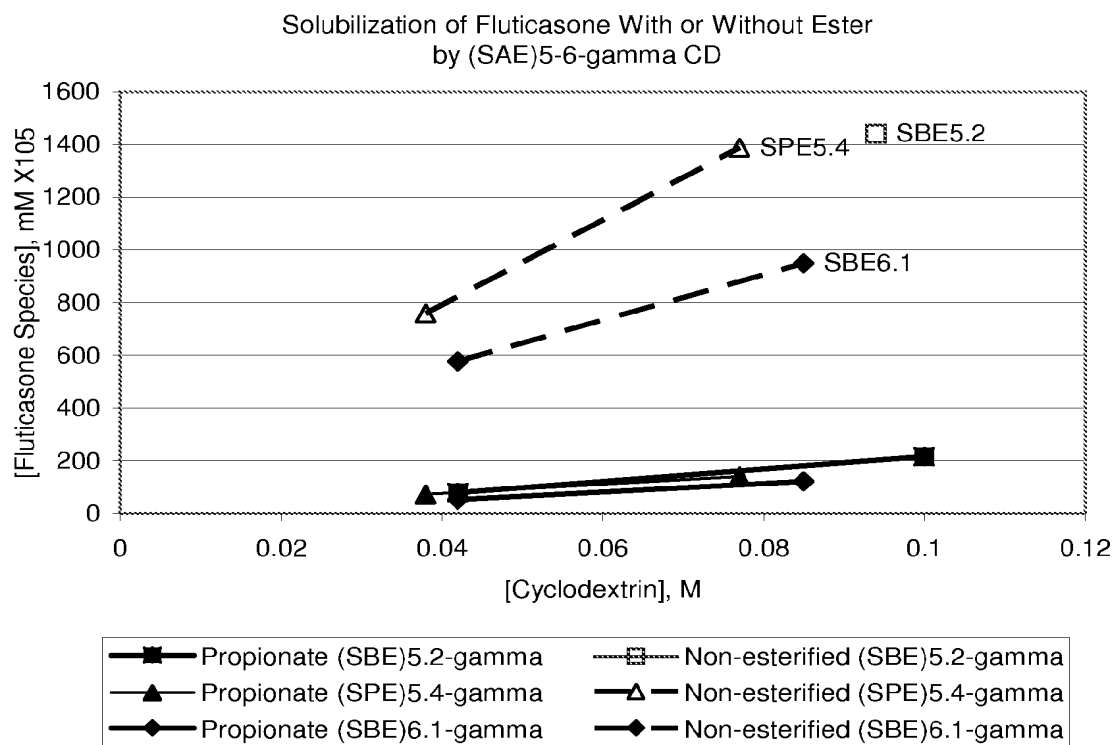


FIG. 16

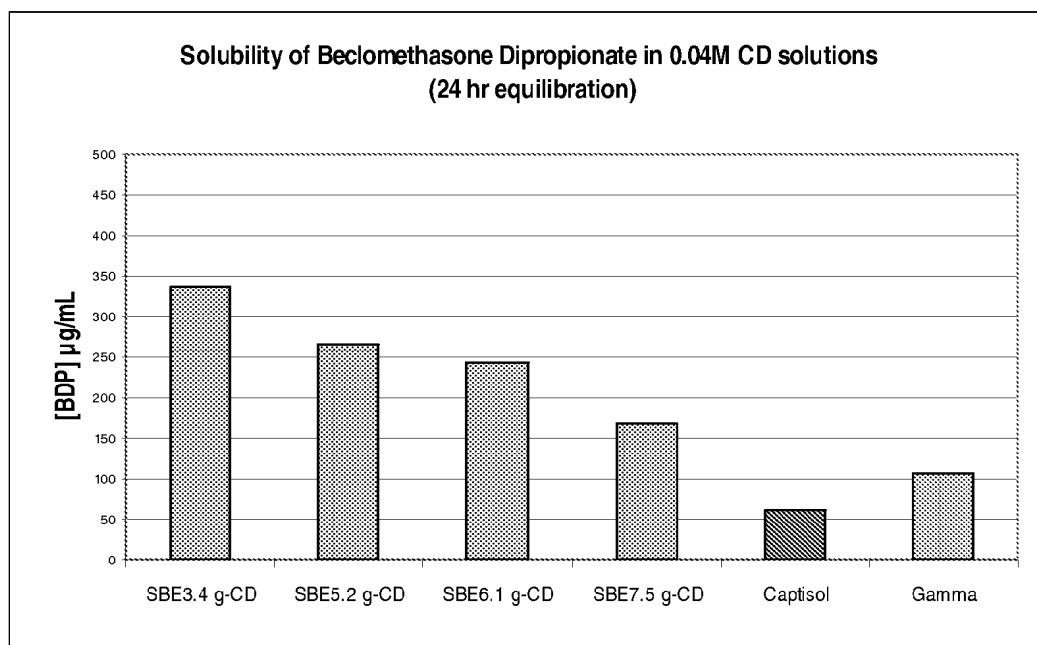


FIG. 17

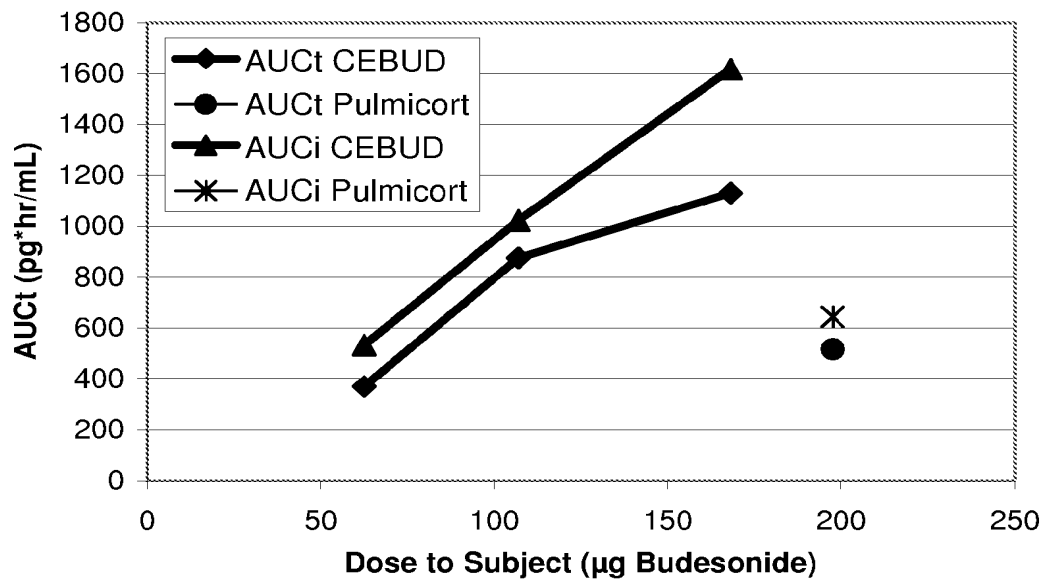


FIG 18.

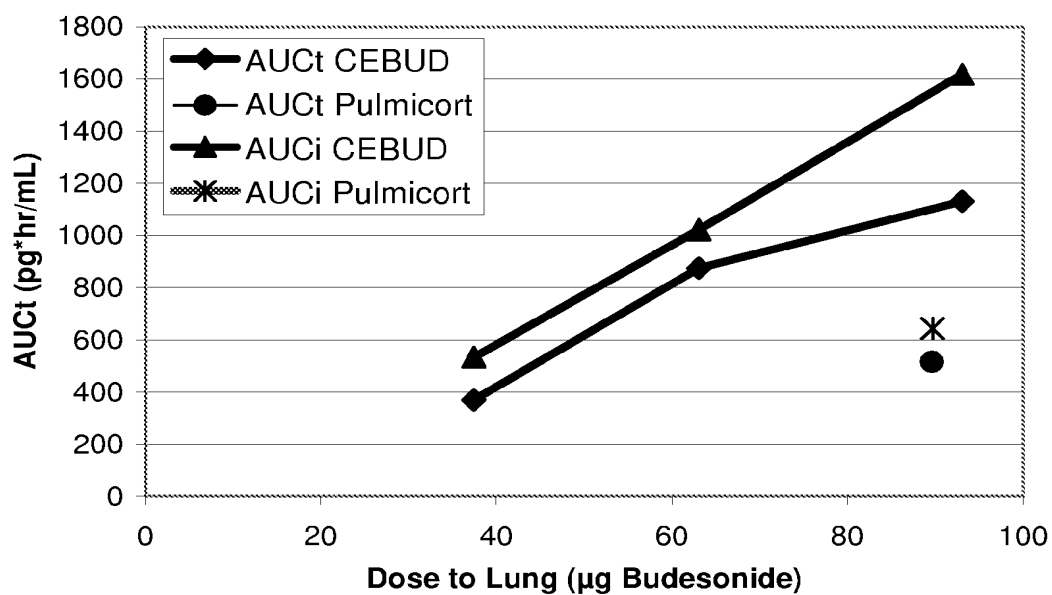


FIG. 19

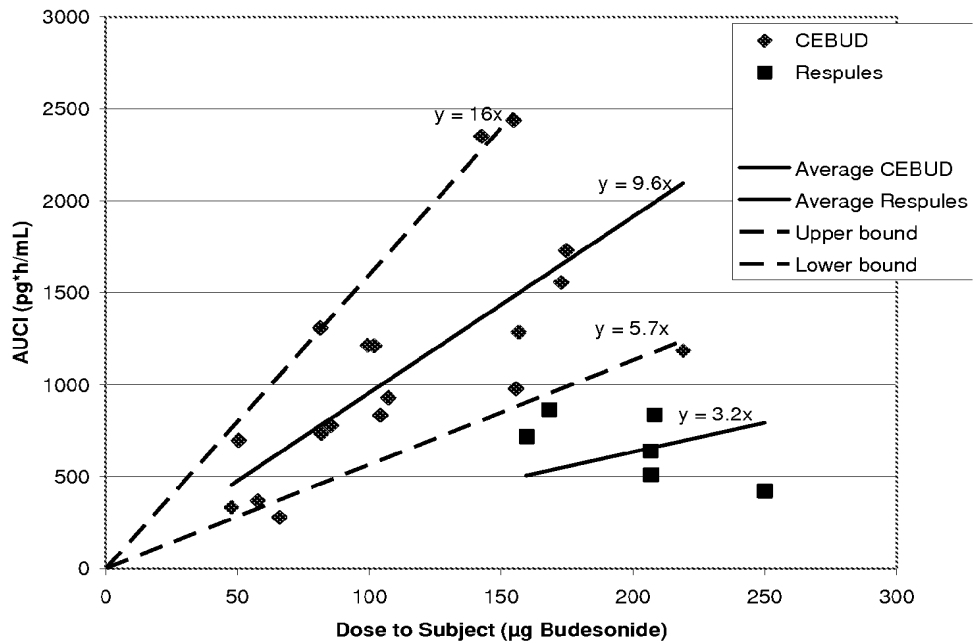


FIG. 20

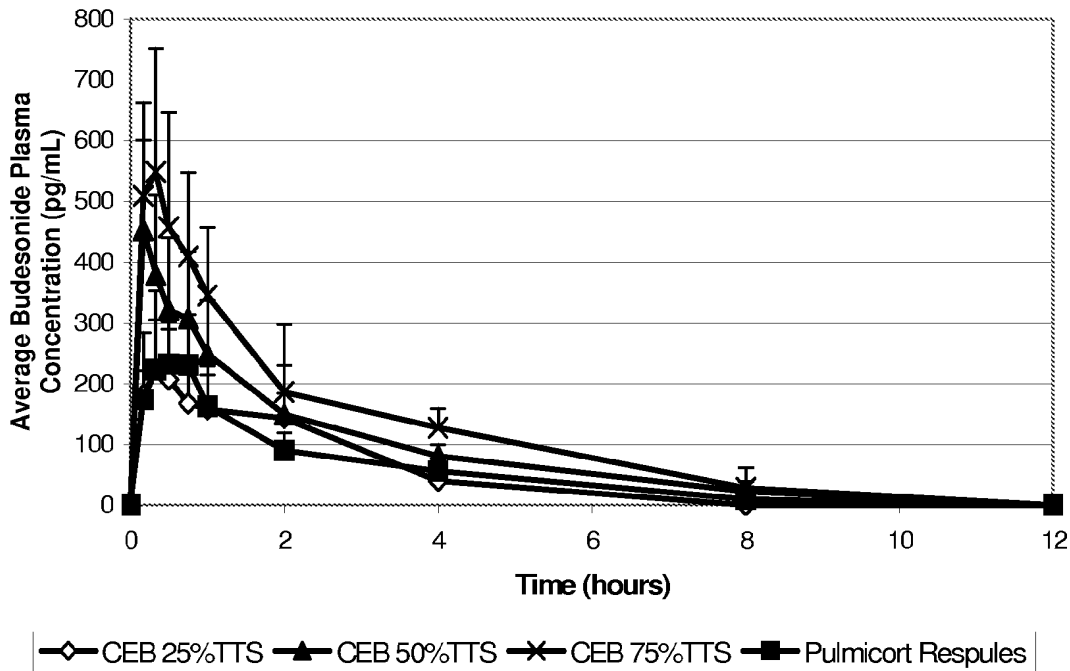


FIG. 21

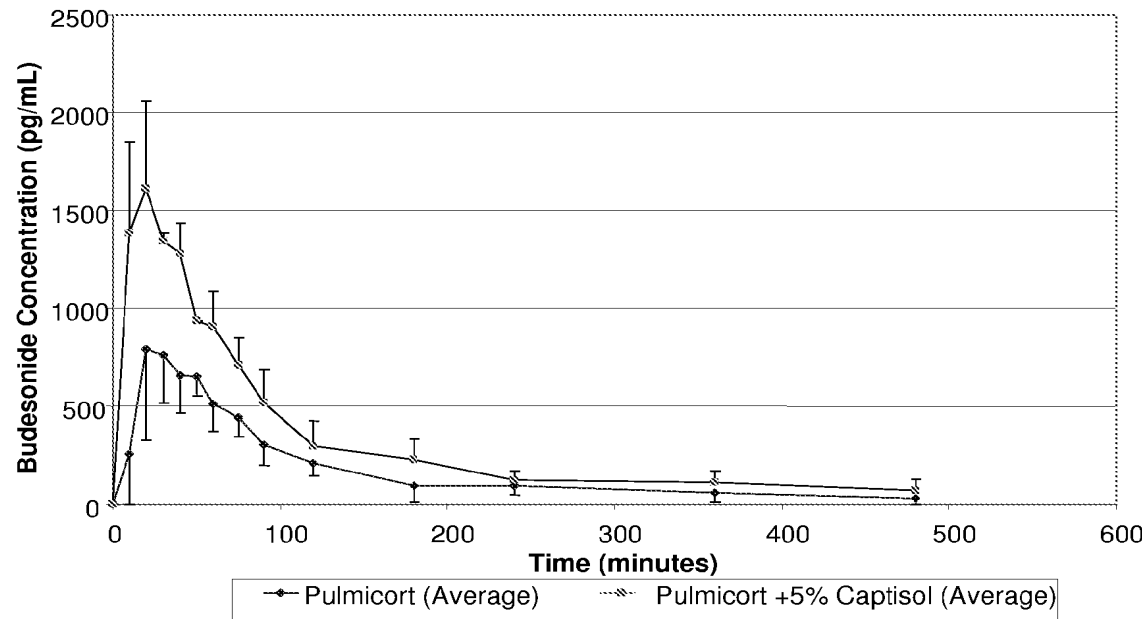
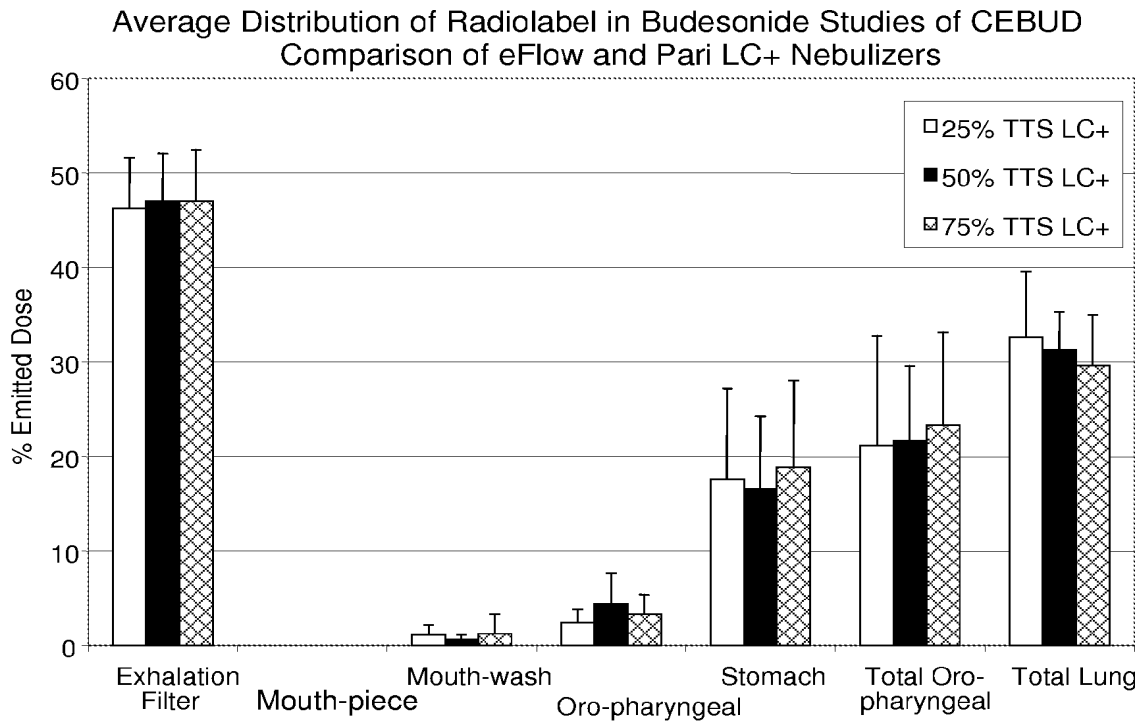


FIG. 22



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US06/62346

A. CLASSIFICATION OF SUBJECT MATTER

IPC: A61K 9/00(2006.01);9/12(2006.01);C08B 37/16(2006.01)

USPC: 424/45,434;514/58,179

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/45, 434; 514/58, 179

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WEST, NPL (SCIRUS), WIPO, EPO, PALM

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,576,645 (STELLA et al) 27 December 1994 (27.12.1994), entire document, especially columns 4, 6, 8, 10 and 12.	1-111

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed		"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family

Date of the actual completion of the international search

17 September 2007 (17.09.2007)

Date of mailing of the international search report

05 OCT 2007

Name and mailing address of the ISA/US

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Alexandria, Virginia 22313-1450

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Inventor(s): BAR-SHALOM DANIEL [SKJOEDE JENSEN SIMON]
Applicant(s): BIONEER AS[DK] | BAR-SHALOM DANIEL[DK] | SKJOEDE JENSEN SIMON[DK]
Classification: A61K9/00AI
Application number: WO2011EP52061 2011-02-11
Priority number: US20100303758P 2010-02-12; DK20100070051 2010-02-12;

Abstract of WO2011098578A2

This invention concerns a pharmaceutical formulation for ocular or intra-ocular administration providing sustained or delayed release of at least one active ingredient, said pharmaceutical formulation comprising liposomes, said liposomes comprising at least part of said at least one active ingredient, at least part of said liposomes exhibiting a charge increasing the adherence of the charged liposomes to the eye after administration, in particular when said pharmaceutical formulation is for ocular administration, and wherein at least one of the lipids in the liposomes is degradable by sPLA2 activity, allowing sustained or delayed release of said at least one active ingredient from said liposomes upon contact with tear fluid or with sPLA2 in the eye or in the vicinity of the eye. The pharmaceutical formulation is useful for ophthalmic indications.

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International Bureau



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(81) Designated States (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

(54) Title: LIPOSOME SYSTEM FOR OCULAR ADMINISTRATION

(57) Abstract: This invention concerns a pharmaceutical formulation for ocular or intra-ocular administration providing sustained or delayed release of at least one active ingredient, said pharmaceutical formulation comprising liposomes, said liposomes comprising at least part of said at least one active ingredient, at least part of said liposomes exhibiting a charge increasing the adherence of the charged liposomes to the eye after administration, in particular when said pharmaceutical formulation is for ocular administration, and wherein at least one of the lipids in the liposomes is degradable by sPLA₂ activity, allowing sustained or delayed release of said at least one active ingredient from said liposomes upon contact with tear fluid or with sPLA₂ in the eye or in the vicinity of the eye. The pharmaceutical formulation is useful for ophthalmic indications.

LIPOSOME SYSTEM FOR OCULAR ADMINISTRATION

FIELD OF THE INVENTION

The present invention relates to liposomes for ocular or intra-ocular administration, allowing sustained or delayed release of an active pharmaceutical ingredient.

5 BACKGROUND OF THE INVENTION

Delivery of drugs to the eye is difficult since most drugs are washed off by lacrimation, tear dilution and tear turnover. This means that retention of drugs in the eye is limited to short periods, resulting in a fast reduction in eye drug concentration, leading to subsequent reduced therapeutic effect.

10 The tear fluid, which protects the eye from bacteria and which lubricates the eye contains several enzymes that participate in the protection against bacteria, one of them being secretory phospholipase A₂ which in particular has a protective effect against infections with gram negative bacteria. sPLA₂ has enzymatic activity towards phospholipids when these lipids are present in certain liposomes.

15 International patent application publication WO 01/58910 in the name of Liplasome Pharma A/S purports to disclose lipid-based prodrugs, which are turned into active drugs by hydrolysis via the extracellular phospholipase. The disclosed drug delivery system is purportedly particularly useful in the treatment or alleviation of diseases which are characterized by localized activity of extracellular PLA₂ activity.

20 International patent application publication WO 07/107161 in the name of Liplasome Pharma A/S purports to disclose a lipid based drug delivery system comprising lipid derivatives which are substrates for extracellular phospholipase A₂, and lipopolymers and/or glycolipids so as to present hydrophilic chains on the surface of the system.

International patent application publication WO 2009/141450 in the name of Liplasome
25 Pharma A/S purports to disclose cholesterol-free liposomes comprising a therapeutic agent, wherein the liposomes have been stabilized by exposure to divalent cations, in particular Calcium ions.

The Liplasome technology was developed for targeted delivery of drugs administered systemically, i.e. the drug or prodrug was supposed to be carried by the blood stream and released or activated at sites with increased levels of extracellular PLA₂ activity, thereby providing targeted delivery to e.g. cancerous tissues. However, the targeting aspect did not live up to expectations, most likely due to degradation of the liplasomes in the systemic circulation.

SUMMARY OF THE INVENTION

The problem of systemic degradation of liposomes i.a. observed when exercising the liplasome technology is eliminated when a liposome is delivered directly to the eye.

Surprisingly, it has been demonstrated that the retention of the sPLA₂ sensitive, charged liposomes is enhanced in the presence of (human) tear fluid comprising sPLA₂ as shown in the examples below.

Prior art liposomes, such as those described in US 4,804,539, typically include high concentrations of cholesterol (the liposomes in US 4,804,539 e.g. include 20-50% mol/mol of cholesterol), thus apparently rendering tear fluid sPLA₂ ineffective in providing the effects disclosed herein.

According to an aspect, the invention concerns a liposome for ocular or intra-ocular administration, said liposome comprising:

lipids and at least one active ingredient;

said liposome preferably exhibiting a charge increasing the adherence of said liposome to the eye after administration, in particular when said liposome is for ocular administration; wherein at least one of the lipids in the liposome is degradable by sPLA₂ activity, thereby allowing sustained or delayed release of said at least one active ingredient from said liposome upon contact with tear fluid (e.g. human tear fluid) or sPLA₂ in the eye or in the vicinity of the eye.

Positively charged liposomes exhibit a superior retention to the cornea due to the electronegative charge of the cornea. Positively charged liposomes therefore seem to be most efficient for ocular drug delivery.

sPLA₂ in tear fluid is present in very high amounts and is able to degrade liposomes administered to the eye either when the liposomes are freely present in the tear fluid, or when the liposomes adhere to the negatively charged cornea, where the liposomes are retained due to electrostatic attraction. Positively charged liposomes are attracted very

efficiently to the cornea, but are degraded relatively slowly by sPLA₂, because sPLA₂ is most active on negatively charged liposomes, which can be disrupted within minutes causing in the subsequent release of the encapsulated drug.

Conversely, in some pathological situations, electropositively charged areas may occur, and in these areas electronegatively charged lipids or liposomes would consequently target themselves. Hence, such electronegatively charged lipids or liposomes are within the scope of the invention according to this aspect.

According to another aspect, the invention concerns a pharmaceutical formulation comprising the liposome according to the first aspect of the invention.

The person skilled in the art is aware of suitable excipients to include in such a pharmaceutical formulation. However, reference is made to the detailed disclosure below.

According to a third aspect, the invention concerns a lipid based delivery system for ocular or intra-ocular administration, said system providing sustained or delayed release of at least one active ingredient,

said system comprising:

(I) lipids comprising:

(a) an organic radical having a least 2 carbon atoms; and

(b) a hydrophilic moiety;

said lipids being a substrate for enzymes in (human) tear fluid or for sPLA₂ to the extent that the organic radical can be hydrolytically cleaved off, leading to an intramolecular cyclization reaction; and

(II) at least one active ingredient;

said lipids allowing the formation of liposomes, said liposomes comprising at least part of said at least one active ingredient;

at least part of said liposomes exhibiting a charge, which increases the adherence of the charged liposomes to the eye after administration; and wherein at least one of the lipids in the liposome is degradable by enzymes in (human) tear fluid or by secretory phospholipase, in particular sPLA₂ activity, allowing sustained or delayed

release of said at least one active ingredient from said liposomes upon contact with secretory phospholipase, in particular sPLA₂.

The sustained or delayed drug release system of the present invention does not depend on increased levels of sPLA₂ induced by the presence of disease or an abnormal condition.

- 5 According to a fourth aspect, the invention concerns a method of ocular and/or intra-ocular treatment, comprising administration of liposomes, a pharmaceutical formulation and/or a lipid based delivery system according to the invention.

According to a fifth aspect, the invention concerns the use of liposomes, a pharmaceutical formulation and/or a lipid based delivery system according to the invention, for the
10 manufacture of a medicament for an indication disclosed herein.

LEGENDS TO THE FIGURE

Fig. 1: Overview of liposomes, liposome size and surface charge. A) List of 5 liposomes composed of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol (POPG) and 1,2-dihexadecanoyl-3-trimethylammonium-propane (DOTAP). Ratio of each component in the liposomes is shown, and their expected
15 sensitivity to PLA₂ mediated hydrolysis shown together with their expected cell binding property. B) Liposome size measured in nanometer (nm) by dynamic light scattering on a ZetaPALS zeta potential analyzer from Brookhaven Instruments. C) Liposome surface charge expressed in mV. Liposome compositions for B and C refer to the numbers from figure 1A.

20 Fig. 2: Maldi-TOF analysis of lipid formulations (liposomes 1-5 from figure 1A) hydrolyzed in human tear fluid dependent on time of incubation. The Maldi-TOF peak intensity of LPC16-Na (m/z=518.4) and POPC-Na (m/z=782.6) were collected and the ratio $R = \frac{PI_{LPC16}}{(PI_{POPC} \times \%_{POPC})}$ was calculated. PI_{LPC16} and PI_{POPC} are the peak intensities of LPC16-Na and POPC-Na and $\%_{POPC}$ denoted fractional content of POPC in the formulation. Zero hydrolysis corresponds
25 to $R=0$ whereas full hydrolysis corresponds to a large/infinite R-value.

Fig.3: Liposome binding to human cells dependent on time of incubation and liposome composition. After the indicated incubation time (minutes) cells were washed three times in cell media and left in fresh media until fluorescence measurement. Total amount of liposome added is seen for $t=0$. Fluorescence measurement was made by to trace liposome presence
30 through incorporation of 0.2% DOPE-rhodamine into the liposome composition.

Fig.4: Liposome binding to human cells dependent on presence of tear fluid or bee venom lipase. A) Liposome association with human cells expressed in relative fluorescence units by measurement of DOPE-rhodamine in the liposome compositions compared to total amount of liposome added. After incubation with cells for 60 min, cells were washed three times in media and remaining liposome (cell associated) was measured by fluorescence intensity of the cells. B) The data expressed as percent fluorescence intensity compared to total amount of liposome added to the cells (data adapted from figure 4A).

Fig.5: Liposome cytotoxicity was measured on human cells using the formulations from figure 1A. The liposomes were incubated with the cells for 10, 30, 60 or 120 min before washing with cell media. After 24 h the liposome induced cytotoxicity was measured by incubation with XTT for 3 hours, allowing live cells to convert XTT. There was no significant cytotoxicity of the cationic liposomes containing DOTAP compared to neutral or negatively charged liposomes. Increased time of incubation did not induce cytotoxicity.

DETAILED DISCLOSURE OF THE INVENTION

Definitions

A "liposome" in the present application and claims denotes an artificial prepared vesicle made of at least one lipid bilayer.

A "hydrophobic" drug in the present context denotes a drug substance, which exhibits a distribution coefficient between 1-octanol and water (measured using the shake flask method at 20°C, 1 bar and pH 7.4) higher than 1, *i.e.* a $\log D_{\text{octanol/water}}$ higher than 0. Preferred hydrophobic drugs of relevance for the present invention exhibit higher $\log D_{\text{octanol/water}}$ values, such as values of at least 0.1, at least 0.2, at least 0.3, at least 0.4, at least 0.5, at least 0.6, at least 0.7, at least 0.8, at least 0.9, at least 1.0, at least 1.1, at least 1.2, at least 1.3, at least 1.4, at least 1.5, at least 1.6, at least 1.7, at least 1.8, at least 1.9, at least 2.0, at least 2.5, and at least 3.0. $\log D_{\text{octanol/water}}$ is calculated as follows:

$$\log \left(\frac{[drug]_{\text{octanol}}}{[drug]_{\text{water}}^{\text{ionized}} + [drug]_{\text{water}}^{\text{non-ionized}}} \right)$$

An "ointment" may be described as a viscous semisolid preparation used topically on a variety of body surfaces. These include the skin and the mucous membranes of the eye (an

eye ointment), vagina, anus, and nose. Hence, these provide optional routes of administration of liposomes according to an embodiment of the invention.

A "cream" may be described as an emulsion of oil and water (both o/w and w/o) in approximately equal proportions, which penetrates the stratum corneum outer layer of skin well.

A gel normally liquefies upon contact with the skin.

A paste usually combines three agents, i.e. oil, water, and powder; it may be described as an ointment in which a powder is suspended.

When using the terms "substantial"/"substantially" or "essential"/"essentially" herein it is intended that the feature which is described by these terms is present in an amount or has an impact which provides for a technical effect with relevance for the exercise of the presently claimed invention. For instance, a "substantial amount" of a substance in a composition is an amount which provides for a technical effect exhibited by the substance to a degree which provides for a technical effect in terms of the present invention. Likewise, if a composition is indicated as comprising "substantially no" of a particular substance, this means that the composition is allowed to include insignificant amounts of the substance, as long as these amounts do not have any technical impact on the other ingredients in the composition and does not in itself "make a difference" – or put in other words, "substantially no" and "essentially no" means that e.g. trace amounts or effects may be present as long as they do not have an overall technical influence.

Specific embodiments of the invention

Additional embodiments of the present invention are described below. It will be clear for the person skilled in the art, that aspects and/or embodiments of the invention may be combined.

For ocular delivery of drugs, positively charged liposomes will likely be degraded by sPLA₂ within 1-12 h, which means that the drug entrapped inside the liposomes will be released over many hours, which allows a much longer presence of the drug in the eye.

It will be understood though that the results reported herein primarily demonstrate that there appears to be a correlation between the sPLA₂ sensitivity of lipids in the liposomes and their ability to adhere to negatively charged cell surfaces. However, it cannot be excluded that the

effects disclosed in the Examples, where presence of human tear fluid dramatically increases the cell-association of liposomes of the invention may be due to the influence of other enzymes or substances in human tear-fluid (which is a complex aqueous composition).

However, the results demonstrate that positively charged liposomes containing a balanced mixture of anionic lipids and cationic lipids provide for surprisingly improved results in terms of adhesion to corneal surfaces and sustained release to the eye and that these effects are the consequence of the action of tear fluid on the liposomes.

Liposome structure and characteristics

In some embodiments of the invention at least part of the lipids in the liposomes exhibit a positive charge, particularly after ocular administration. It is contemplated that up to 100% (mol/mol) may be positively charged, but lower amounts are preferred. In some embodiments at most 90%, such as at most 80%, at most 70%, at most 60%, at most 50% and at most 40%. Especially preferred embodiments entail that at most 30% (mol/mol) lipids exhibit a positive charge. The amount of positively charged lipids is hence at most 30%, at most 29%, at most 28%, at most 27%, at most 26%, at most 25%, at most 24%, at most 23%, at most 22%, at most 21% and at most 20%. On the other hand, the amount of positively charged lipids is at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 11%, at least 12%, at least 13%, at least 14%, at least 15%, at least 16%, at least 17%, at least 18%, at least 19%, and at least 20%.

According to an embodiment of the invention the liposome comprises an amount of 10-60%, preferably 20-50% positively charged/cationic lipids (mol/mol), typically selected among 0-5%, 5%-15%, 10-20%, 20-25%, 25-30%, 30-40%, 40-50%, 50-60%, 60-70%, 70-80%, 80-90%, 90-100% (mol/mol) positively charged/cationic lipids

At any rate, irrespective of the exact amount of positively charged lipids, some embodiments of the present invention entail that the liposome exhibits a net positive charge, in particular after administration to the normal human eye. For instance, these embodiments include those where the liposome has a zeta potential in the range from 10-60 mV. The zeta potential is typically at most 55 mV, such as at most 50 mV, or at most 45 mV, e.g. at most 40 mV. Also, the zeta potential is typically at least 15 mV, such as at least 20 mV, or at least 25 mV, e.g. at least 30 mV.

In other embodiments of the invention the lipids in the liposomes are substantially not charged, and in certain embodiments the net charge of the liposomes is essentially neutral.

In accordance with the findings herein, particularly preferred liposomes of the invention are those which exhibit an improved binding to normal corneal tissue and the ability to sustainedly release a (hydrophobic) drug to the eye after having being exposed to human tear fluid. An important feature of the liposomes of the invention is their ability to, when contacted with 50% human tear fluid for 60 minutes, subsequently exhibit at least 20% of maximum possible association with HT1080 cells after one hour of incubation. The maximum possible association is in this context the complete association of all liposomes administered to the eye in a situation where a pharmaceutically relevant and acceptable amount of liposomes are administered, *i.e.* in a situation where the association to the eye is not inhibited by administration of excessive amounts of liposomes. It is preferred that the liposome, when contacted with 50% human tear fluid for 60 minutes, subsequently exhibits at least 30%, such as between 50 and 60%, of maximum possible association with HT1080 cells after one hour of incubation. When testing this ability, the conditions set forth in Example 5 are used.

As is apparent from the examples below, the exact lipid composition of the liposomes of the invention dictates the kinetics of the liposomes' release of drugs to the eye, in particular for hydrophobic drugs. If administering a hydrophobic drug such as a corticosteroid to the eye in a simple aqueous solution, the release will be immediate – on the other hand, use of a lipid-based delivery system such as a liposome will delay or even prevent release, since the drug will remain in the hydrophobic environment of the delivery system. The present invention provides for an intermediate between these two extremes and utilises the ability of tear fluid to degrade certain but not all lipids in e.g. liposomes. So, in some embodiments, the liposome of the invention exhibits a release of hydrophobic active ingredient when said liposome is applied to the normal human eye, which is slower than release from an aqueous solution and faster than from reference liposomes comprising >20% mol/mol cholesterol. In these embodiments the liposome preferably releases hydrophobic active ingredient more slowly than a reference liposome having a net negative charge. The aqueous solution and the reference liposomes in these situations comprise the same amount of hydrophobic active ingredient as does the liposome. Expressed in terms of bioavailability, there will be an increase in bioavailability when using the liposomes of the invention which is at least twice as high as that provided by the reference liposomes. However, higher increases in bioavailability are contemplated, such as at least 2.5, at least 3, and at least 4 times.

The above-described embodiments entail that the liposome preferably comprises lipids that exhibit a negative charge as well as lipids that exhibit a positive charge, in particular after ocular administration – as shown in the examples, POPG and derivatives are interesting anionic/negatively charged lipids in the liposomes. The liposomes may consequently also include electroneutral lipids which are e.g. used to balance the charge of the liposome – for

instance, POPC and derivatives are, as seen in the examples, interesting neutral lipids to include.

In general, lipids that are electroneutral and which are useful in the liposomes of the invention comprise head groups selected from phosphatidylcholine and

5 phosphatidylethanolamine to which are coupled hydrophobic groups such as alkyl chains.

Lipids that are negatively charged and which are useful in the liposomes of the invention comprise head groups selected from phosphatidylserine, phosphatidylglycerol, phosphatidic acid, and phosphatidylinositol, to which are coupled hydrophobic groups such as alkyl chains.

10 The hydrophobic groups that form part of the lipids used in the liposomes of the invention are typically di-acyl or tri-acyl carbon chains having lengths from 8-24 hydrocarbons, cf. the description of alkyl groups below. The hydrocarbons may be saturated or include one or more double bonds.

The lipids in the liposomes may comprise or constitute phospholipids. The lipids may comprise a group selected from phosphatidylglycerol (PG), phosphatidylethanolamine (PE),
15 phosphatidylserine (PS), phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidic acid (PA), DPG (bisphosphatidyl glycerol), PEOH (phosphatidyl alcohol) and cholesterol.

As mentioned above, the liposome may comprise a cationic lipid, which is preferably selected from the group consisting of stearylamine (SA), dimethyldioctadecylammonium bromide (DDAB), 3 β -[N-(N',N'-dimethylaminoethane)-carbonyl]cholesterol (DC-Cholesterol), 1,2-
20 ditetradecanoyl-3-trimethylammonium-propane (DMTAP), DOTAP derivatives (1,2-dioctadecanoyl-3-trimethylammonium-propane, 1,2-di-(9Z-octadecenoyl)-3-trimethylammonium-propane, 1,2-dihexadecanoyl-3-trimethylammonium-propane), DODAP derivatives (1,2-di-(9Z-octadecenoyl)-3-dimethylammonium-propane, 1,2-ditetradecanoyl-3-dimethylammonium-propane, 1,2-dihexadecanoyl-3-dimethylammonium-propane, 1,2-
25 dioctadecanoyl-3-dimethylammonium-propane), 1,2-di-O-octadecenyl-3-trimethylammonium propane (DOTMA), dioctadecylamide-glycylspermine, SAINT-2, polycationic lipid 2,3-dioleoyloxy-N-[2(spermine-carboxamido)ethyl]-N,N-dimethyl-1-propanaminiumtrifluoroacetate (DOSPA), GL67TM, 1,2-dioctadecanoyl-sn-glycero-3-ethylphosphocholine (Etyl PC), and 1,2-dioctadecanoyl-sn-glycero-3-phosphoethanolamine (DSPE).

30 Preferred cationic lipids are DOTAP and DOTAP derivatives. Additional examples of cationic lipids and lipid components may be found in or made according to US 4,804,539.

According to the present invention, it is preferred that the liposomes comprise less than 20% (mol/mol), such as less than 10%, preferably less than 5%, more preferred less than 2% cholesterol, most preferred substantially no cholesterol. However amounts of cholesterol up to about 10% appear to be acceptable, so amounts in the range between 0 and 10% are also within the invention, such as amounts between 1 and 9%, 2 and 8% and 3 and 7%.

The liposomes of the invention preferably include lipids, which when relevant, contain alkyl chains that are C8–C24, preferably C10–C22, more preferred C12–C20, preferably C14–C18, most preferred C16–C18 saturated chains or unsaturated chains, preferably saturated chains the two latter mentioned species may have chain lengths of C12–C18, and may also include double bonds.

Preferred liposomes of the invention are in the form of Large Unilamellar Vesicle (LUV), meaning that LUVs are preferred components of composition comprising liposomes of the invention.

Typically, the liposome of the present invention has a diameter of 50–10,000 nm, preferably 60–5,000 nm, more preferred 70–1,000 nm, preferably 80–120 nm, more preferred 100–500 nm, preferably 200–7,500 nm, more preferred 300–3,000 nm, preferably 500–2,000 nm, more preferred 1,000–1,500 nm.

In some embodiments, the liposome does not comprise a hydrophilic polymer conventionally used in preparation of liposomes. For instance, it is preferred that the liposome does not comprise a polymer selected among PEG [poly(ethylene glycol)], PAcM [poly(N-acryloylmorpholine)], PVP [poly(vinylpyrrolidone)], PLA [poly(lactide)], PG [poly(glycolide)], POZO [poly(2-methyl-2-oxazoline)], PVA [poly(vinyl alcohol)], HPMC (hydroxypropylmethylcellulose), PEO [poly(ethylene oxide)], chitosan [poly(D-glucosamine)], PAA [poly(aminoacid)], polyHEMA [Poly(2-hydroxyethylmethacrylate)] and co-polymers thereof. In preferred embodiments, the liposome contains essentially no hydrophilic polymer.

In other embodiments, the liposome comprises that at least part of the lipids comprise or is conjugated to a hydrophilic polymer, such as the hydrophilic polymers discussed above, subject to the proviso that the average molecular weight of hydrophilic polymer for the totality of the lipids is less than 2,000 Dalton, preferably less than 1,500 Dalton, more preferred less than 1,000 Dalton, preferably less than 500 Dalton, more preferred less than 300 Dalton, preferably less than 200 Dalton, more preferred less than 100 Dalton.

The liposome according to any one of the preceding claims, wherein at least one lipid is a substrate for enzyme(s) in human tear fluid or for sPLA₂.

The liposome of the invention include an active ingredient, typically a drug substance or composition. Typically, the liposome comprises substantially all of said at least one active ingredient. Normally, one of the compartments selected among the interior aqueous compartment, a hydrophobic bilayer, and a polar inter-phase of the inner and outer leaflet of the liposome carry said at least one active ingredient.

The at least one active ingredient is generally selected from drugs or drug composition useful in therapeutic or prophylactic treatment or amelioration of conditions of the eye. The active ingredient may also be one which may be administered to the eye but which exhibits its intended clinical effect in a different anatomical location. The skilled person will generally be knowledgeable about the choice of active ingredient and the correct dosage thereof. Typical drugs are antimicrobial drugs/antibiotics/antiviral drugs as well as immunomodulating or anti-inflammatory drugs as well as drugs that exert their effects on nerve tissue or on vasculature. A special group of active ingredients are relevant in the treatment of glaucoma.

In embodiments, said at least one active ingredient is selected from the group consisting of acetazolamide, acyclovir, indomethacin, verapamil, rapamycin, ascomycin, ciprofloxacin, ofloxacin, fusidin, gentamicin, chloramphenicol, levofloxacin, oxytetracyclin, tobramycin, acyclovir, prednisolon, dexamethason, chloramphenico, brimonidin, brimonidintartrat, dorzolamid, timolol, latanoprost, tetrahydropyridinehydrochlorid, and natriumcromoglicat.

Also the at least one active ingredient is a non-steroidal anti-inflammatory drug (NSAID), preferably selected from the group consisting of ketoprofen, flurbiprofen, ibuprofen, diclofenac, ketorolac, nepafenac, amfenac and suprofen.

In preferred embodiments, the active ingredient is in the form of a hydrophobic active ingredient, such as a hydrophobic drug (substance or composition). Particularly interesting hydrophobic drugs are selected from the group consisting of Diclofenac, Fusidine, Levocabastin, Indometacin, Latanoprost, Travoprost, Opatadin, Azelastin, Rimexolon, ketotifen, naphazolin, Bimatoprost, Betaxolol, emedastin, Ketorolac, Resorcinol, Fluormetolon, Atropine, Apraclonidin, Cyclopentolat, Dexamethasone, Lidocain, Prednisolone, Nepafenac, Timolol, Tropicamid, Brimonidine, Chloramphenicol, Pilocarpine, Vancomycin, Fluconazol, Sulfamethizol, and Moxifloxacin.

Pharmaceutical compositions of the invention

As discussed above, the liposomes of the present invention are useful as constituents of pharmaceutical formulations of the invention. Any form of such a formulation conventionally used for pharmaceuticals for direct application to the human or animal eye are contemplated

and a general reference to the requirements for and preparation of such formulations can be found in Bartlett & Jaanus: Clinical Ocular Pharmacology, Section 1 (chapters 2-4), 2007, ISBN 10: 0-7506-7576-4, ISBN 13: 978-0-7506-7576-5.

The pharmaceutical formulation of the invention is preferably in the form of a cream, an ointment, a gel, a paste or eye-drops.

It is preferred that the pharmaceutical formulation provides release of said at least one active ingredient during a period of 0.5-24 hours, preferably 1-12, more preferred 2-8, preferably 3-6 hours. As detailed above, the precise kinetics of the liposomes forming part of the pharmaceutical composition may be adjusted to aim precisely at a suitable release profiled. For instance, said pharmaceutical formulation can e.g. comprise a mixture of liposomes of the invention with different release characteristics, whereby the pharmaceutical formulation provides sustained release of said at least one active ingredient.

Therapeutic uses or methods

Also part of the invention is the liposome or the lipid-based drug delivery system or the pharmaceutical composition of the invention for use as a pharmaceutical. In particular, these aspects of the invention may be for use in prophylactic or therapeutic treatment or amelioration of an ophthalmic indication, such as post-operative pain or ocular inflammation, such as an ocular inflammation which results from iritis, conjunctivitis, seasonal allergic conjunctivitis, acute and chronic endophthalmitis, anterior uveitis, uveitis associated with systemic diseases, posterior segment uveitis, chorioretinitis, pars planitis, masquerade syndromes including ocular lymphoma, pemphigoid, scleritis, keratitis, severe ocular allergy, corneal abrasion or blood-aqueous barrier disruption. Also, they may be for use in treatment or amelioration of the indication post-operative ocular inflammation, such as inflammation resulting from photorefractive keratectomy, cataract removal surgery, intraocular lens implantation or radial keratotomy. Finally, they may also be for use in treatment or amelioration of an indication selected from the group consisting of fungal infection; microbial infection; inflammatory disease; non-ocular inflammatory disease, such as rheumatoid arthritis (RA); dry eye disease and dry eye condition, such as episodic or chronic dry eye condition and aqueous tear deficiency (ATD); keratoconjunctivitis sicca (KCS), such as Sjögren syndrome (SS), associated KCS and non-SS associated KCS; mucin deficiency; Stevens-Johnson syndrome; ocular injury such as chemical burn; chronic blepharitis; contact lens intolerance; chronic ocular surface inflammation; inflammation of ocular surface disease; glaucoma, such as open-angle glaucoma, primary open-angle glaucoma (POAG) and exfoliation glaucoma (AG); conjunctival inflammation; and corneal disease, such as allergy, conjunctivitis (Pink Eye), corneal infection, Fuchs' Dystrophy, Herpes Zoster (Shingles),

iridocorneal endothelial syndrome, keratoconus, lattice dystrophy, map-dot-fingerprint dystrophy, ocular herpes, and pterygium.

Consequently, the present invention also relates to a method for prophylactic or therapeutic treatment or amelioration of an ocular or intraocular disease or condition, the method comprising administering, ocularly or intraocularly, to a subject in need thereof an effective amount of a liposome, lipid based drug delivery system, or a pharmaceutical composition of the invention; the ocular or intraocular disease or condition is preferably identical to or associated with any one of the indications discussed in the previous paragraph.

All cited references are incorporated in their entirety herein.

10 EXAMPLE 1

Liposome preparation

Unilamellar fully hydrated liposomes were made from 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol (POPG), 1,2-dihexadecanoyl-3-trimethylammonium-propane (DOTAP). As a fluorescence marker to measure presence of liposomes in biological systems, 0.2% DOPE-rhodamine was mixed with the lipids as a tracer. The molar ratios of each lipid in the liposomes are outlined in figure 1A. POPC, POPG and DOTAP were all obtained from Avanti Polar lipids. Briefly, weighed amounts of POPC, POPG and DOTAP were dissolved in chloroform. The solvent was removed by a gentle stream of N₂ and the lipid films were dried overnight under low pressure to remove trace amounts of solvent. Multilamellar vesicles were prepared by dispersing the dried lipids in a buffer solution containing: 150 mM KCL, 10 mM HEPES (pH = 7.5), 1 mM NaN₃, 30 μM CaCl₂ and 10 μM EDTA. The multilamellar vesicles were extruded ten times through two stacked 100 nm pore size polycarbonate filters as described by Mayer et al., Biochim. Biophys. Acta, 858, 161-168.

25 EXAMPLE 2

Characterization of liposome size and surface charge dependent on composition

Liposomes prepared as outlined in figure 1A were prepared with the attempt to design liposomes with ability to adhere to the ocular surface of the eye as demonstrated by L. Guo, C.T. Redmann and R. Radhakrishnan, US patent No. 4,804,539, 1986. Charged liposomes

with up to 40% positively charged lipids show an increased adherence to ocular tissue, most likely due to presence of the negatively charged glycoprotein mucin, possibly combined with the negative surface charge of mammalian cells. Therefore, we designed liposomes with 10 or 20% net positive charge (formulation 4 and 5 in figure 1A), together with control liposomes with negative or nearly neutral charge (formulation 1-3 in figure 1A). The liposomes were prepared as described in example 1, and their size measured in nanometer (nm) by dynamic light scattering on a ZetaPALS zeta potential analyzer from Brookhaven Instruments. The liposomes showed sizes between 130-180 nm in diameter (Figure 1B). The surface charge (Zeta potential) of the liposomes, was measured in mV and showed surface charge dependent on lipid composition (figure 1C). High content of the negatively charged POPG (60 molar percent) in the liposome, showed negative surface charge of -35 mV (figure 1C, composition 1). High content of the neutral lipid POPC (100 molar percent) in the liposome showed weak negative charge (figure 1C, composition 2). With increased negative and positively charged lipids with the neutral POPC (molar percent 80:10:10), the charged lipids counteracted each other, and resulted in a net weak negative charge (figure 1C, composition 3). Increased amounts of DOTAP to 20% (molar percent 70:10:20), showed a net positive charge of nearly 30 mV (figure 1C, composition 4), whereas the highest amount of DOTAP at 30 mol% (molar percent 60:10:30), showed a net positive charge of 40 mV (figure 1C, composition 5). Formulation 4 and 5 are therefore expected to be the liposomes with adherence to ocular and cellular surfaces due to their electrostatic attraction.

EXAMPLE 3

Liposome hydrolysis in human tear fluid dependent on lipid composition

Tear fluid derived secretory phospholipase A2 type IIA (sPLA₂) which is present in very high amounts in human tear fluid (ranging from 49.7 µg/ml in men to 55,7 µg/ml in women) (O. Kari, V.V. Aho et al., Acta Ophthalmologica Scandinavia, 2005, 83, 483-486), was used to analyse liposome degradation. Human tear fluid was collected from the eye of a number of adults, pooled and distributed in tubes and stored frozen at -20 C. The liposomes were incubated in 50% tear fluid and PBS for 1 and 24 h at 37 C, and frozen until further analyses. For analyses of lipid degradation by sPLA₂, the liposome samples were mixed with 2:9 with DHB matrix in methanol and Maldi-TOF spectra were recorded. The Maldi-TOF peak intensity of (lyso-phosphatidyl choline C16) LPC16-Na (m/z=518.4) and POPC-Na (m/z=782.6) were collected and the ratio $R = \frac{PI_{LPC16}}{(PI_{POPC} \times \%_{POPC})}$ was calculated. PI_{LPC16} and PI_{POPC} are the peak intensities of LPC16-Na and POPC-Na and $\%_{POPC}$ denoted fractional content of POPC in the formulation. Zero hydrolysis of the liposome by sPLA₂ corresponds to $R=0$ whereas full hydrolysis corresponds to a large/infinite R-value (Fig. 2). All five formulations can be

hydrolyzed by sPLA₂ in tear fluid, and the level of hydrolysis increases from 1h to 24h incubation. Formulation 1 shows the largest level of hydrolysis which is expected due to the high POPG content, POPG being a good substrate for sPLA₂. The level of hydrolysis of formulation 2 is low, which corresponds to the fact that POPC is a poor sPLA₂ substrate. When POPG is included in the formulations, sPLA₂ has the ability to hydrolyse the lipids to larger extent as seen for formulation 3, but unexpectedly, the hydrolysis profile increases with increased DOTAP content and net positive surface charge. This is unexpected because sPLA₂ is known to have higher preference for negatively charged membranes. One explanation may be that the very high amounts of sPLA₂ causes hydrolysis of otherwise poor substrates.

10 EXAMPLE 4

Liposome association with cells dependent on lipid composition and charge properties

Liposomes prepared according to the composition set forth in Fig. 1 were added to human cells with negative charge properties similar to the negatively charged corneal surface. The human fibrosarcome cell line HT1080 was used as a model system for a negatively charged biological membrane. HT1080 cells were seeded with 10.000 cells/well, and allowed to grow to confluency over 4 days, where the cell layer was completely dense, mimicking the well differentiated and dense corneal surface. The cells were washed with serum free media 3 times, and serum free media added to the cells in a volume of 25 µl/well. 5 µl liposome (of a 5 mM stock) was added to each well, and incubated for 10, 30, 60 and 120 min prior to washing in cell media three times. The total absorbance was measured at time of addition of liposome as a measure of total amount of added liposome (Fig. 3, t=0). After 10 min incubation and subsequent washing, formulation 4 was the only one that was associated with the cells, where 20% of total amount added was associated with the cells. Formulation 4 increased cell association to a maximum of 40% after 120 min of incubation. Formulation 5 was associated with the cells to nearly 10% of added liposome after 30 min of incubation, and increased to a maximum of 20% after 60 min of incubation (Fig. 3). It can be concluded that liposomes with a net positive charge most efficiently associates with the cells, whereas negatively charged or neutral liposomes showed no or poor cell association.

EXAMPLE 5

Enhancement of liposome cell association in presence of human tear fluid dependent on lipid composition and charge properties

Positively charged liposomes show a strongly enhanced retention on ocular surfaces, associated with enhanced bioavailability and stronger therapeutic effect of the associated drug (L. Guo, C.T. Redmann and R. Radhakrishnan, US patent No. 4,804,539, 1986, and R. M. Hathout et al., AAPS PharmSciTech 2007, 8(1), E1-E12.). For hydrophilic drugs like acetazolamide, which are encapsulated inside the liposome, the release profile, bioavailability and therapeutic effect was improved. However, for lipophilic drugs like e.g. glucocorticoids and prostaglandins, the drugs will remain in the liposome or lipid structures and not become bioavailable to the same extent as hydrophilic drugs. Therefore, a drug-release mechanism is required in order to make lipophilic drugs active for treatment of ocular diseases from the liposomes which adhere to the corneal surface. The combination of cationic liposomes which adheres to the ocular surface with the release mechanism provided by the tear fluid derived sPLA₂ mediated liposome degradation, will provide a drug delivery system which will allow better ocular surface retention, higher ocular bioavailability and increased therapeutic activity of lipophilic drugs in the eye.

The effect of tear fluid sPLA₂ on liposomal association with human cells as model system for ocular surface retention was investigated. Liposomes were incubated without lipase, with 50% tear fluid or with 50% bee venom lipase (figure 4A and B). After 60 min incubation, liposomes were added to the cells for another 60 min, washed 3 times and fluorescence intensity remaining in the cells measured. After addition of liposomes the total fluorescence intensity was measured (figure 4A). Normalized to the total amount of fluorescence intensity, the liposomes without lipase associated with cells similar to what was seen in figure 3, with mainly cationic liposomes associated with the cells (figure 4B, formulation 4 and 5). Incubated with tear fluid, formulation 4 and 5 showed a dramatic increase in cell associated fluorescence intensity, indicating that these liposomes were further activated by sPLA₂, and showed that 50-60% of total added liposome associated with the cells. Incubation with bee venom lipase did not have this effect. This shows that the increased effect was a specific effect of tear fluid and the human sPLA₂ subtype, and that this effect can not be obtained using other types of sPLA₂ such as the bee venom sPLA₂, which has much broader substrate specificity. None of the tested liposomes showed signs of cytotoxicity towards the cells. There was no change in viability when cells were incubated for 10, 30, 60 or 120 min with liposomes, subsequently washed and cultured for 3 h in XTT to measure cell viability (figure 5). Neither was there any change in viability between incubation with the different liposomes.

EXAMPLE 5

Release of lipophilic drugs in cell culture model systems with e.g. glucocorticoids or prostaglandins from cationic liposomes with adherence to ocular or cellular surfaces and sensitivity towards tear fluid sPLA₂

- 5 Liposomes are prepared using cationic liposomes with a lipid composition of net 0-30% positive charge, e.g. POPC:POPG:DOTAP compositions of 70:10:20, 60:10:30, 50:10:40 or between these ratios. Liposomes are prepared as in EXAMPLE 1, but with addition of 0.1-5% of dexamethasone or prostaglandin or their analogues, and with 0.2% DOPE-rhodamine. The liposomes are added to cultured cells where the cells are seeded in 96 well plates 4 days
- 10 prior to the experiment with a cell density of 10.000 cells/well in cell culture media containing 10% FCS. Prior to the experiment the cells are washed 3 times with media with no serum. 10 µl of serum free media is added to the cells, 15 µl of human tear fluid and 5 µl of liposome (5 mM) are added to the cells for a certain time, typically between 5-60 min. The cells are washed three times with media and cell-associated liposomes measured as fluorescence
- 15 intensity in each well. The cells are assayed for presence of drug in several ways. 1) Dexamethasone is measured in cell lysate using an ELISA kit against dexamethasone. 2) Dexamethasone or its analogues can be assayed for biological activity where a human cell line or primary cells after 1-24 h of liposome contact and washing are exposed to an immune stimulating reagent like LPS, PMA, TNFα, Poly I:C or another TLR-ligand. After approximately
- 20 24 h the conditioned media is analysed for suppression of cytokine secretion compared to non-liposome treated cells, or cells treated with liposomes which do not adhere to the cells, but which contains dexamethasone. For a prostaglandin, a similar approach is used. A biological endpoint can be assayed using agonists or antagonists towards the relevant prostaglandin receptor, and measurement of the prostaglandin itself. This can be done using
- 25 Mass spectrometry, HPLC, RIA, EIA or ELISA kits.

Example 6

Analyses of retention of liposomes on human eyes

- Liposomes are prepared using cationic liposomes with a lipid composition as in EXAMPLE 5. Liposomes are prepared as in EXAMPLE 1, but with addition of fluorophore like rhodamine,
- 30 fluorescein. This can be 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(DOPE rhodamine) or 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(carboxyfluorescein) (DOPE-fluorescein) which are both headgroup labelled phospholipids. Alternatively, a fluorophore on the acyl chain can be used like e.g. 1-oleoyl-2-{6-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]hexanoyl}-sn-glycero-3-phosphoethanolamine (18:1-06:0 NBD

PE), where NBD is located at the sn-2 position of the phospholipid. The liposomes are prepared according to GMP in clinical grade quality. The liposomes are added to the ocular surface of healthy volunteers, where the liposome formulation is added to one eye, and a control fluorophore to the other eye. The fluorophores must be the same, added in same amount at the same time. Subsequently, the fluorescence signal is measured over time in both eyes, and the difference in signal intensity is registered. If the liposome formulated fluorophore is present for longer time on the cornea, the liposome carrier system causes a longer retention time on the cornea, and is thus an attractive carrier system for ocular drug delivery. Similar experiments may be carried out on laboratory animals like mice, rats, rabbits, minipigs, guinea pigs or other species.

EXAMPLE 7

Analyses of therapeutic effect of glucocorticoid containing liposomes on animal or human eyes

Clinical grade liposomes are prepared according to EXAMPLE 1 and 5. A suitable drug is formulated with the liposome. Suitable drugs are lipophilic drugs belonging to the glucocorticoid class like dexamethasone or its analogues (e.g. fluormetolon, prednisolone or rimexolon). Glucocorticoid containing liposomes are useful for treatment of non-infectious inflammatory conditions in the eye like e.g. iritis, scleritis or episcleritis. Non-liposome formulated glucocorticoids are recommended applied 3-6 times per day, and in severe conditions every hour. The present liposome formulated glucocorticoids will be applied once or twice daily to obtain a similar therapeutic effect. Human volunteers with non-infectious inflammatory conditions in the eye like e.g. iritis, scleritis or episcleritis are included in a clinical test, and treated twice daily with liposomal glucocorticoids. A control group is treated with same amounts of non-liposome formulated glucocorticoids two to three times daily. The two groups are compared for therapeutic effect, and tear fluid is obtained after treatment to measure bioavailability dependent on time after treatment, typically 30, 60, 120, 240 minutes after administration. Similar experiments may be carried out on rabbits or other laboratory animals.

EXAMPLE 8

Analyses of therapeutic effect of prostaglandin containing liposomes on animal or human eyes

Liposomes are prepared according to EXAMPLE 1 and 5. Example of another suitable drug group for the present technology are prostaglandin or its analogues (bimatoprost, tafluprost, latanoprost, travoprost) which are useful for treatment of glaucoma. The prostaglandin of interest is formulated with the lipids in the liposome in clinical grade quality. Human volunteers with glaucoma are allocated to two groups, one treated with liposomal prostaglandins, the other with non-liposomal standard prostaglandin. Both groups are treated once daily in the evening. The two groups are clinically examined for side effects after 1-4 weeks treatment. The liposome treated group are expected to show fewer side effects, either because a lower dose is required, or because additives for the liposomal formulation are different from additives in the free formulation. In particular the presence of benzalkoniumchloride in the free formulation causes side effects.

EXAMPLE 9

Test of release of encapsulated drug in animal model

Liposomes are prepared as described in EXAMPLE 1. The liposomes are added to the cornea of a laboratory animal, typically a mouse, rat, rabbit, dog or pig. Controls for such study is the same free drug at the same dose as used in the encapsulated drug in liposomes. Eye fluid is removed from the animal eye at appropriate time points within the first 24 h after administration, and the amount of drug determined at each time point for each formulation. The release profile which is optimal for the specific drug can then be optimized by adjusting the amount of positive and negative charges in the liposome. A slow release can be prepared using liposomes with 10-50% positive charge, potentially with amounts of cholesterol (0-10 mol%). A faster release profile can be achieved by introducing negatively charged phospholipids in the positively charged liposome (e.g. POPG) and reducing the amount of cholesterol.

EXAMPLE 10

Eye drop formulation

An eye formulation may be made as indicated in the patent US 5145680, by interchanging the active ingredient with liposomes of the present invention carrying an active ingredient.

CLAIMS

1. A liposome for ocular or intra-ocular administration, said liposome comprising:
lipids and at least one active ingredient;
said liposome preferably exhibiting a charge increasing the adherence of said liposome to the
5 eye after administration, in particular when said liposome is for ocular administration;
wherein at least one of the lipids in the liposome is degradable by sPLA₂ activity, thereby
allowing sustained or delayed release of said at least one active ingredient from said
liposome upon contact with tear fluid or with sPLA₂ in the eye or in the vicinity of the eye.
2. The liposome according to any claim 1, wherein at least part of the lipids exhibit a
10 positive charge, particularly after ocular administration.
3. The liposome according to claim 2, wherein at most 30% (mol/mol) lipids exhibit a
positive charge.
4. The liposome according to claim 1 or 2, which has a net positive charge after
administration.
- 15 5. The liposome according to claim 4, wherein the liposome has a zeta potential in the
range from 10-60 mV.
6. The liposome according to claim 1, wherein the lipids are substantially not charged.
7. The liposome according to any one of the preceding claims, which when contacted with
50% human tear fluid for 60 minutes under the conditions described in Example 5
20 subsequently exhibits at least 40% of maximum possible association with HT1080 cells after
one hour of incubation.
8. The liposome according to claim 7, wherein the liposome when contacted with 50%
human tear fluid for 60 minutes subsequently exhibits at least 50%, such as between 50 and
60%, of maximum possible association with HT1080 cells after one hour of incubation.
- 25 9. The liposome according to any one of the preceding claims, wherein the release of
hydrophobic active ingredient when said liposome is applied to the normal human eye is
slower than from an aqueous solution and faster than from reference liposomes comprising
>20% mol/mol cholesterol, and where the liposome preferably releases hydrophobic active
ingredient more slowly than a reference liposome having a net negative charge, where the

aqueous solution and the reference liposomes comprise the same amount of hydrophobic active ingredient as does the liposome.

10. The liposome according to any one of the preceding claims, which comprises lipids that exhibit a negative charge as well as lipids that exhibit a positive charge, in particular after
5 ocular administration.

11. The liposome according to any one of the preceding claims, which also comprise lipids that are electroneutral, in particular after ocular administration.

12. The liposome according to any one of the preceding claims, which comprise substantially all of said at least one active ingredient.

10 13. The liposome according to any one of the preceding claims, wherein one of the compartments selected among the interior aqueous compartment, a hydrophobic bilayer, and a polar inter-phase of the inner and outer leaflet of the liposome carry said at least one active ingredient.

14. The liposome according to any one of the preceding claims, wherein said at least one
15 active ingredient is selected from the group consisting of acetazolamide, acyclovir, indomethacin, verapamil, rapamycin, ascomycin, ciprofloxacin, ofloxacin, fusidin, gentamicin, chloramphenicol, levofloxacin, oxytetracyclin, tobramycin, acyclovir, prednisolon, dexamethason, chloramphenico, brimonidin, brimonidintartrat, dorzolamid, timolol, latanoprost, tetrazyolinhydrochlorid, and natriumcromoglicat.

15. The liposome according to any one of the preceding claims, wherein said at least one active ingredient is a non-steroidal anti-inflammatory drug (NSAID), preferably selected from the group consisting of ketoprofen, flurbiprofen, ibuprofen, diclofenac, ketorolac, nepafenac, amfenac and suprofen.

16. The liposome according to any one of the preceding claims, wherein the active
25 ingredient is in the form of a hydrophobic active ingredient, such as a hydrophobic drug.

17. The liposome according to claim 16, wherein the hydrophobic active ingredient is selected from the group consisting of Diclofenac, Fusidine, Levocabastin, Indometacin, Latanoprost, Travoprost, Opatadin, Azelastin, Rimexolon, ketotifen, naphazolin, Bimatoprost, Betaxolol, emedastin, Ketorolac, Resorcinol, Fluormetolon, Atropine,
30 Apraclonidin, Cyclopentolat, Dexamethasone, Lidocain, Prednisolone, Nepafenac, Timolol,

Tropicamid, Brimonidine, Chloramphenicol, Pilocarpine, Vancomycin, Fluconazol, Sulfamethizol, and Moxifloxacin.

18. The liposome according to any of the preceding claims, wherein the lipids comprise or are phospholipids.

19. The liposome according to any of the preceding claims, where the lipid comprises or constitutes phosphatidylglycerol (PG), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidic acid (PA), DPG (bisphosphatidyl glycerol), PEOH (phosphatidyl alcohol) and cholesterol.

20. The liposome according to any of the preceding claims, wherein at least part of the lipids is a cationic lipid, preferably selected from the group consisting of stearylamine (SA), dimethyldioctadecylammonium bromide (DDAB), 3β -[N-(N',N'-dimethylaminoethane)-carbamoyl]cholesterol (DC-Cholesterol), 1,2-ditetradecanoyl-3-trimethylammonium-propane (DMTAP), DOTAP derivatives (1,2-dioctadecanoyl-3-trimethylammonium-propane, 1,2-di-(9Z-octadecenoyl)-3-trimethylammonium-propane, 1,2-dihexadecanoyl-3-trimethylammonium-propane), DODAP derivatives (1,2-di-(9Z-octadecenoyl)-3-dimethylammonium-propane, 1,2-ditetradecanoyl-3-dimethylammonium-propane, 1,2-dihexadecanoyl-3-dimethylammonium-propane, 1,2-dioctadecanoyl-3-dimethylammonium-propane), 1,2-di-O-octadecenyl-3-trimethylammonium propane (DOTMA), dioctadecylamide-glycylspermine, SAINT-2, polycationic lipid 2,3-dioleoyloxy-N-[2(spermine-carboxamido)ethyl]-N,N-dimethyl-1-propanaminiumtrifluoroacetate (DOSPA), GL67TM, 1,2-dioctadecanoyl-sn-glycero-3-ethylphosphocholine (Etyl PC), and 1,2-dioctadecanoyl-sn-glycero-3-phosphoethanolamine (DSPE).

21. The liposome according to any one of the preceding claims, wherein the liposomes comprise 0-60%, preferably 10-60%, such as 20-50% (mol/mol) cationic lipids.

22. The liposome according to any one of the preceding claims, wherein the liposomes comprise less than 20%, such as less than 10%, preferably less than 5%, more preferred less than 2% cholesterol, most preferred substantially no cholesterol.

23. The liposome according to any one of the preceding claims, wherein lipids that are electroneutral comprise head groups selected from phosphatidylcholine and phosphatidylethanolamine.

24. The liposome according to any one of the preceding claims, wherein lipids that are negatively charged comprise head groups selected from phosphatidylserine, phosphatidylglycerol, phosphatidic acid, and phosphatidylinositol.

25. The liposome according to any one of the preceding claims, wherein the alkyl chains of the lipids are C8–C24, preferably C10–C22, more preferred C12–C20, preferably C14–C18, most preferred C16–C18 saturated chains or unsaturated chains, preferably saturated chains.

26. The liposome according to any one of the preceding claims, wherein at least one liposome is a Large Unilamellar Vesicle (LUV).

27. The liposome according to any one of the preceding claims, wherein the liposomes have a diameter of 50–10,000 nm, preferably 60–5,000 nm, more preferred 70–1,000 nm, preferably 80–120 nm, more preferred 100–500 nm, preferably 200–7,500 nm, more preferred 300–3,000 nm, preferably 500–2,000 nm, more preferred 1,000–1,500 nm.

28. The liposome according to any one of the preceding claims, which does not comprise a hydrophilic polymer, such as a polymer selected among PEG [poly(ethylene glycol)], PAcM [poly(N-acryloylmorpholine)], PVP [poly(vinylpyrrolidone)], PLA [poly(lactide)], PG [poly(glycolide)], POZO [poly(2-methyl-2-oxazoline)], PVA [poly(vinyl alcohol)], HPMC (hydroxypropylmethylcellulose), PEO [poly(ethylene oxide)], chitosan [poly(D-glucosamine)], PAA [poly(aminoacid)], polyHEMA [Poly(2-hydroxyethylmethacrylate)] and co-polymers thereof.

29. The liposome according to any one of claims 1-27, wherein at least part of the lipids comprise or is conjugated to a hydrophilic polymer, such as the polymers defined in claim 28, subject to the proviso that the average molecular weight of hydrophilic polymer for the totality of the lipids is less than 2,000 Dalton, preferably less than 1,500 Dalton, more preferred less than 1,000 Dalton, preferably less than 500 Dalton, more preferred less than 300 Dalton, preferably less than 200 Dalton, more preferred less than 100 Dalton.

30. The liposome according to any one of the preceding claims, wherein at least one lipid is a substrate for enzymes in tear fluid, such as human tear fluid, e.g. a substrate for tear fluid sPLA₂.

31. Lipid based drug delivery system for ocular or intra-ocular administration, said system providing sustained or delayed release of at least one active ingredient, said system comprising:

(I) lipids comprising:

(a) an organic radical having a least 2 carbon atoms; and

(b) a hydrophilic moiety;

said lipids being a substrate for enzymes in tear fluid or for extracellular sPLA₂ to the extent that the organic radical can be hydrolytically cleaved off, leading to an intramolecular cyclization reaction; and

(II) at least one active ingredient;

said lipids allowing the formation of liposomes, said liposomes comprising at least part of said at least one active ingredient;

at least part of said liposomes exhibiting a charge, which increases the adherence of the charged liposomes to the eye after administration; and

wherein at least one of the lipids in the liposome is degradable by secretory phospholipase, in particular sPLA₂ activity, allowing sustained or delayed release of said at least one active ingredient from said liposomes upon contact with secretory phospholipase, in particular sPLA₂.

32. A pharmaceutical formulation suitable for ocular or intra-ocular administration and providing sustained or delayed release of at least one active ingredient, said pharmaceutical formulation comprising liposomes, said liposomes comprising at least part of said at least one active ingredient, and at least part of said liposomes are liposomes according to any one of claims 1-30.

33. The pharmaceutical formulation for ocular administration according to claim 32, in the form of a cream, an ointment, a gel, a paste or eye-drops.

34. The pharmaceutical formulation according to claim 32 or 33, providing release of said at least one active ingredient during a period of 0.5-24 hours, preferably 1-12, more preferred 2-8, preferably 3-6 hours.

35. The pharmaceutical formulation according to any one the claims 32-34-, wherein said pharmaceutical formulation comprises a mixture of liposomes with different release characteristics, whereby the pharmaceutical formulation provides sustained release of said at least one active ingredient.

36. The Liposome according to any one of claims 1-30 or the lipid-based drug delivery system according to claim 31 or the pharmaceutical composition according to any one of claims 32-35 for use as a pharmaceutical.

37. The Liposome according to any one of claims 1-30 or the lipid-based drug delivery system according to claim 31 or the pharmaceutical composition according to any one of claims 32-35 for use in treatment or amelioration of an ophthalmic indication, such as post-operative pain or ocular inflammation, such as an ocular inflammation which results from iritis, conjunctivitis, seasonal allergic conjunctivitis, acute and chronic endophthalmitis, anterior uveitis, uveitis associated with systemic diseases, posterior segment uveitis, chorioretinitis, pars planitis, masquerade syndromes including ocular lymphoma, pemphigoid, scleritis, keratitis, severe ocular allergy, corneal abrasion or blood-aqueous barrier disruption.

38. The Liposome according to any one of claims 1-30 or the lipid-based drug delivery system according to claim 31 or the pharmaceutical composition according to any one of claims 32-35 for use in treatment or amelioration of the indication post-operative ocular inflammation, such as inflammation resulting from photorefractive keratectomy, cataract removal surgery, intraocular lens implantation or radial keratotomy.

39. The Liposome according to any one of claims 1-30 or the lipid-based drug delivery system according to claim 31 or the pharmaceutical composition according to any one of claims 32-35 for use in treatment or amelioration of an indication selected from the group consisting of fungal infection; microbial infection; inflammatory disease; non-ocular inflammatory disease, such as rheumatoid arthritis (RA); dry eye disease and dry eye condition, such as episodic or chronic dry eye condition and aqueous tear deficiency (ATD); keratoconjunctivitis sicca (KCS), such as Sjögren syndrome (SS), associated KCS and non-SS associated KCS; mucin deficiency; Stevens-Johnson syndrome; ocular injury such as chemical burn; chronic blepharitis; contact lens intolerance; chronic ocular surface inflammation; inflammation of ocular surface disease; glaucoma, such as open-angle glaucoma, primary open-angle glaucoma (POAG) and exfoliation glaucoma (AG); conjunctival inflammation; and corneal disease, such as allergy, conjunctivitis (Pink Eye), corneal infection, Fuchs' Dystrophy, Herpes Zoster (Shingles), iridocorneal endothelial syndrome, keratoconus, lattice dystrophy, map-dot-fingerprint dystrophy, ocular herpes, and pterygium.

40. A method for prophylactic or therapeutic treatment or amelioration of an ocular or intraocular disease or condition, the method comprising administering, ocularly or intraocularly, to a subject in need thereof an effective amount of a liposome according to any one of claims 1-30, a lipid based drug delivery system according to claim 31 or a pharmaceutical composition according to any one of claims 32-35.

41. The method according to claim 40, wherein the ocular or intraocular disease or condition is associated with any one of the indications set forth in claims 36-39.

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<u>Liposomes</u>	<u>Expected:</u>	
	<u>PLA2 sensitivity</u>	<u>Cell binding</u>
1) 30:60:10 POPC:POPG:DOTAP	+++++	-
2) 100:0:0 POPC:POPG:DOTAP	+/-	-
3) 80:10:10 POPC:POPG:DOTAP	+/-	-
4) 70:10:20 POPC:POPG:DOTAP	-	+
5) 60:10:30 POPC:POPG:DOTAP	-	++

Fig. 1A

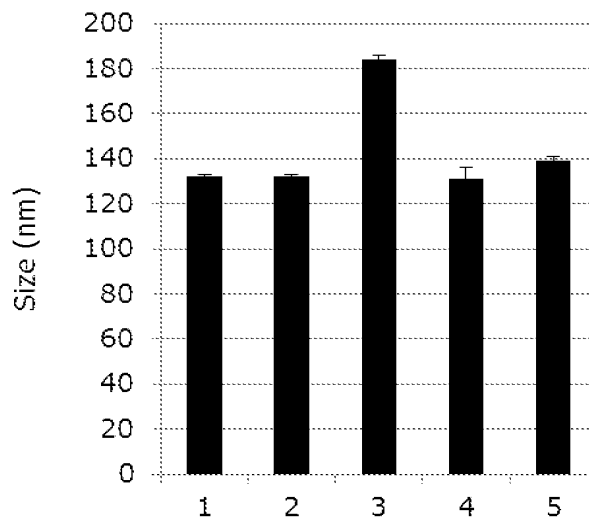


Fig. 1B

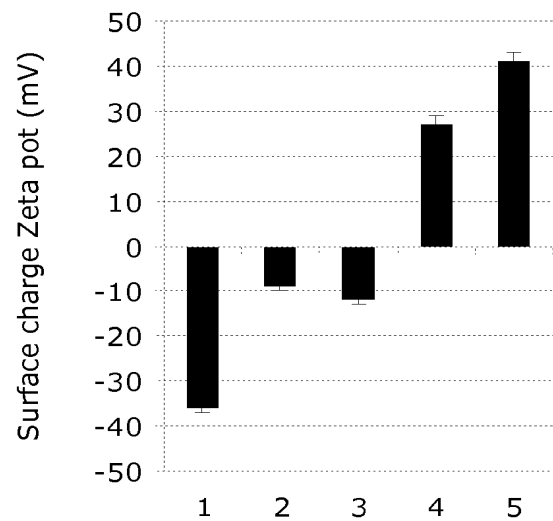


Fig. 1C

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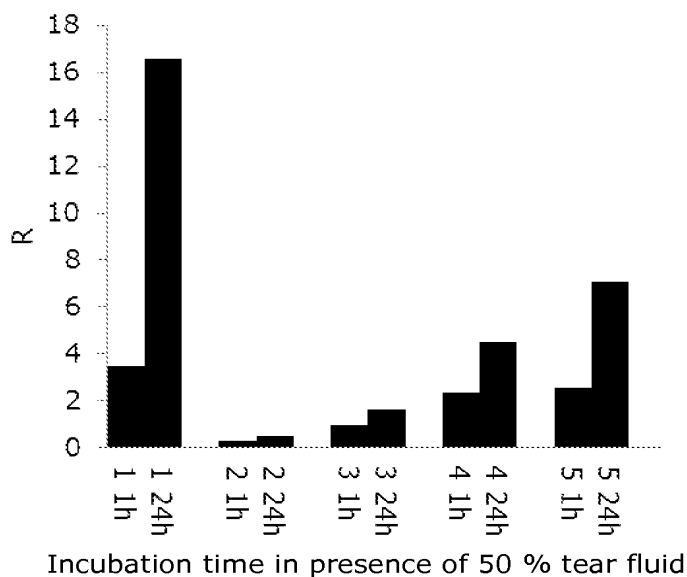


Fig. 2

$$R = \text{PI}_{\text{LPC16}} / (\text{PI}_{\text{POPC}} \times \%_{\text{POPC}})$$

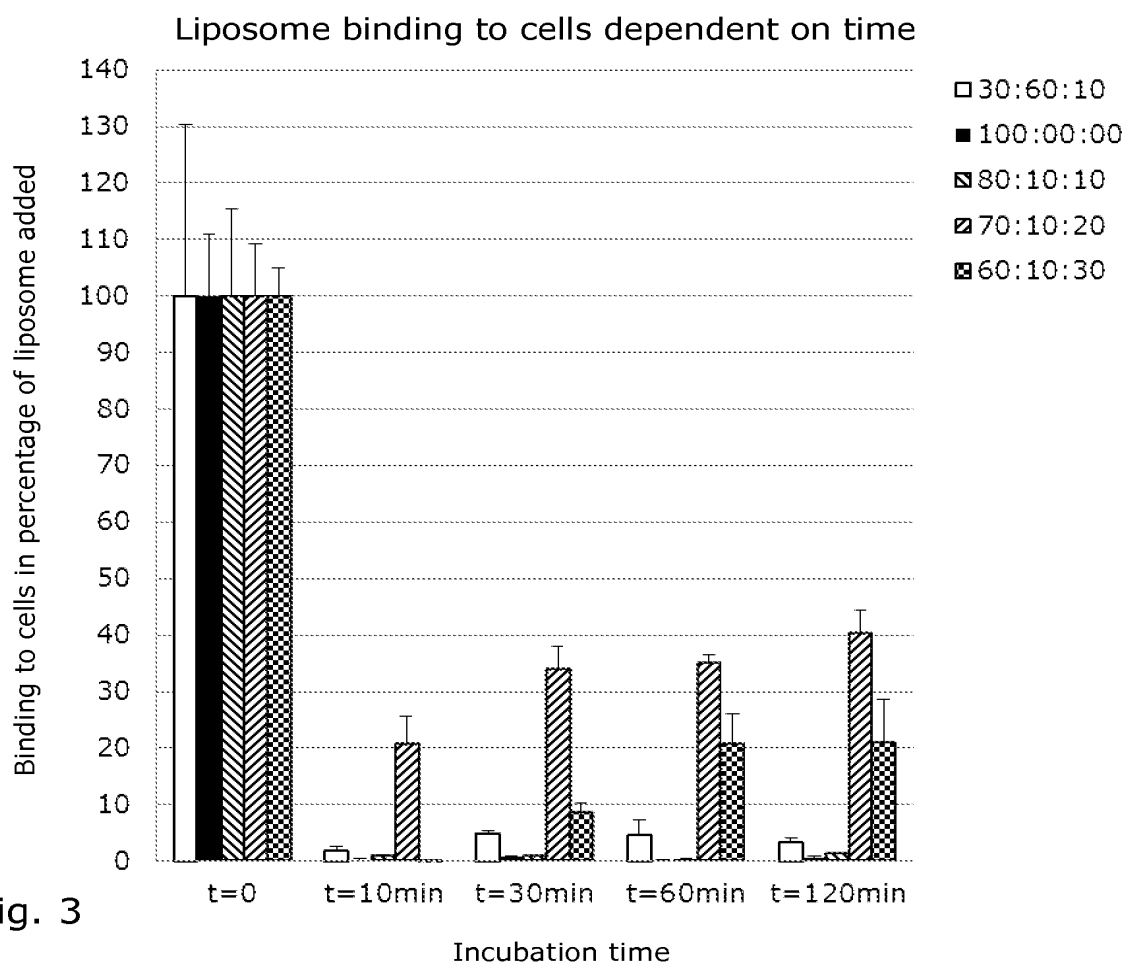


Fig. 3

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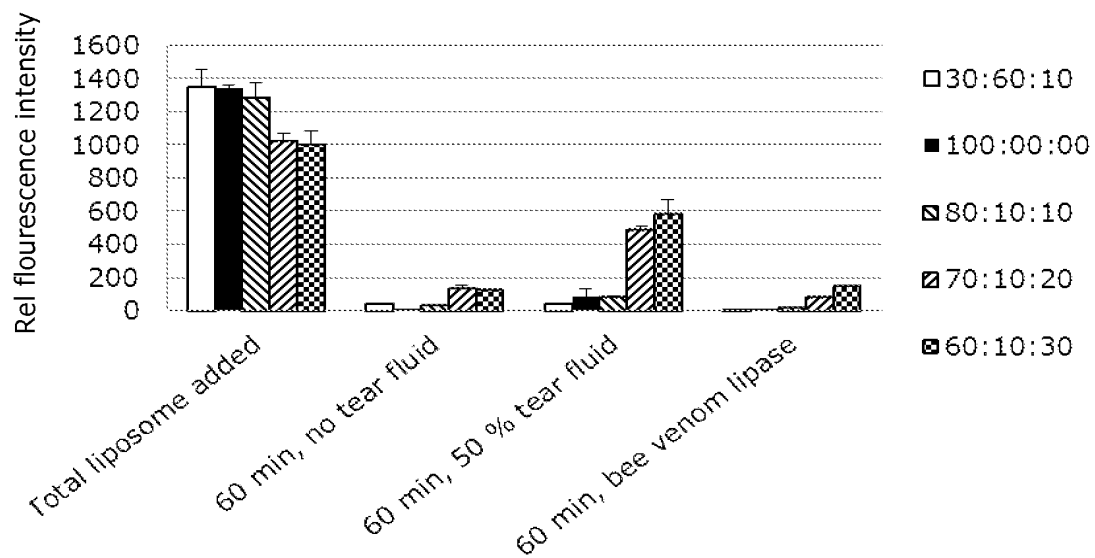


Fig. 4A

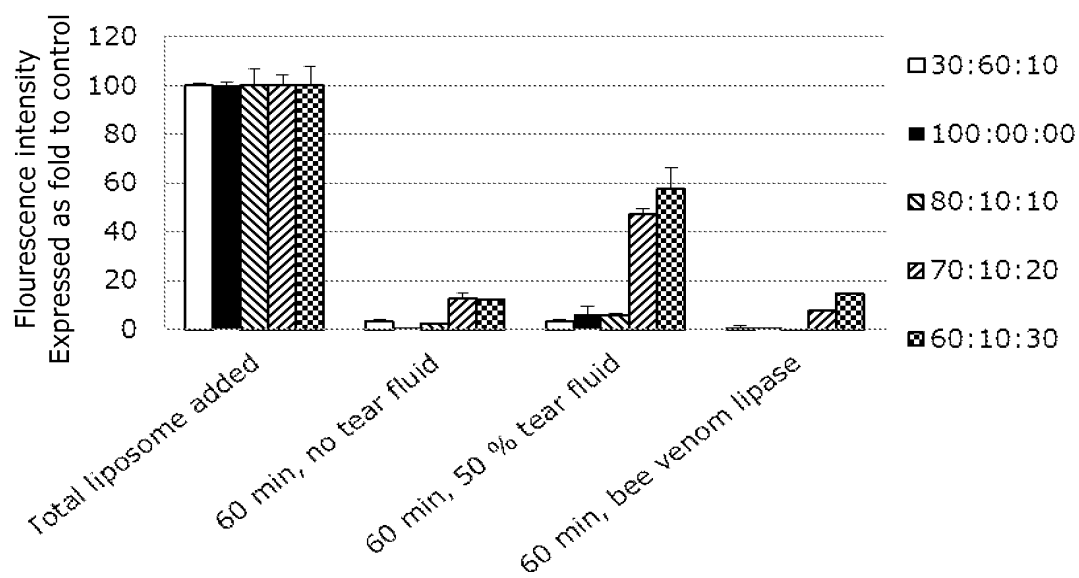


Fig. 4B

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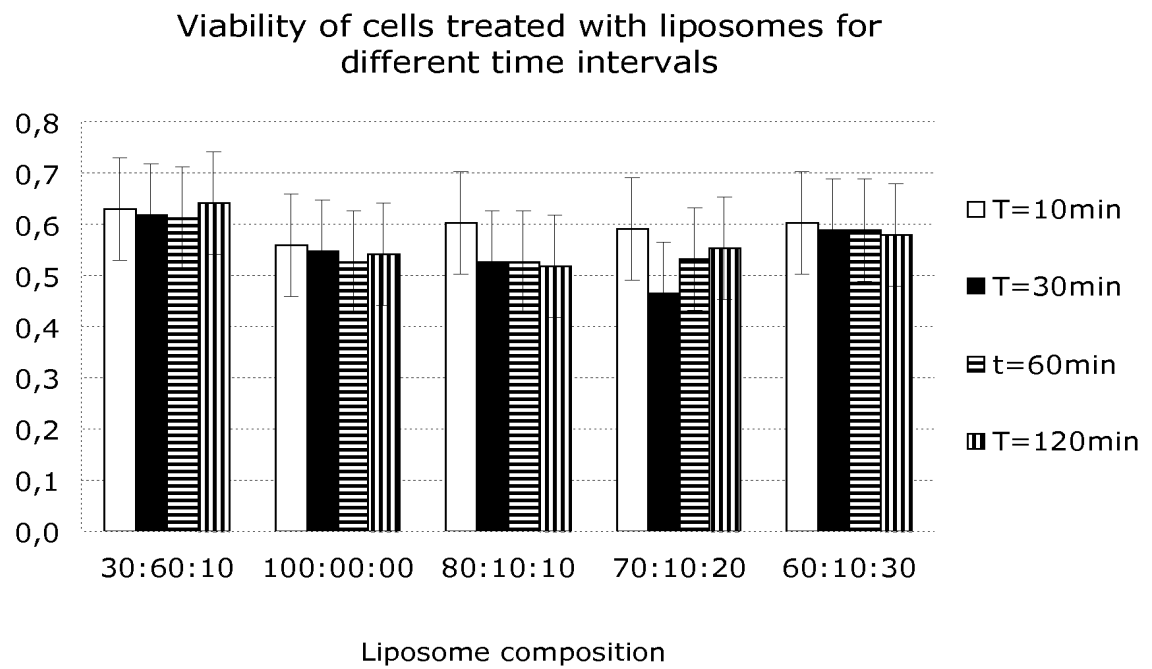


Fig. 5

Bibliographic data

Title: COMPOSITION AND/OR METHOD FOR REDUCING AND/OR PREVENTING MYOPIA PROGRESSION COMPRISING ATROPINE

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Abstract of WO2012161655A1

The invention relates to a composition and/or method for reducing and/or preventing myopia progression. The composition comprises an ultra-low atropine concentration. The composition comprises less than 0.025% atropine. For example, the composition comprises about 0.01% atropine. The ultra-low atropine composition reduces and/or prevents myopia progression with negligible side effects, such as loss of accommodation, mydriasis, near-vision blur, allergic conjunctivitis and dermatitis.



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(54) Title: COMPOSITION AND/OR METHOD FOR REDUCING AND/OR PREVENTING MYOPIA PROGRESSION COMPRISING ATROPINE

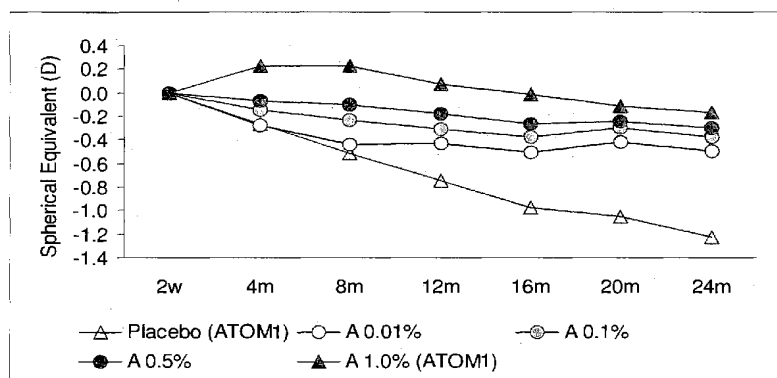


Figure 5

(57) Abstract: The invention relates to a composition and/or method for reducing and/or preventing myopia progression. The composition comprises an ultra-low atropine concentration. The composition comprises less than 0.025% atropine. For example, the composition comprises about 0.01% atropine. The ultra-low atropine composition reduces and/or prevents myopia progression with negligible side effects, such as loss of accommodation, mydriasis, near-vision blur, allergic conjunctivitis and dermatitis.

COMPOSITION AND/OR METHOD FOR REDUCING AND/OR PREVENTING MYOPIA PROGRESSION COMPRISING ATROPINE

Field of the invention

The present invention relates to a composition and/or method for reducing and/or preventing myopia progression. In particular, the invention relates to an ultra-low
5 concentration atropine solution for reducing and/or preventing myopia progression.

Background of the invention

Myopia (nearsightedness or short sightedness) is a type of refractive error of the eye, in which the visual image is focused in front of the retina, typically resulting in blurred vision of distant objects. Myopia is especially prevalent among Asians and has been
10 reported to be as high as 70-90% in Asian countries. Myopia may be corrected by prescription lenses (for example, spectacles or contact lenses) or refractive surgery (for example, LASIK or phakic intraocular lens implantation).

After onset of myopia, which typically occurs during early childhood, its progression is often difficult to control. Atropine is a non-specific muscarinic antagonist. Atropine at
15 1.0% and 0.5% has been demonstrated through randomised trials to be effective in slowing myopia progression (Shih *et al.*, 1999). The safety profile, and associated side-local and systemic effects of atropine (i.e. its effect on pupil size and accommodation), however, has often been a source of concern, and has greatly deterred the use of this medication for myopia control. Every unit increase in pupil size results in an
20 exponential increase in the amount of light entering the eye, and this may cause glare and photophobia. The long term effect of excessive amounts of ultraviolet light entering an eye which has been chronically dilated with atropine may potentially increase the risk of cataract or macular degeneration. Atropine also decreases accommodation amplitude (ability to focus on near objects), thus reducing near vision so that children
25 may require bifocal or progressive glasses to read or see close objects.

In atropine in the treatment of myopia study 1 (ATOM1), 1 % atropine was reported to be effective in slowing the progression of myopia (Chua *et al.*, 2006). Side effects were recognised with 1 % atropine treated eyes, including blurring of near vision in the

atropine-treated eye (likely due to cycloplegia) and anisocoria or uneven pupil size, (likely due to mydriasis).

5 In a retrospective non-randomized study, Lee *et al.*, (2006) found that myopia in 21 children aged 6-12 years on 0.05% atropine progressed at a rate of 0.28 ± 0.26 D per year, compared to 0.75 ± 0.35 D per year in 36 consecutive untreated clinic patients. Negative side effects including photophobia and hampered near vision were reported in the subjects.

10 In another retrospective review of 50 pre-myopic children, 24 of whom were started on 0.025% atropine, Fang *et al.*, (2010) noted that subsequent myopia shift was less (-0.14 ± 0.24 D) in the 0.025% atropine group, compared to controls (-0.58 ± 0.34 D). Both the control group and the 0.025% atropine group exhibited photophobia although the difference was not significant. None of the subjects complained of near-vision blur.

It is desirable to provide an atropine composition for reducing and/or preventing myopia progression with negligible or minimal side effects.

15 Summary of the invention

The present composition provides an ultra-low concentration atropine composition for reducing and/or preventing myopia progression.

According to a first aspect, the present invention provides a composition for reducing and/or preventing myopia progression comprising less than 0.025% atropine.

20 According to another aspect, the invention relates to the use of atropine in the preparation of a composition for reducing and/or preventing myopia progression, wherein the composition comprises less than 0.025% atropine.

The invention also provides a method for reducing and/or preventing myopia progression comprising administering to a subject a composition comprising less than
25 0.025% atropine.

Brief description of the figures

Figure 1 is a flow chart illustrating the study design of ATOM1.

Figure 2 is a graph showing mean spherical equivalent change from baseline from ATOM1.

- 5 Figure 3 is a graph showing mean axial length change from baseline from ATOM1.

Figure 4 is a flow chart illustrating the study design of ATOM2.

Figure 5 is a graph showing the mean change in spherical equivalent for groups from baseline 2 to 24 months with 0.01%, 0.1% and 0.5% atropine from ATOM2 and 1% atropine and placebo from ATOM1.

- 10 Figure 6 is a graph illustrating progression of myopia according to severity (pooled eyes) with 0.01%, 0.1% and 0.5% atropine from ATOM2 and 1% atropine and placebo from ATOM1. Myopia progression from baseline 2 is classified as severe (if > 1 D), moderate (0.5-0.99 D) and mild (if < 0.5 D).

- 15 Figure 7 is a graph illustrating the main change in axial lengths from baseline 2 to 24 months from ATOM2.

Figure 8 is a graph illustrating the changes in spherical equivalent in groups from ATOM2 after stopping atropine for 1 year.

Figure 9 is a graph illustrating the changes in axial length in groups from ATOM2 after stopping atropine for 1 year.

- 20 Definitions

The term "about" when used in conjunction with a value, for example about 0.01%, means a value reasonably close to the value. For example, for 0.01%, 0.006%, 0.007%, 0.008%, 0.009%, 0.0095%, 0.0099%, 0.011%, 0.0115%, 0.012%, 0.013%, 0.014% would be included. In particular, it would include the value itself.

- 25

“Accommodation” refers to the process by which the eye adjusts to a near focus, to maintain a clear image (focus) on an object as its distance changes.

“Amblyopia” (also known as “lazy eye”) refers to a decrease of vision, either unilaterally or bilaterally, with no apparent structural abnormality in the eye. In amblyopia, visual stimulation either fails to transmit or is poorly transmitted through the optic nerve to the brain.

“Anisocoria” refers to a condition where the pupils of the eyes are uneven in size.

“Cycloplegia” refers to paralysis of the ciliary muscle of the eye, resulting in a loss of accommodation.

“Mydriasis” refers to a condition where the pupil of the eye is dilated due to disease, trauma or the use of drugs. Mydriasis may be associated with glare intolerance and photophobia.

“Photophobia” refers to a condition of sensitivity to light. In ordinary medical terms, photophobia is not a morbid fear or phobia, but an experience of discomfort or pain to the eyes due to light exposure.

“Strabismus” (also known as “crossed eyes”) refers to a condition in which the two eyes do not line up in the same direction, and therefore do not look at the same object at the same time.

Detailed description of the invention

The composition of the present invention comprises an ultra-low concentration of atropine, i.e. less than 0.025% atropine. The concentration of atropine in the composition may be any value less than 0.025%, for example, the composition may comprise 0.001 to 0.0249% atropine; 0.005% to 0.0249% atropine, 0.01 to 0.0249% atropine, 0.005 to 0.02% atropine, 0.05 to 0.015% atropine or 0.008 to 0.012% atropine.

Accordingly, the composition may comprise 0.001%, 0.002%, 0.003%, 0.004%, 0.005%, 0.006%, 0.007%, 0.008%, 0.009%, 0.01%, 0.011%, 0.012%, 0.013%, 0.014%, 0.015%, 0.016%, 0.017%, 0.018%, 0.019%, 0.02%, 0.021%, 0.022%, 0.023%, 0.024%, 0.0245% or 0.0249% atropine. In particular, the composition comprises about 0.01%

atropine. More in particular, the composition comprises 0.01%. The composition may further comprise at least one pharmaceutically acceptable excipient.

The composition according to any aspect of the invention may be for use in reducing and/or preventing myopia progression and/or treating myopia. Accordingly, the invention includes a method of reducing and/or preventing myopia progression comprising administering to a subject a composition according to any aspect of the invention. The composition is for administration to the eye. In particular, the composition is for topical administration to the eye. More in particular, the composition is an eye-drop composition

- 10 After onset, myopia typically progresses during childhood and may only stabilise in adulthood. Accordingly, the composition is suitable for reducing and/or preventing myopia progression in subjects where myopia is still progressing and/or has not stabilised, even in late adulthood. For example, the composition is suitable for reducing and/or preventing myopia progression in subjects from 3 to 30 years old, where myopia
- 15 is still progressing and/or has not stabilised. In particular, the composition is suitable for reducing and/or preventing myopia in children from 6 to 12 years old.

Accordingly, the invention also provides a method for reducing and/or preventing myopia progression comprising administering to a subject a composition according to any aspect of the invention.

- 20 In a study (Example 2), 400 children were administered either 0.01%, 0.05% or 0.1% atropine nightly to both eyes and myopia progression was monitored over a period of 2 years. The results of this study showed that 0.01% atropine was found to induce negligible side-effects (for example: loss of accommodation, mydriasis, allergic conjunctivitis, dermatitis) compared to atropine at 0.1% and 0.05% and retains
- 25 comparable efficacy in controlling myopia progression.

Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the present invention.

EXAMPLES

Example 1 First atropine in the treatment of myopia study (ATOM1)

In ATOM1, 400 children aged 6-12 years with spherical equivalents of -1.00 D and -6.00 D were randomly assigned to having 1% atropine or placebo medication in one eye (Chua *et al.*, 2006). The design of the study is illustrated in Figure 1 and summarised below. The results from ATOM1 are compared and also used in Example 2.

Summary of ATOM 1

Purpose: To evaluate the efficacy and safety of topical atropine, a nonselective muscarinic antagonist, in slowing the progression of myopia and ocular axial elongation in Asian children.

Design: Parallel-group, placebo-controlled, randomized, double-masked study.

Subjects: 400 children aged 6 to 12 years with refractive error of spherical equivalent -1.00 to -6.00 D and astigmatism of -1.50 D or less.

Intervention: Subjects (the children) were assigned with equal probability to receive either 1 % atropine or vehicle eye-drops once nightly for 2 years. Only 1 eye of each subject was chosen through randomization for treatment.

Main Outcome Measures: The main efficacy outcome measures were change in spherical equivalent refraction as measured by cycloplegic autorefraction and change in ocular axial length as measured by ultrasonography. The primary safety outcome measure was the occurrence of adverse events.

Results: 346 (86.5%) subjects completed the 2-year study. After 2 years, the mean progression of myopia and of axial elongation in the placebo-treated control eyes was -1.20 ± 0.69 D (Figure 2) and 0.38 ± 0.38 mm (Figure 3), respectively. In the atropine-treated eyes, myopia progression was -0.28 ± 0.92 D (Figure 2), whereas the axial length remained essentially unchanged compared with baseline (-0.02 ± 0.35 mm, Figure 3). The changes in refractive error and axial length in the non-treated eyes of

subjects in both the atropine group and placebo-control group paralleled that of the placebo-treated eyes (Figures 2 and 3). The differences in myopia progression and axial elongation between the 2 groups were -0.92 D (95% confidence interval, -1.10 to -0.77 D; $P < 0.001$) and 0.40 mm (95% confidence interval, 0.35-0.45 mm; $P < 0.001$), respectively. No serious adverse events related to atropine were reported. In this study, glare and photophobia were minimized with the use of photochromatic lenses. 54 subjects withdrew from the study. Reasons for withdrawal were: allergic or hypersensitivity reactions or discomfort (4.5%), glare (1.5%), blurred near vision (1%), logistical difficulties (3.5%), and others (0.5%).

- 10 **Conclusions:** Topical 1% atropine was effective in slowing the progression of low and moderate myopia and ocular axial elongation in Asian children.

Example 2: Second atropine in the treatment of myopia study (ATOM2)

- 15 In ATOM2, the effect of lower doses of atropine in preventing myopia progression and the visual side effects (pupil dilation, loss of accommodation and near-vision blur) were assessed.

(i) Summary of ATOM 2

Purpose: To compare efficacy and visual side-effects of 3 lower doses of atropine: 0.5%, 0.1% and 0.01%.

- 20 **Design:** Single-center, double-masked, randomized study. The design of ATOM2 is illustrated in Figure 4.

Subjects: 400 children aged 6-12 years with myopia of at least -2.0 D and astigmatism of -1.50 D or less.

- 25 **Intervention:** Subjects (the children) were randomly assigned in a 2:2:1 ratio to 0.5%, 0.1% and 0.01% atropine, to be administered once nightly to both eyes for 2 years. Cycloplegic refraction, axial length, accommodation amplitude, pupil diameter and visual acuity were noted at baseline, 2 weeks and then every 4 months for 2 years.

Main outcome measure: Myopia progression at 2 years. Changes were noted and differences between groups were compared using the Huber-White robust standard error to allow for data clustering of two eyes per subject.

Results: The mean myopia progression at 2 years was -0.30 ± 0.60 D, -0.38 ± 0.60 D and -0.49 ± 0.63 D in the 0.5%, 0.1% and 0.01% atropine groups respectively ($p=0.02$ between 0.01 and 0.5% groups; other $p>0.05$). In comparison, myopia progression in ATOM1 was -1.20 ± 0.69 D in the placebo group and -0.28 ± 0.92 in the 1% atropine group. The mean increase in axial length was 0.27 ± 0.25 mm, 0.28 ± 0.28 mm and 0.41 ± 0.32 mm in the 0.5%, 0.1% and 0.01% groups ($p<0.01$ between the 0.01% and 0.1% groups and between the 0.01% and 0.5% groups). Differences in myopia progression (0.19 D) and axial length change (0.14 mm) between groups, however, were small and may be considered clinically insignificant. 0.01% atropine had negligible effect on accommodation and pupil size and no effect on near visual acuity. Allergic conjunctivitis and dermatitis were the most common adverse effect noted; with 16 cases in 0.1% and 0.5% atropine groups, none in the 0.01% group.

Conclusion: 0.01% atropine has negligible side-effects compared to atropine at 0.1% and 0.5% and retains comparable efficacy in controlling myopia progression.

(ii) ATOM2 in detail

Methods

Children aged between 6-12 years with myopic refraction of at least 2.0 D in both eyes, astigmatism of less than 1.5 D and a documented myopic progression of at least 0.5 D in the past year were enrolled in a double-masked single-centre clinical trial. Excluded were those with ocular pathology (eg. amblyopia, strabismus), previous use of atropine or pirenzepine, an allergy to atropine, or systemic ill health (eg. cardiac or respiratory illness). Written informed consent was obtained from parents or guardians and verbal assent was obtained from children. The study was conducted according to the tenets of the Declaration of Helsinki, with ethics approval from the Singapore Eye Research Institute Review Board.

Subjects (the children) were randomized to receive 0.5%, 0.1% or 0.01% atropine once nightly in both eyes at an allocation ratio of 2:2:1 in six strata defined by gender and age-groups of 6-7, 8-10 and 11-12 years to ensure gender and age balance across the three treatment arms. Trial medications were pre-packaged so that bottles were pre-labelled with subject number and of similar appearance. Trial medication consisted of
5 the appropriate dose of atropine sulfate with 0.02% of 50% benzalkonium chloride as preservative (Ashwood Laboratories Ltd, Macau).

After assessment at the time of enrolment (baseline), the subjects were re-assessed 2 weeks after starting atropine (baseline 2), and then at 4, 8, 12, 16, 20 and 24 months.
10 At each visit, best-corrected distance logMar visual acuity (BCVA) was assessed by an optometrist using the Early Treatment Diabetic Retinopathy study chart. Near visual acuity was assessed using best-corrected distance spectacle correction with a reduced LogMar reading chart placed at 40cm under well-lit conditions. The near point of accommodation (NPA) was measured using a RAF rule using best-corrected distance
15 spectacle correction. Subjects were instructed to move the target inwards till the N5 print became slightly blurred, and then outwards till it just became clear. Accommodation amplitude was calculated as the inverse of NPA. Mesopic pupil size was measured with the Procyon 3000 pupillometer (Lion House, Red Lion Street, London, UK), using the Meso-Hi (4 lux) setting. Photopic pupil size was measured
20 using the Neuroptics pupillometer (Neuroptics Inc, Irvine, CA, USA) while the subjects were viewing a target placed at 3m, after at least 10second of exposure to lamps providing 300 lux of luminance. In both cases, at least 5 pupil size readings (with range <0.5 mm) were recorded and averaged.

Cycloplegic autorefraction was determined 30 minutes after 3 drops of cyclopentolate
25 1% (Cyclogyl, Alcon-Convreur) were administered at 5 minutes apart using a Canon RK-F1 autorefractor (Canon Inc. Ltd, Tochigiken, Japan). Five readings, all of which had to be less than 0.25 D apart, were obtained and averaged. Spherical equivalent was calculated as sphere plus half cylinder power. The Zeiss IOL Master (Carl Zeiss Meditec Inc, CA, USA), a non-contact partial coherence interferometry, was used to
30 measure the ocular axial length. Five readings, with a maximum-minimum deviation of 0.05 mm or less, were taken and averaged.

Parents or guardians, subjects (the children) and study investigators were kept masked to the assigned dosage of trial medications. Each subject (child) kept a diary of usage of the trial medication. Compliance level of each subject was classified according to mean number of frequency of using atropine per week as reported by subjects over the first 24 months. Subjects with 75% compliance rate (≥ 5.25 days/week) were considered compliant.

The primary end-point was myopia progression over 2 years. Since a hyperopic shift may occur after commencing atropine, myopic progression was calculated from the second baseline, when the subjects had been on trial medication for 2 weeks. Level of myopia progression in each eye was further categorized as being mild (<0.5 D), moderate (0.5 to 0.99 D) or severe (>1.0 D).

Secondary end-points included myopia progression at one year, change in axial length at one and two years, and side-effect parameters such as changes in accommodation amplitude, mesopic and photopic pupil size and best-corrected distance and near visual acuity. Myopia and axial changes were noted from second baseline, while accommodation, pupil size and visual acuity were monitored from first baseline. Any adverse events, regardless of whether they appeared relevant to atropine use, were documented.

Statistical analysis

All analyses were based on intention to treat principle and performed in statistical software Stata (version 10.1, Stata Corp., College Station, TX). For demographic and other person-level data such as compliance and ever experiencing adverse events, Fisher's exact test was used to test for difference in proportion of subjects between treatment groups and ANOVA for difference in means between treatment groups. Endpoints from both eyes were pooled in a combined analysis using the Huber-White robust standard errors to allow for the correlation between eyes within person. Results on left and right eyes were very similar. For example, mean difference (95% CI) in 2-year myopia progression between left and right eyes were -0.01 (-0.06 , 0.03). For brevity and better precision, this report shows analyses pooling both eyes with robust standard errors for clustered data. The global null hypothesis of no difference

between 3 treatment groups was tested first, followed by pair-wise comparisons. Nominal level of statistical significance (p-value) was reported, i.e. no adjustment for multiple comparison. Interpretation will begin with considering the global null hypothesis between 3 groups in order to prevent inflated type I error rate. Placebo and
5 atropine treated eyes in ATOM1 were used for reference in secondary analyses.

Results

A total of 400 children were recruited into the study, with 161, 155 and 84 children in the 0.5%, 0.1% and 0.01% atropine treatment arms respectively (Figure 4). There were almost equal numbers of males and females and 91% of children were of ethnic
10 Chinese origin (Table 1). No differences were noted in demographics, baseline refractive error, accommodation, pupil diameter or best corrected visual acuity between groups (Table 1). Correlation between change in spherical equivalent and axial length over 2 years was high (correlation coefficient=0.82, $p<0.001$), and suggests good measurement validity.

Table 1: Characteristics at baseline and second baseline (ie. 2 weeks after starting trial medication)

Variables	Atropine(A) dose			p-value ^a
	A 0.01% (n 84)	A 0.1% (n 155)	A 0.5% (n 161)	
Age (yr), mean (sd)	9.5 (1.5)	9.7 (1.6)	9.7 (1.5)	0.95
Female, %	48.8	46.5	47.2	0.95
Chinese %	90.5	92.3	90.0	0.99
Spherical equivalent (D)				
- baseline	-4.5 (1.5)	-4.8 (1.5)	-4.7 (1.8)	0.40
- second baseline	-4.5 (1.5)	-4.5 (1.4)	-4.3 (1.8)	0.67
Axial length (mm)				
- baseline	25.1 (1.0)	25.2 (0.8)	25.2 (0.9)	0.94
- second baseline	25.2 (1.0)	25.1 (0.8)	25.1 (0.9)	0.93
Accommodation (D)				
- baseline	16.2 (3.4)	16.7 (3.0)	15.8 (3.4)	0.01
- second baseline	11.3 (4.3)	3.8 (2.5)	2.2 (1.2)	<0.001
Mesopic pupil diameter (mm)				
- baseline	3.9 (0.6)	3.9 (0.6)	4.0 (0.7)	0.21
- second baseline	5.2 (0.8)	7.2 (0.7)	7.8 (0.5)	<0.001
Photopic pupil diameter (mm)				
- baseline	4.7 (0.7)	4.6 (0.7)	4.6 (0.7)	0.63
- second baseline	5.8 (0.8)	7.4 (0.7)	7.9 (0.6)	<0.001
BCVA distant (logMar)				
- baseline	0.01 (0.05)	0.01 (0.06)	0.02 (0.06)	0.56
- second baseline	0.01 (0.05)	0.01 (0.05)	0.01 (0.06)	0.86
Near vision (logMar)				
- baseline	0.04 (0.09)	0.04 (0.08)	0.04 (0.07)	0.38
- second baseline	0.06 (0.08)	0.29 (0.18)	0.48 (0.16)	<0.001

a. Fisher's exact test for binary demographic variables; ANOVA for age; Huber-White robust standard error for clustered data (both eyes pooled) on ocular parameters

- 5 Two-year primary endpoint data were available for 355 of 400 (88.8%) subjects. 44 subjects withdrew participation on their own accord; 9 (10.7%), 14 (9.0%) and 21 (13.0%) from the 0.01%, 0.1% and 0.5% treatment group respectively (p=0.43); 1 subject did not turn up for the second year assessment. Compliance, defined as >75% expected usage was 98.7%, 96.8% and 98.8% in the 0.5%, 0.1% and 0.01% arms
- 10 (p=0.53) in the 2 year period.

Change in myopic progression and axial length

A clinically small, dose related response on myopic progression was noted between the 3 treatment arms (Figure 5). An initial hyperopia shift of 0.3 to 0.4 D was noted in the 0.1% and 0.5% groups but not in the 0.01% group (Table 1). At the end of 1 year, there

was a significant difference in myopia progression between the 0.5% atropine group and the 0.01% ($p < 0.001$) and 0.1% ($p = 0.01$) groups, but no statistical significant difference between 0.01% and 0.1% groups. The final myopia progression over 2 years was -0.49 ± 0.60 D, -0.38 ± 0.60 D and -0.30 ± 0.63 D in the atropine 0.01%, 0.1% and 5 0.5% groups respectively ($p = 0.07$), with significant difference only between the 0.01% and 0.5% groups (Table 2). There was no significant difference in spherical equivalent levels between groups ($p = 0.20$). Fifty percent of the 0.01% group had progressed by less than 0.5 D, compared to 58% and 63% in the 0.1 and 0.5% group respectively, with approximately 18% progressing by > 1.0 D in all 3 groups (Figure 6).

Table 2: Ophthalmology parameters at 2nd annual visit

	Atropine (A) dose (mean (SD))			p-value
	A 0.01%	A 0.1%	A 0.5%	
Spherical equivalent (D)				
- at 1 year	-4.9 (1.5)	-4.8 (1.4)	-4.6 (1.9)	0.26
- at 2 years	-5.1 (1.5)	-4.9 (1.3)	-4.7 (1.7)	0.20
- mean change over 1 year	-0.43 (0.52)	-0.31 (0.50)	-0.17 (0.47)	<0.001 ^{A,C}
- mean change over 2 years	-0.49 (0.63)	-0.38 (0.60)	-0.30 (0.60)	0.07 ^A
Axial length (mm)				
- at 1 year	25.4 (1.0)	25.3 (0.8)	25.3 (0.9)	0.36
- at 2 years	25.7 (1.0)	25.4 (0.8)	25.4 (1.0)	0.08 ^B
- mean change over 1 year	0.24 (0.19)	0.13 (0.18)	0.11 (0.17)	<0.001 ^{A,B}
- mean change over 2 years	0.41 (0.32)	0.28 (0.27)	0.27 (0.25)	0.002 ^{A,B}
Accommodation (D)				
- at 1 year	11.7 (4.3)	6.0 (3.4)	3.6 (3.2)	<0.001 ^{A,B,C}
- at 2 years	11.8 (3.2)	6.8 (3.4)	4.0 (2.6)	<0.001 ^{A,B,C}
- mean change over 1 year	-4.4 (4.9)	-10.9 (4.0)	-12.4 (3.3)	<0.001 ^{A,B,C}
- mean change over 2 years	-4.6 (4.2)	-10.1 (4.3)	-11.8 (4.4)	<0.001 ^{A,B,C}
Mesopic pupil size (mm)				
- at 1 year	5.1 (0.9)	6.7 (1.0)	7.5 (1.1)	<0.001 ^{A,B,C}
- at 2 years	5.1 (0.9)	6.7 (1.1)	7.5 (1.2)	<0.001 ^{A,B,C}
- mean change over 1 year	1.15 (0.78)	2.77 (1.03)	3.50 (1.05)	<0.001 ^{A,B,C}
- mean change over 2 years	1.15 (0.71)	2.71 (1.12)	3.56 (1.14)	<0.001 ^{A,B,C}
Photopic pupil size (mm)				
- at 1 year	5.6 (0.8)	7.0 (1.0)	7.7 (1.0)	<0.001 ^{A,B,C}
- at 2 years	5.5 (0.8)	6.9 (1.0)	7.8 (1.1)	<0.001 ^{A,B,C}
- mean change over 1 year	0.91 (0.78)	2.42 (0.91)	3.11 (1.08)	<0.001 ^{A,B,C}
- mean change over 2 years	0.74 (0.75)	2.25 (1.01)	3.11 (1.10)	<0.001 ^{A,B,C}
BCVA distant (logMar)				
- at 1 year	-0.005 (0.042)	-0.003 (0.054)	-0.003 (0.054)	0.99
- at 2 years	-0.001 (0.057)	0.005 (0.054)	0.011 (0.057)	0.25
- mean change over 1 year	-0.02 (0.05)	-0.02 (0.06)	-0.03 (0.05)	0.21
- mean change over 2 years	-0.02 (0.06)	0.01 (0.06)	-0.01 (0.06)	0.44
Near vision (logMar)				
- at 1 year	0.03 (-0.06)	0.15 (0.15)	0.35 (0.18)	<0.001 ^{A,B,C}
- at 2 years	0.01 (0.07)	0.10 (0.13)	0.29 (0.18)	<0.001 ^{A,B,C}
- mean change over 1 year	-0.01 (0.10)	0.10 (0.16)	0.32 (0.19)	<0.001 ^{A,B,C}
- mean change over 2 years	-0.02 (0.08)	0.06 (0.13)	0.25 (0.19)	<0.001 ^{A,B,C}

BCVA: best-corrected visual acuity

Myopia progression and axial length: change from second baseline; other parameters: change from initial baseline

- 5 P-values for test of global null hypotheses of all groups being the same are shown. Pairwise comparison P-values are represented by A: significant (P<0.05) difference between 0.01% and 0.5% atropine, B: significant difference between 0.01% and 0.1% atropine, and C: significant difference between 0.1% and 0.5% atropine.

10 With respect to axial length, change at 1 year was larger in the 0.01% group (0.24 ± 0.19 mm) than in the 0.1% (0.13 ± 0.18 mm) and 0.5% (0.11 ± 0.17 mm) groups (p<0.001) (Figure 7). Pair-wise comparison showed statistically significant difference

between 0.01% and the other two groups ($p < 0.001$). This difference persisted till the end of the 24 month period (Table 2).

Changes in accommodation, pupil diameter and visual acuity

There was no difference in accommodation, mesopic and photopic pupil diameter between groups at baseline (Table 1). However, significant dose-related differences quickly became evident by second baseline visit (Table 1). Changes within the 0.01% group were significantly less than in the 2 other groups. Accommodation amplitude in the 0.01% group was only reduced to 11.3 D compared to 3.8 D and 2.2 D in the 0.1% and 0.5% groups (Table 1). In functional terms, this meant that near visual acuity was not significantly impaired in the 0.01% group, while deficiencies were noted in the 2 other groups. Mean best-corrected distant visual acuity was not affected by atropine use (Table 2), although 10% of subjects did encounter mild distance blur (Table 3).

Table 3. Adverse event and serious adverse events

	Atropine (A) dose			Exact test p-value ^a
	A 0.01% (n=84)	A 0.1% (n=155)	A 0.5% (n=161)	
	Number of episode /number of children (% children)			
Adverse Events				
Allergic conjunctivitis	0/0 (0)	7/6 (4)	7/7 (4)	0.16
Dermatitis involving eyelids	0/0 (0)	2/1 (1)	4/3 (2)	0.54
Stye/chalazion	2/2 (2)	16/12 (8)	16/12 (7)	0.22
Loss of distant BCVA > 1 line	11/11 (13)	20/20 (13)	13/13 (8)	0.38
Others, eye-related	2/1 (1)	2/2 (2)	3/3 (2)	1.00
Others, non eye-related	306/69 (82)	470/122 (78)	477/132 (82)	0.73
Severe Adverse Events				
Events requiring hospitalisation	1/1 (1)	3/3 (2)	3/3 (2)	1.00

a. Fisher's exact test for proportion of children with adverse events

Pupil size, under both photopic and mesopic conditions, in the 0.01% group increased by only 1 mm, while pupils in the 0.1% and 0.5% groups were about 3 mm larger (Table 2). While the atropine effect on pupil diameter remained unchanged over time,

the accommodation appeared to improve in the 0.1% and 0.5% groups over time (Table 2). The mean accommodation amplitude in the 0.5% group, for example, fell from 15.8 D at baseline to 2.2 D at the second baseline visit but rose to 3.6 D and 4.1 D by the end of the first and second years. Changes in 0.01% group were less, varying from 16.2 D to 11.3 D, 11.7 D and 11.8 D over the same time-period.

Changes in spherical equivalent (myopia) and axial length after stopping atropine for 1 year (between 24 and 36 months)

356 children (89%) entered into the wash-out phase of the ATOM2 study. On cessation of atropine, there was a rebound increase in myopia was noted in all 3 groups which was greater in 0.5% atropine eyes (-0.87 ± 0.52 D) than in 0.1% atropine (-0.68 ± 0.45 D) and 0.01% atropine (-0.28 ± 0.33 D) eyes ($p < 0.001$). This resulted in overall progression over the 36 month period being significantly less in the 0.01% atropine eyes (-0.72 ± 0.72 D) than in the 0.1% atropine (-1.04 ± 0.83 D) and 0.5% atropine eyes (-1.15 ± 0.81 D) (Figure 8).

During the same period, there was a significantly greater increase in axial length in the 0.1% atropine and 0.5% atropine eyes compared to the 0.01% atropine eyes (Figure 9). Overall change in axial length, however, was not significantly different between groups.

Adverse events

Majority of the adverse events were deemed to be unrelated to study treatment (eg. flu-like illness) (Table 3). Adverse reactions directly attributable to atropine included allergic conjunctivitis, which occurred in 13 subjects (4.1%) in 0.1% atropine and 0.5% atropine groups. In 3 (1.2%) subjects, symptoms were severe enough to warrant ceasing trial medication. Four subjects in the 0.1% and 0.5% groups (1.3%) had allergy related dermatitis of the eyelids. Six subjects had other eye symptoms, 5 of which could be attributed to atropine including 1 case of irritation and another of blur in the 0.01% atropine group, and 2 cases of ocular irritation and 1 with intolerable glare in the 0.5% atropine group.

Seven subjects had a severe adverse event requiring hospitalization. In the 0.01% group, one subject had acute gastric pain. In the 0.1% atropine group, there was 1 case each of appendicitis, respiratory infection and Ewing's sarcoma. In the 0.5% atropine group, there was 1 case each of tachycardia, dengue fever and gastroenteritis.

5 None of these events are thought to be associated with atropine.

Discussion

At the end of 2 years, the mean myopia and axial length progression in the ATOM1 study was -0.28 ± 0.92 D and -0.02 ± 0.35 mm in the 1% atropine eyes compared to -1.20 ± 0.69 D and 0.38 ± 0.38 mm in the placebo eyes. The progression of myopia in
10 the ATOM2 subjects lie in a dose-related manner between these two extremes (Figure 5).

In ATOM2, the progression of myopia in subjects on 0.5% atropine was -0.17 ± 0.47 D over 1 year, and -0.30 ± 0.60 D over 2 years. This was similar to the progression noted in subjects on 1% atropine in the ATOM1 study (Figure 5).

15 Changes in myopia and axial lengths outcome in the 0.1% atropine group were very similar to the 0.5% atropine group. The myopia progression was initially larger in the 0.1% atropine at 1 year (-0.31 D vs -0.17 D, $p=0.01$) but this gap had closed by the second year (-0.38 D vs -0.30 D, $p=0.25$). In terms of effect on other ocular parameters, accommodation (-10.9 D vs -12.4 D), mesopic pupil diameter (2.7mm vs
20 3.5mm) and photopic pupil diameter (2.2 D vs 3.1 D) were also less in the 0.1% atropine compared to the 0.5% atropine group, making the overall efficacy-side-effect profile of 0.1% atropine better than 0.5% atropine.

The changes in spherical equivalent and axial lengths upon stopping atropine is interesting. There was a rebound increase in myopia and axial length which was
25 greater in higher dose atropine. The change in the 0.01% group, however, was more gradual with overall change in spherical equivalent being less in the 0.01% group compared to all other groups.

The subjects from ATOM1 were slightly younger (mean age: 9.2 versus 9.6 years), had lower spherical equivalents (mean spherical equivalent -3.4 versus -4.7 D) and smaller

axial lengths (mean axial length 24.8 versus 25.2 mm) than those in the ATOM2 group. Nevertheless, the reduction in myopia progression with atropine eyedrops observed in both studies with otherwise similar study profiles and methodologies was consistent and should be regarded as definitive outcomes in these studies. Axial lengths were
5 measured differently between the two studies with A-scan ultrasonography used in ATOM1 (Chua *et al.*, 2006) and IOLmaster in ATOM2. Thus, the axial lengths of ATOM1 and ATOM2 have not been compared in Figure 7.

In designing ATOM2, 0.01% atropine was initially assumed to have minimal effect, and thus act as a potential control; hence the lower allocation of subjects to this group.
10 However, contrary to expectations, 0.01% atropine also had significant clinical efficacy on reducing myopia progression not dissimilar to the higher concentrations, but unlike the other concentrations, had negligible effect on accommodation and pupil size. The myopia progression rate in this group ($-0.49 \text{ D} \pm 0.63 \text{ D}$ at 2 years) was much less than the $-1.20 \pm 0.69 \text{ D}$ at 2 years in the ATOM1 placebo groups. Compared with the 2
15 higher doses, the difference in myopia progression at 2 years in the 0.01% group was statistically significant compared to the 0.5% group and similarly, the difference in axial length increase was statistically larger than both the 0.1% and 0.5% group. However, absolute differences between groups were clinically small with differences in myopic progression and axial length increase of only 0.19 D and 0.13 mm over 2 years (Table
20 2, Figures 5 and 7). Most importantly, the ocular side-effect profile was significantly better with accommodation remaining at 11.8 D, mean pupil size of 5 mm and a mean near logMar vision of 0.01. In other words, 0.01% atropine was still clinically effective in reducing myopia progression, but did not cause side-effects normally associated with higher concentrations of atropine.

25 Overall, atropine-related adverse effects were uncommon at the 0.01% dose. Allergic reactions were most frequent; with 3.2% experiencing allergic conjunctivitis and 0.8% experiencing an allergy-associated dermatitis, all of which were in the 0.1% or 0.5% groups. It should be noted that the benzalkonium preservative in the 0.01% atropine eyedrop formulation may be at least partly responsible for these allergies. A number of
30 subjects (11%) also noted at least 1 line loss in distance best-corrected vision (Table 3). These effects are reversible on stopping medication (Tong *et al.*, 2009). There are
~~no long-term studies on the effect of atropine on the eye and continued vigilance is~~

necessary, but so far there are no known long-term adverse effects associated with its use (North and Kelly, 1987).

The strength of ATOM2 was its randomised double-blind design and low drop out rate. Instead of a placebo group, the comparison was based on external (historical and
5 population) controls. The non-inclusion of a placebo group was a decision based on findings from the ATOM1 study, which clearly showed the efficacy of atropine treatment compared to placebo, rendering a placebo arm unethical. The more important aspect of this trial remained the comparison of low dose versus high dose not only in terms of efficacy, but also in terms of the visual side effects of atropine. ATOM2 was otherwise
10 designed to have largely similar study parameters so that direct comparison with ATOM1 was deemed appropriate.

In conclusion, the results of ATOM2 suggest that 0.5%, 0.1% and 0.01% atropine remain effective in reducing myopia progression, as compared to placebo treatment, and that the clinical differences in myopia progression between these 3 groups are
15 small. In terms of side-effects, 0.5% and 0.1% atropine formulations were still noted to cause visual side-effects of accommodation loss and pupil dilation, but the 0.01% atropine formulation had no or negligible effect on accommodation or pupil dilation. The lowest concentration of 0.01% atropine appears to retain efficacy and is thus a viable concentration for reducing myopia progression in children, whilst attaining a
20 clinically significant improved safety profile in terms of accommodation, pupil size and near visual acuity, and subsequently much reduced adverse impact on visual function. Moreover, the 0.01% formulation exhibited fewer adverse events, and exhibited much less myopic rebound after it was stopped. These findings collectively suggest that 0.01% atropine nightly appears to be a safe and effective regime for slowing myopia
25 progression in children, with minimal impact on visual function in children.

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Claims

1. A composition for reducing and/or preventing myopia progression comprising less than 0.025% atropine.
2. The composition according to claim 1; comprising 0.001% to 0.0249% atropine.
- 5 3. The composition according to any one of the preceding claims; comprising 0.005% to 0.02% atropine.
4. The composition according to any one of the preceding claims, comprising 0.005% to 0.015 % atropine.
- 10 5. The composition according to claim 1, wherein the composition comprises 0.001%, 0.002%, 0.003%, 0.004%, 0.005%, 0.006%, 0.007%, 0.008%, 0.009%, 0.01%, 0.011%, 0.012%, 0.013%, 0.014%, 0.015%, 0.016%, 0.017%, 0.018%, 0.019%, 0.02%, 0.021%, 0.022%, 0.023%, 0.024%, 0.0245% or 0.0249% atropine.
- 15 6. The composition according to any one of the preceding claims, wherein the composition comprises about 0.01% atropine.
7. The composition according to any one of the preceding claims, wherein the composition comprises 0.01% atropine.
8. The composition according to any one of the preceding claims, further comprising at least one pharmaceutically acceptable excipient.
- 20 9. The composition according to any one of the preceding claims, wherein the composition is for administration to the eye.
10. The composition according to any one of the preceding claims, wherein the composition is an eye-drop composition.
- 25 11. The composition according to any one of claims 1 to 10, for use in reducing and/or preventing myopia progression and/or treating myopia.

12. A method for reducing and/or preventing myopia progression comprising administering to a subject a composition according to any one of claims 1 to 10.
13. Use of atropine in the preparation of a composition for reducing and/or preventing myopia progressssion, wherein the composition comprises less than 0.5% atropine.
14. Use according to claim 13, wherein the composition comprises 0.001% to 0.0249% atropine.
15. Use according to claim 13 or 14, wherein the composition comprises 0.005 to 0.02% atropine.
16. Use according to any one of claims 13 to 15, wherein the composition comprises 0.005 to 0.015% atropine.
17. Use according to any one of claims 13 to 16, wherein the composition comprises 0.008 to 0.012% atropine.
18. Use according to claim 13 , wherein the composition comprises 0.001%, 0.002%, 0.003%, 0.004%, 0.005%, 0.006%, 0.007%, 0.008%, 0.009%, 0.01%, 0.011%, 0.012%, 0.013%, 0.014%, 0.015%, 0.016%, 0.017%, 0.018%, 0.019%, 0.02%, 0.021%, 0.022%, 0.023%, 0.024%, 0.0245% or 0.0249% atropine.
19. Use according to any one of claims 13 to 18, wherein the composition comprises about 0.01% atropine.
20. Use according to any one of claims 13 to 19, wherein the composition comprises 0.01% atropine.
21. Use according to any one of claims 10 to 15, wherein the composition further comprises at least one pharmaceutically acceptable excipient.
22. Use according to any one of claims 10 to 16, wherein the composition is for administration to the eye.

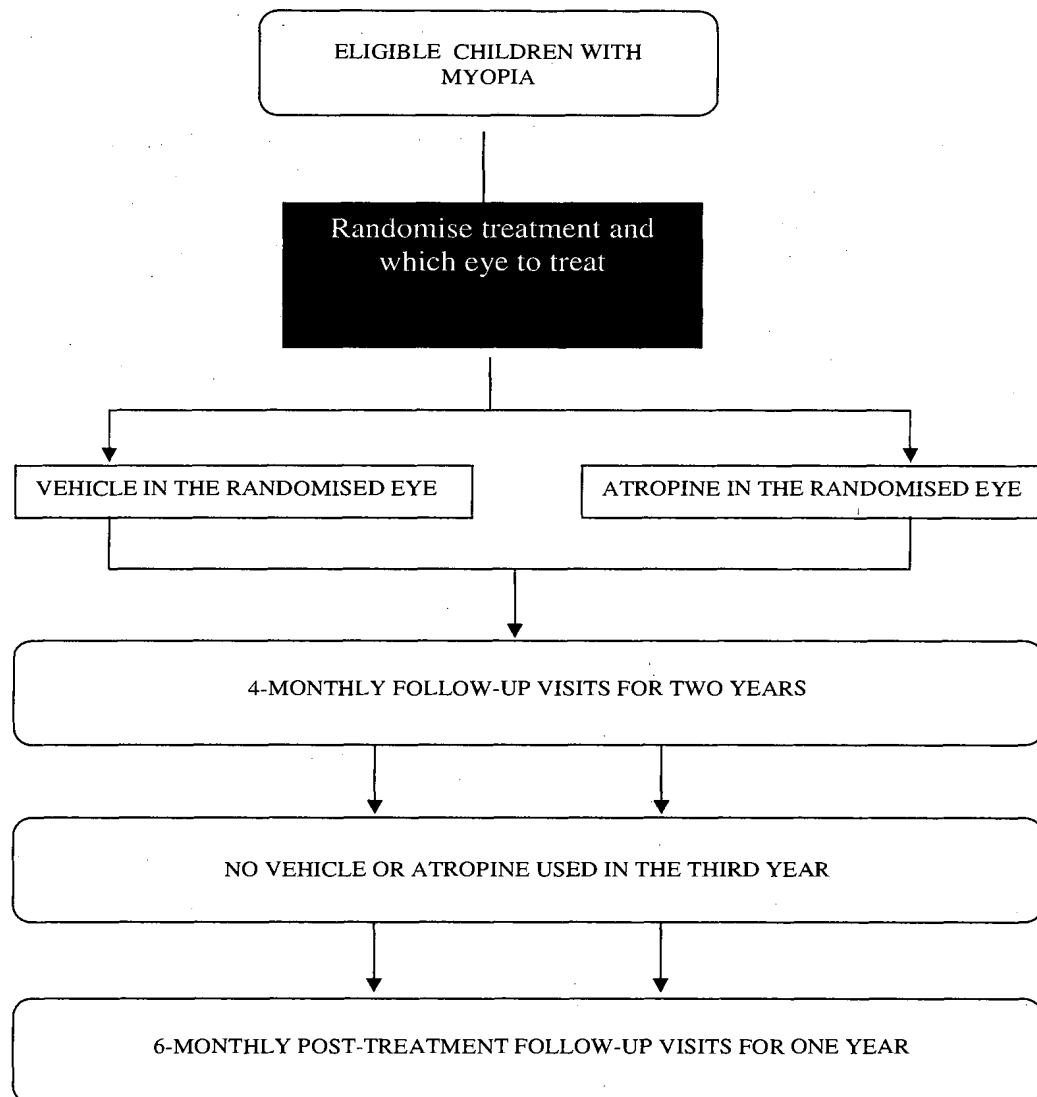


Figure 1

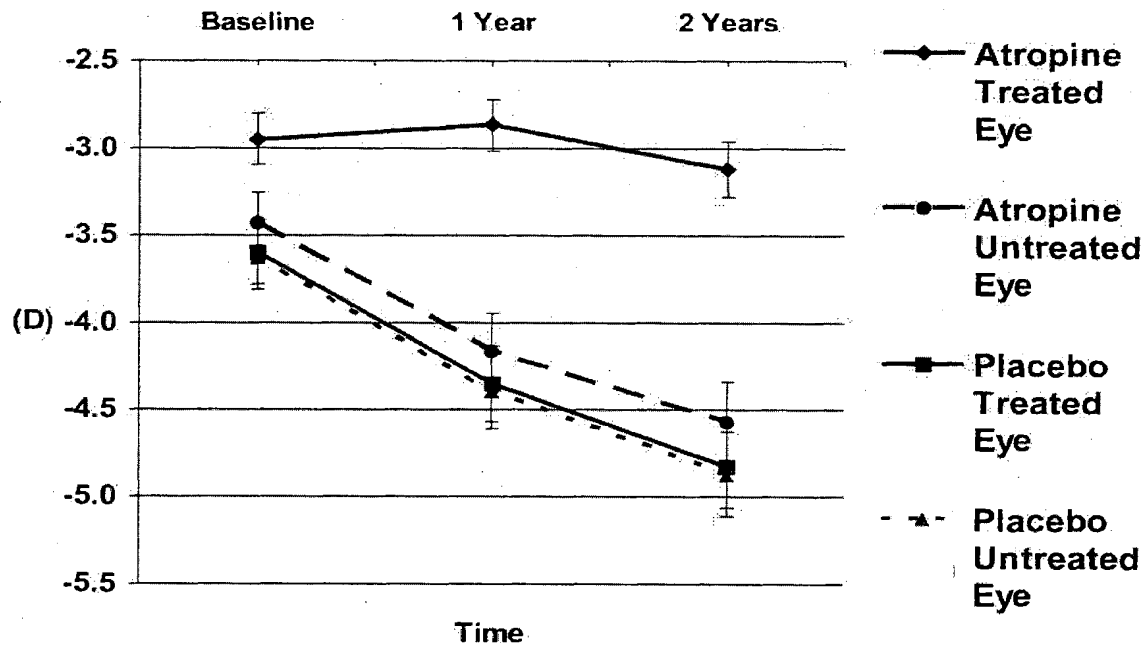


Figure 2

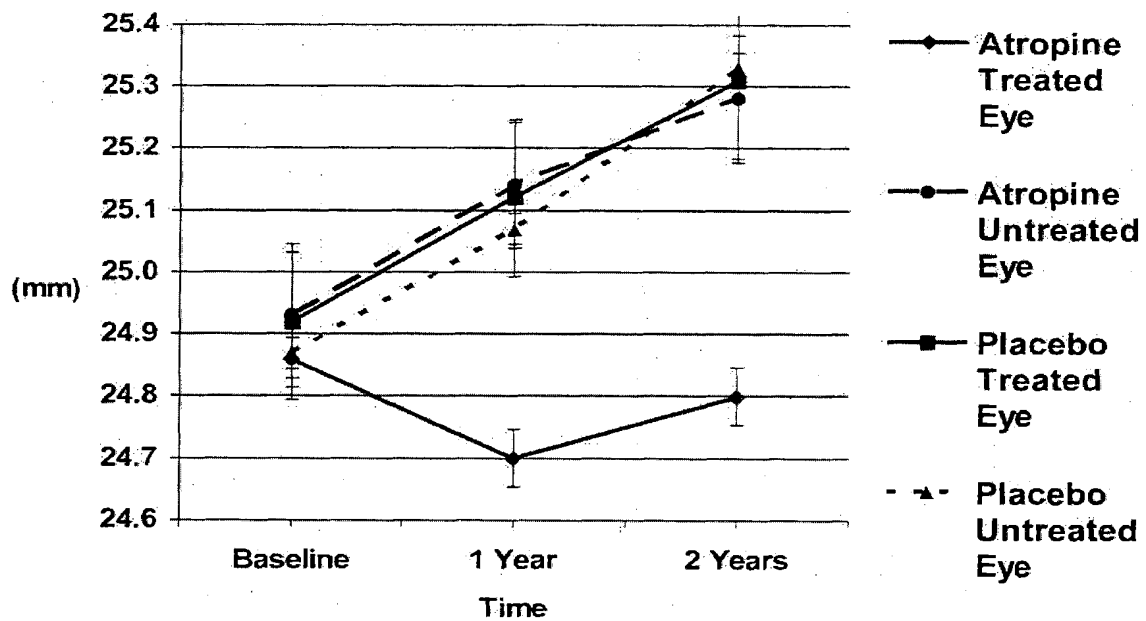


Figure 3

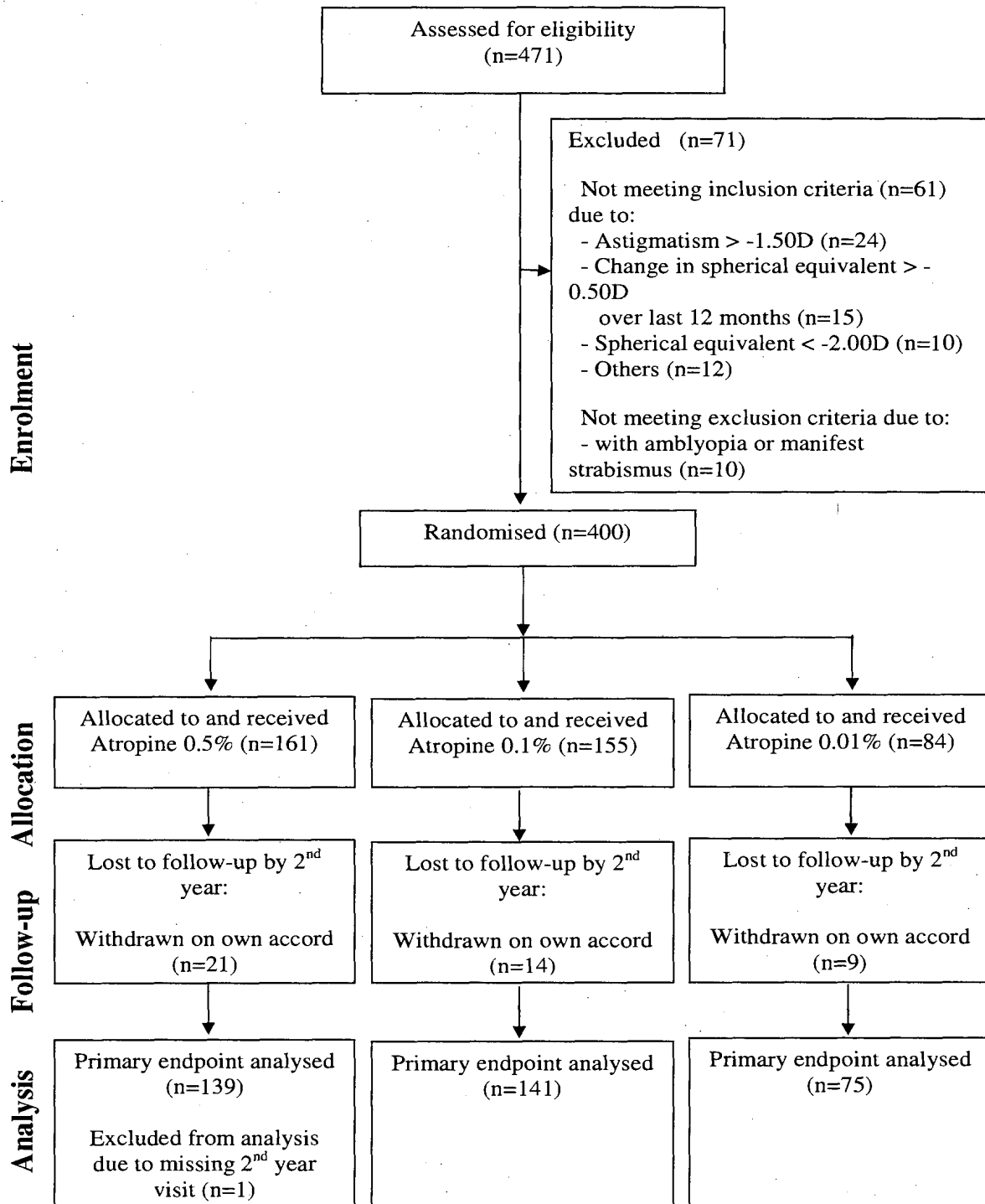


Figure 4

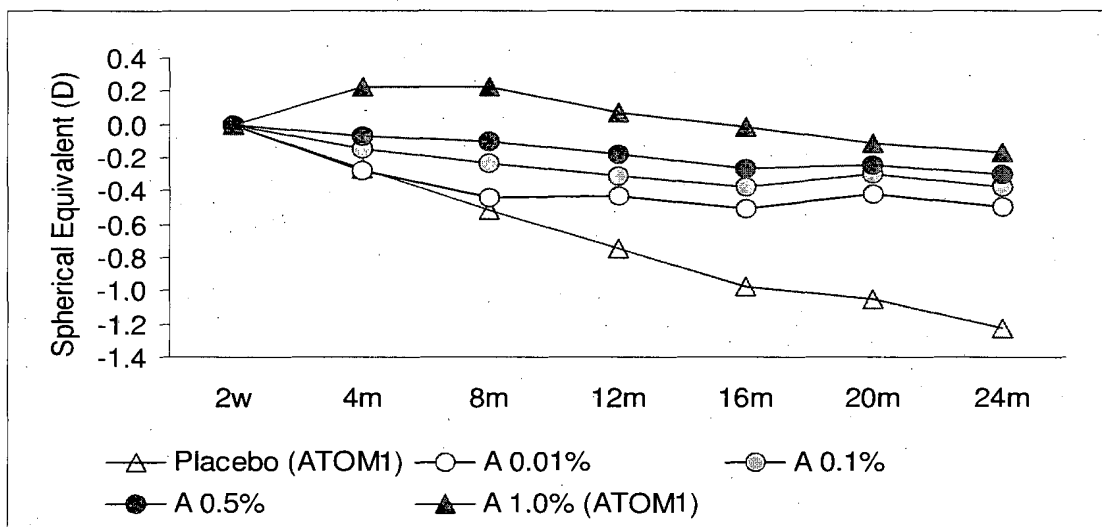


Figure 5

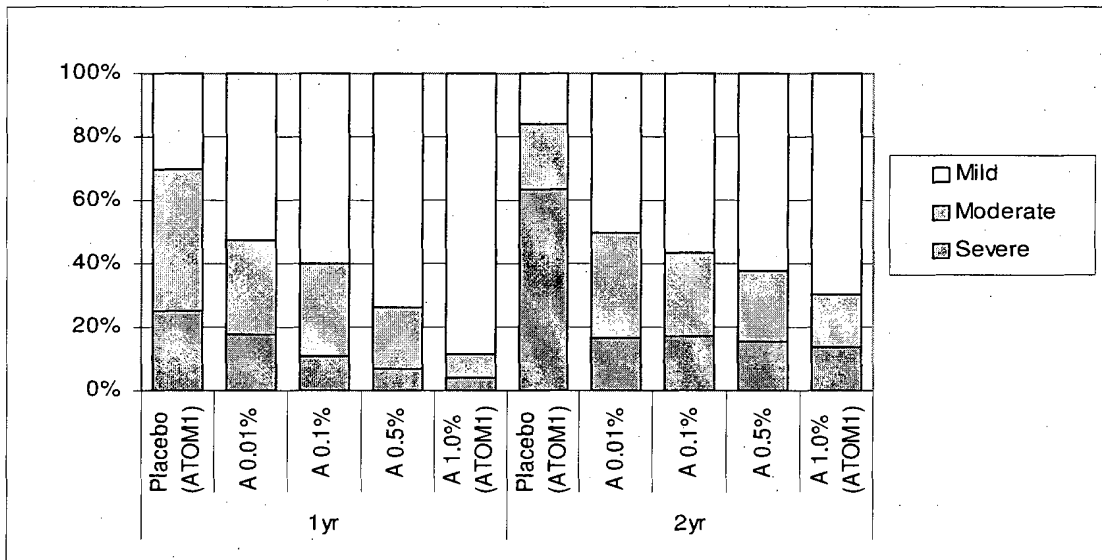


Figure 6

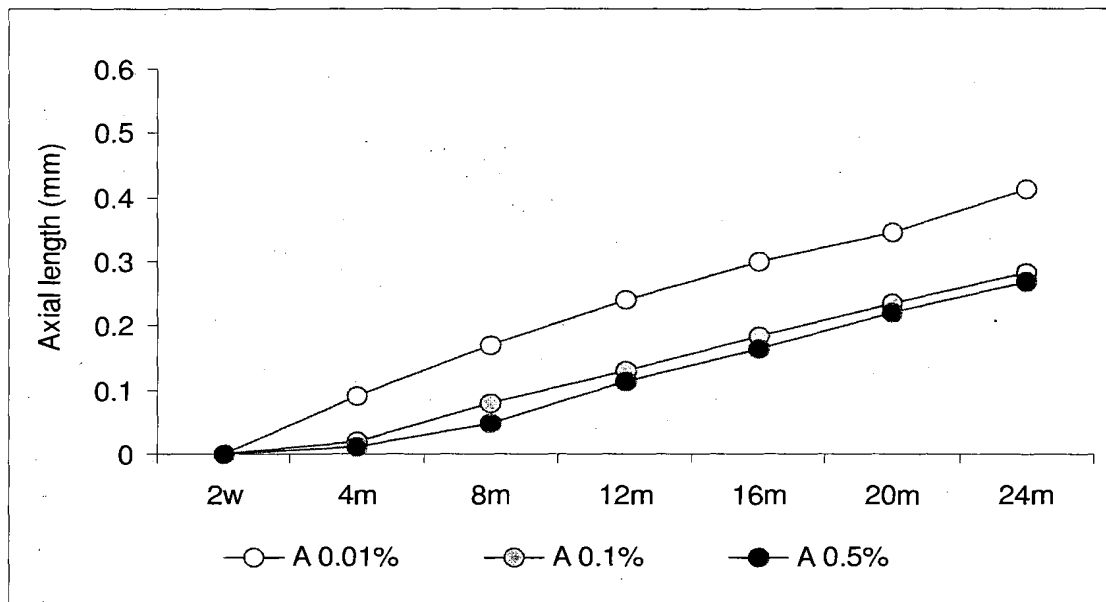


Figure 7

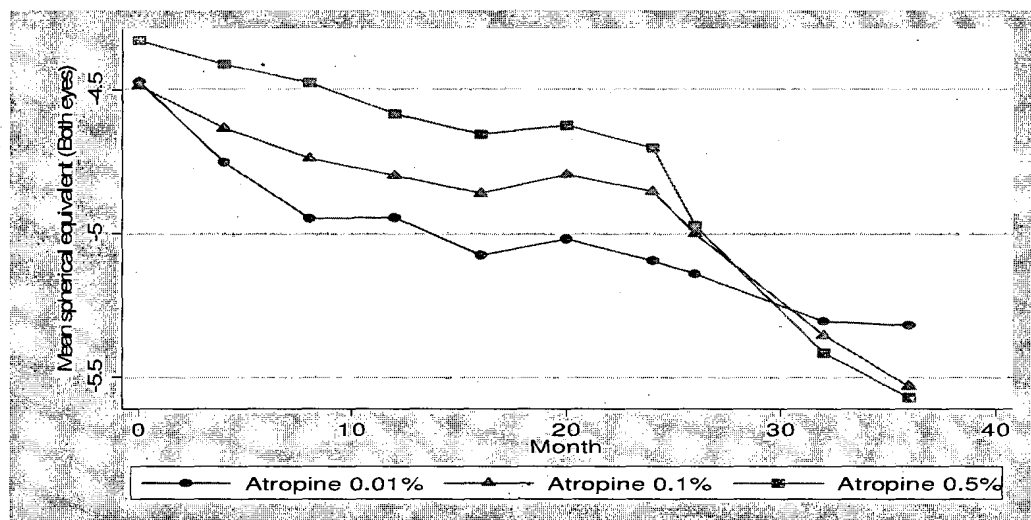


Figure 8

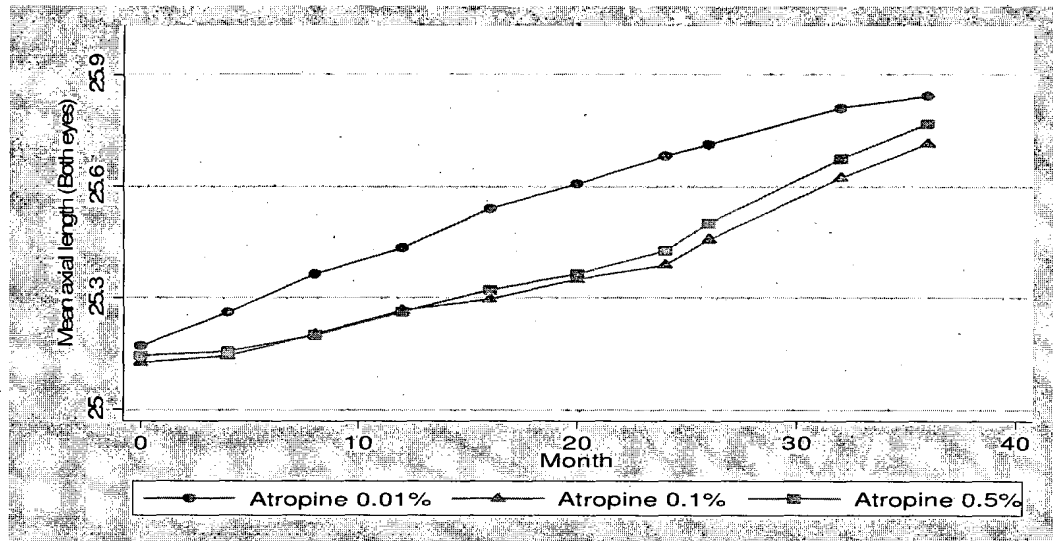


Figure 9

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SG2012/000174

A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/46 (OCT 2005) A61P 27/10 (OCT 2005)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Medline, WPI, EPODOC: atropine, atropen, hyoscyamine, cytopaz, anaspaz, myopia, short sightedness, near sightedness; Clinical trials.gov website: myopia, atropine

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	



Further documents are listed in the continuation of Box C



See patent family annex

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 09 July 2012	Date of mailing of the international search report 12 July 2012
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustalia.gov.au Facsimile No.: +61 2 6283 7999	Authorized officer Suzanne Malik AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. 0262256152

INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		PCT/SG2012/000174
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	'Safety and Efficacy Study of 0.5%, 0.1% & 0.01% Atropine Treatment to Both Eyes in Treatment of Myopia in Children.' [retrieved 28 June 2012] Retrieved from the internet, URL: http://clinicaltrials.gov/ct2/show/NCT00371124?term=ATROPINE&rank=18 published 30 August 2010. whole document	1-22
X	US 2007/0254914 A1 (WU, P-C. et al.) 01 November 2007 paragraphs 0015, 0017	1-22
X	SHIH, Y-F. et al. 'Effects of Different Concentrations of Atropine on Controlling Myopia in Myopic Children.' Journal of Ocular Pharmacology and Therapeutics. 1999. 15(1):85-90 page 86, 2nd paragraph	13, 21-22
X	FANG, P-C. et al. 'Prevention of Myopia Onset with 0.025% Atropine in Premyopic Children'. 'Journal of Ocular Pharmacology and Therapeutics. 2010 Aug; 26(4):341-5. page 342	13, 21-22
P,X	CHIA, A. et al. 'Atropine for the Treatment of Childhood Myopia: Safety and Efficacy of 0.5%, 0.1%, and 0.01% Doses (Atropine for the Treatment of Myopia 2).' Ophthalmology. Feb 2012. 119(2):347-54. whole document	1-22

INTERNATIONAL SEARCH REPORT Information on patent family members		International application No. PCT/SG2012/000174	
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.			
Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	Publication Date	Publication Number	Publication Date
US 2007/0254914 A1	01 Nov 2007	US 2007254914 A1	01 Nov 2007
End of Annex			
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001. Form PCT/ISA/210 (Family Annex)(July 2009)			

Bibliographic data

Title: MATERIALS AND COMPOSITIONS FOR DENTAL CEMENTS
AND FILLER MATERIALS

Pub/Pat no: WO2014140105A1

Pub/Issue Date: 2014-09-18

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Applicant(s): D2 BIOSCIENCE GROUP LTD| BAYERL THOMAS MARIA
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Classification: A61K6/083A1

Application number: WO2014EP54846 2014-03-12

Priority number: US201361780166P 2013-03-13;

Abstract of WO2014140105A1

The invention relates to dental cement or filler material composition comprising graphene and/or deuterium oxide or deuterium and their uses in direct or indirect dental restoration and/or prevention.

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61/780,166 13 March 2013 (13.03.2013) US(71) Applicant: **D2 BIOSCIENCE GROUP LTD.**; 129 Front
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18, 80538 München (DE).(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY,
BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM,
DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT,
HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR,
KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME,
MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ,
OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA,
SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM,
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ZW.(84) Designated States (unless otherwise indicated, for every
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UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ,
TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK,
EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV,
MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM,
TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,
KM, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) Title: MATERIALS AND COMPOSITIONS FOR DENTAL CEMENTS AND FILLER MATERIALS

FIGURE 1

Table A below shows the results of Example 10:

Sample	Cement type	surface hardness MH [N/mm ²]
KME	GIC	215 ± 20
D-KME	GIC	240 ± 17
KME-G 0.1	GIC	265 ± 22
KME-G 0.3	GIC	270 ± 20
D-KME-G 0.1	GIC	255 ± 25
D-KME-G 0.3	GIC	280 ± 23
KC	GIC	208 ± 15
D-KC	GIC	235 ± 10
KC-G 0.1	GIC	240 ± 16
KC-G 0.3	GIC	245 ± 12
D-KC-G 0.1	GIC	270 ± 20
D-KC-G 0.3	GIC	285 ± 15
KCP	RMGIC	135 ± 20
KCP-G	RMGIC	170 ± 15
NX3	RBC	175 ± 20
NX3-G	RBC	210 ± 20
CMGIC	GIC	175 ± 15
D-CMGIC	GIC	205 ± 15

(57) Abstract: The invention relates to dental cement or filler
material composition comprising graphene and/or deuterium
oxide or deuterium and their uses in direct or indirect dental
restoration and/or prevention.



Published:

— with international search report (Art. 21(3))

— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))

Materials and compositions for dental cements and filler materials

Field of the invention

The field of the invention relates to dental cements or filler materials for direct or indirect dental restoration and/or prevention.

Background of the invention

Dental cements and filler materials can be divided into 3 classes: i) Glass-ionomer cements (GIC), ii) resin modified glass ionomer cements (RMGIC) and iii) resin based cements (RBC). While GIC's (Naasan MA et al., Am J Dent 11:36–45, 1998) have advantages regarding their easy handling, continuing release of fluoride ions for prevention of enamel demineralization and excellent binding to enamel and dentine, RBC's (Summers A et al., American Journal of Orthodontics and Dentofacial Orthopedics 126: 200–206, 2004) exhibit better mechanical parameters (mostly regarding surface hardness, bending stiffness and compressive strength as compared to GIC's) and RMGIC's (Sidhu SK et al., Am J Dent 8:59–67, 1995) represent the attempt to have the advantages of the other two classes unified in one dental cement or filler for better long term wear and strength. All three classes expose, to a different degree, the feature of shrinkage during the hardening (curing) process which poses a general problem because of the possible formation of tiny fissures and micro fractures. Although GIC's exhibit, because of their inorganic nature, several advantages particularly as dental filling materials, (fluoride release, dentine binding, colour retention, acid and base resistance), their use is limited because of inferior mechanical parameters (Zhen Chun Lia et al., Journal of Prosthetic Dentistry 81: 597–609, 1999). While GIC's cure in a chemical acid-base reaction without the addition of further components, RMGIC's and RBC's require a source of free radicals for initiation of the polymerization of the resin which is either provided by a chemical initiator (self-cure) or energy, preferably in the form of UV-light (light-cure). If both is used, they are called dual-cure RBC's and dual-cure RMGIC's, respectively.

In the field of dental cements and filler materials, no material has been devised in the state of the

art which achieve adequate reinforcement. The presence of such a material would reduce the probability of the progression of micro fractures and fissures through the dental cement or filler material during its curing owing to plastic shrinkage and thus contribute to the prevention of faults formation and the improvement of mechanical strength.

Summary of the invention

The invention provides materials and compositions for the improvement of the mechanical parameters, in particular, without being restricted to these, tensile strength, surface hardness and compressive strength, of conventional dental cements and filler materials.

In one aspect, the invention uses graphene (strictly two dimensional single layer carbon sheets with material parameters which are in virtually all aspects superior to steel) and its chemically functionalized forms as a material to reinforce dental cements and filler materials by inclusion of graphene into the dental cement or filler material matrix, resulting in a composite of higher mechanical strength.

In another aspect, the invention uses deuterium oxide or deuterium to replace the water in conventional glass ionomer dental cement or filler material preparations to improve the mechanical parameters of glass ionomer dental cements or filler materials. According to the invention, deuterium oxide or deuterium is used alone or in combination with graphene in the glass ionomer dental cement or filler material materials.

Consequently, in a further aspect, the invention provides composite glass ionomer dental cements or filler materials which include both graphene and deuterium oxide.

In yet another aspect, the invention provides glass ionomer dental cements (GIC) or filler materials which include either deuterium oxide alone (GIC) or graphene and deuterium oxide (composite GIC) in combination with one or more water soluble polymers.

Within the scope of this invention, the term „graphene“ comprises all types of single layer sp^2 -bonded carbon sheets forming a honeycomb crystal lattice, without or with functional groups bound to it, in particular with $-OH$, $-NH_2$ or $-COOH$ groups or any other, hydrogen bond formation enabling functional groups bound to its edges. The said honeycomb crystal lattice may

have holes in it, resulting in a two-dimensional mesh. Furthermore, the term graphene comprises both rod like and slab like structures which can be single or layered on top of each other.

The term "dental cement" as used in this invention comprises all materials which can be used as a cement in dentistry, in particular resin based cements, resin modified glass ionomer cements and glass ionomer cements.

The term "filler material" as used in this invention comprises all materials which can be used as a filler material in dentistry, in particular resin based filler materials, resin modified glass ionomer filler materials and glass ionomer filler materials.

The term "direct" dental restoration and/or prevention as used in this invention, comprises all restoration and/or prevention works which are performed on site, i.e. directly in the mouth of the patient.

The term "indirect" dental restoration and/or prevention as used in this invention comprises all restoration and/or prevention works which are performed off site, i.e. outside the mouth of the patient, including, but not restricted to, all works to produce inlays, onlays, veneers, bridges and crowns by both reductive (e.g. milling) and/or additive (e.g. 3-dimensional printing) techniques.

According to the present invention, the terms "dental cement", "cement", "filler material", "filler" and "filler material matrix" and "filler matrix" are used synonymously.

The term "shrinkage" or "shrinking" as used in this invention comprises all physical, chemical and physico-chemical processes which lead to an effective volume reduction of a specimen made of dental cement or filler material during its curing, including the formation of fractures and fissures in the specimen.

The term "bulk filling" as used in this invention comprises all methods and/or techniques of direct dental restoration and/or prevention where a cavity is filled with a suitable filling material.

The term "one-step bulk filling" as used in this invention comprises all methods and/or techniques of direct dental restoration and/or prevention where the filling is performed in a way that the total volume of the cavity is filled up completely with the filling material in one step during

the setting of the filling material.

The term "deuterium oxide" as used in this invention comprises deuterium oxide in all possible isotopic enrichments above the natural abundance of the deuterium isotope, it further comprises all semi-deuterated water molecules (H-O-D) where one hydrogen and one deuterium atom are bound to the oxygen atom.

Deuterium is one of two stable isotopes of hydrogen. It has a natural abundance in Earth's oceans of about one atom in 6,420 of hydrogen. Thus deuterium accounts for approximately 0.0156% (or on a mass basis: 0.0312%) of all the naturally occurring hydrogen in the oceans, while the most common isotope (hydrogen-1 or protium) accounts for more than 99.98%.

The term "deuterium" comprises all deuterium atoms which originated from deuterium oxide.

According to the invention, the terms "deuterium oxide", "D₂O", "deuterium" and "heavy water", are used synonymously and can be replaced by each other.

The term "carbon nanotubes" as used in this invention is synonymous for single walled and multi walled carbon nanotubes.

The term "fibers" as used in this invention, comprises all types of fiber forming materials which can be included or embedded into a cement, such as a portland cement or dental cement, with the aim of improving the the mechanical stability of said cements.

The term "reinforcement" as used in this invention is used synonymously for all suitable methods and techniques where a substance or material forms microscopic or macroscopic structures of its own inside a matrix (e.g. a dental cement or filler material) which improve the mechanical stability of the compound material (e.g. a composite of graphene and dental cement or filler material)

The term "composite" as used in the invention relates generally to all materials which are made of a combination of different substances, in the stricter sense of dental materials it relates to all kinds of mixtures of materials which can be used to fill a cavity or to glue dental parts and/or implants.

The term "setting" of a material, mostly a dental cement or filler material, as used in this invention, relates to the sum of all physical and chemical processes which are initiated by the mixing of its components and which result in a change of the physical state of the dental cement or filler material, in most cases the transformation to the solid physical state. The "setting time" is the time span from initial mixing until the material, preferably present in the form of a paste-like mixture, has become a solid.

The term "curing" as used in this invention comprises part(s) or all of the setting but includes all physical, chemical or physico-chemical changes the material undergoes post-setting, in particular, but not restricted to, the first 100 hours of a material after the initial mixing of its components with the initiation of a setting reaction.

The term "matrix" as used in this invention relates to all types of host materials (a substance or a mixtures of several substances) which allow the inclusion or embedding of another material which is enabled to form structures of its own within the host material and forms a quasi-continuous fluid, semi-fluid or solid phase around the embedded material or structure.

The term "mechanical strength" as used in this invention relates to all physical parameters which can be measured by dedicated techniques to describe qualitatively and quantitatively the mechanical state and stability of a material, in particular, but not restricted to, tensile strength, bending stiffness, compressive strength and surface hardness.

The term "improvement of mechanical strength" or the term "improvement of mechanical parameters" as used in this invention relates to all methods and compositions which improves one or several of the physical parameters which describe the mechanical strength of a material in the sense that said parameters change by the improvement measurably to give the material additional mechanical stability and/or resilience.

The term "glass-ionomer cement" as used in this invention comprises conventional glass ionomer cements based on the reaction of silicate glass and polyalkenoic acid or other agents suitable for an acid-base reaction which results in a said cement as well as metal reinforced glass ionomer cements which additionally comprise a metal component to release metal ions.

The term "resin based cement" as used in this invention relates to all dental cements or filler materials based on a hardenable resin which self cures or light cures or dual cures and with the resin selected preferably, but not restricted to, from acrylic resin, methacrylic resin, epoxy resin,

vinyl resin, urethane resin or mixtures of these resins.

The term "resin modified glass ionomer cement" as used in this invention comprises all glass-ionomer cements which contain additionally a resin (such as hydroxymethylmethacrylate) and a photoinitiator. It encompasses hybrid ionomer cements, dual-cured and tri-cured glass ionomer cements.

The term "silane groups" or "silane chains" as used in this invention relates to all silanating agents capable of chemically silanting graphene and include those having at least one polymerizable double bond and at least one group that easily hydrolyses with water, preferably 3-methacryloxypropyltrimethoxysilane, 3-methacryloxypropyldimethoxy-monochlorosilane, 3-methacryloxypropyldichloromonomethoxysilane, methacryloxypropyltri-chlorosilane, 3-methacryloxypropyldichloromonomethyl-silane, 3-methacryloxypropylmonochlorodimethylsilane, and mixtures thereof.

Brief description of the drawings

The present invention is illustrated on the basis of Fig. 1 to 3, although, these do not restrict the scope and subject-matters of the invention.

Figure 1 shows a table (Table A) presenting the results from the surface hardness measurements performed according to **Example 10**.

Surface hardness (Martens-hardness, MH) measured according to DIN EN ISO 14577 of commercially available dental glass-ionomer cements (KC) and filler materials (KME), resin based dental cements (NX3), resin modified glass ionomer cement (KCP) and carbomer – modified glass ionomer cement (CMGIC). MH values are shown for the unmodified cements (KME, KC, NX3, RXU, CMGIC) and for cements modified by addition of graphene (-G) and/or deuterium oxide (D-). The number in the sample name following the -G represents the graphene concentration in wt.%. For example, D-KME-G0.1 denotes deuterium enriched Ketac Molar Easymix containing 0.1 wt% (% w/w) graphene.

KC: Ketac Cem[®] (3M ESPE, Seefeld, Germany); KME: Ketac Molar Easymix[®] (3M ESPE, Seefeld, Germany); KCP: Ketac Cem Plus[®] (3M ESPE, Seefeld, Germany); NX3: NX3[®] dual cure resin cement (Kerr Corp. CA, USA).

Figure 2 shows a table (Table B) presenting the results from the bending stiffness measurements performed according to **Example 11**.

Bending stiffness measured according to DIN EN ISO 4049:2009(E) of commercially available dental glass-ionomer cements (KC) and filler materials (KME), resin based dental cements (NX3), resin modified glass ionomer cement (KCP) and carbomer –modified glass ionomer cement (CMGIC). MH values are shown for the unmodified cements (KME, KC, NX3, RXU, CMGIC) and for cements modified by addition of graphene (-G) and/or deuterium oxide (D-). The number in the sample name following the -G represents the graphene concentration in wt.% (% w/w). For example, D-KME-G0.1 denotes deuterium enriched Ketac Molar Easymix containing 0.1 wt% (% w/w) graphene.

KC: Ketac Cem® (3M ESPE, Seefeld, Germany); KME: Ketac Molar Easymix® (3M ESPE, Seefeld, Germany); KCP: Ketac Cem Plus® (3M ESPE, Seefeld, Germany); NX3: NX3® dual cure resin cement (Kerr Corp. CA, USA)

Figure 3 shows a table (Table C) presenting the results from the compressive strength measurements performed according to **Example 12**.

Compressive strength measured according to DIN EN ISO 9917 of commercially available dental glass-ionomer cements (KC) and filler materials (KME), resin based dental cements (NX3), resin modified glass ionomer cement (KCP) and carbomer –modified glass ionomer cement (CMGIC). MH values are shown for the unmodified cements (KME, KC, NX3, RXU, CMGIC) and for cements modified by addition of graphene (-G) and/or deuterium oxide (D-). The number in the sample name following the -G represents the graphene concentration in wt.% (% w/w). For example, D-KME-G0.1 denotes deuterium enriched Ketac Molar Easymix containing 0.1 wt% (% w/w) graphene.

KC: Ketac Cem® (3M ESPE, Seefeld, Germany); KME: Ketac Molar Easymix® (3M ESPE, Seefeld, Germany); KCP: Ketac Cem Plus® (3M ESPE, Seefeld, Germany); NX3: NX3® dual cure resin cement (Kerr Corp. CA, USA)

Detailed description of the invention

The present invention provides means and compositions to improve the essential mechanical stability parameters of GIC's , RMGIC's and RBC's by the formation of reinforcement structures within the dental cement or filler material resulting, among other things, in the prevention of microscopic fractures and fissures during the curing process and improvement of mechanical

strength.

Preferred candidate material to successfully achieve the task of reinforcing dental cements or filler materials in accordance with the present invention would possess one or more of the following characteristics: (i) The material is one (like a rod) or two dimensional (like a sheet, slab or platelet) and its spatial extension is in the order of micrometers to millimeters in order to span a distance inside the dental cement or filler material which is in a reasonable relation to the external dimensions of the composite. (ii) The material has superior mechanical parameters which exceed those of the dental cement or filler material. (iii) The material exhibits superior resistance to acids, bases, metal ions and any other liquids and solids (organic and anorganic) which occur regularly or even temporary in the vicinity of dental cement or filler material constructs in the mouth. Finally, (iv) the material forms tight bonds with the dental cement or filler material matrix in order to truly reinforce the composite rather than forming a (demixed or isolated) phase of its own which would destabilize the composite and to prevent the formation of micro-fissures or cracks during the curing of the dental cement.

In a first aspect, the present invention provides materials with these characteristics by the first time introduction of graphene (strictly two dimensional single layer carbon sheets with material parameters which are in virtually all aspects superior to steel) and its chemically functionalized forms as a material to reinforce dental cements or filler materials.

Graphene is a single layer sp^2 -bonded carbon sheet forming a honeycomb crystal lattice, reported first by Mouras (Mouras, S. et al., *Revue de Chimie Minerale*, 1987:24:572) as the two dimensional (2D) form of graphite.

Graphene is optically transparent and can be readily produced, among other preparation methods, from graphite by exfoliation techniques (Hernandez, Y. et al., *Nature Nanotechnol.* 3, 563–568, 2008), the resulting graphene platelets have a size of up to 100 μm . The edges of the graphene platelets can be modified by $-OH$, $-COOH$, $-NH_2$ groups, by Fluoride (F) atoms, or functionalized with silane groups, which allows to dissolve graphene in aqueous and non-aqueous solutions as well as the controlled modification of its solution properties. This enables the use of graphene in GIC's where a hydrophilic graphene is required for the formation of a composite, but also in RMGIC's and RBC's where a hydrophobic graphene is the prerequisite for its dissolution and binding to the resin matrix.

According to the present invention, graphene is used at a concentration of 0.00001 weight/weight (% w/w) to 90 % w/w, preferred 0.01 % w/w to 10 % w/w, more preferred 0.01 % w/w to 5 % w/w, most preferred 0.05 % w/w to 3 % w/w in terms of the dry phase.

In a second aspect, the invention provides a dental cement or filler material, preferably a GIC, comprising of a substance (deuterium oxide or deuterium) to partly or totally replace the water in the fluid component of GIC preparations, to improve the mechanical parameters of dental cements/filler materials with or without graphene even further.

Deuterium oxide (D₂O) or heavy water is a water-like molecule where the two hydrogens bound to the oxygen atom are replaced by a stable isotope of hydrogen called deuterium. Deuterium contains an additional neutron in its nucleus and as a result D₂O is about 10% heavier than normal water (H₂O). This in turn results in a higher density and different vapor pressure. Heavy water occurs in natural water, roughly every 6000th molecule in natural water is a heavy water molecule. It is produced by very energy intensive distillation procedures of natural water and can be obtained commercially at a very high degree of purity (degree of deuterium enrichment) of up to 99.9%. Highly enriched deuterium oxide exhibits a approximately 10% higher viscosity than normal water at the same temperature. Because the setting reaction of a GIC depends, among other factors, on the viscosity of the fluid phase, the replacement of H₂O by D₂O in the fluid component of a GIC provides means of manipulation or modulation of the setting reaction. This may result in improvements of the mechanical stability of the cured GIC.

A salient feature of D₂O is its higher strength to bind to other atoms or molecules via so-called hydrogen bonds. This enables D₂O or deuterons in general to bind significantly stronger to hydrogen bonding sites than hydrogen (or H₂O) itself.

For example, in GIC formation starting from its two components (an acidic liquid phase containing water and a solid phase of essentially silicate containing metal ions), hydrogen bonds play an essential role because water is the medium where the acid base reaction in the setting process of the dental cement/filler material takes place. Hence all water soluble GIC components (silicate, ions, acid polymers) will be surrounded by a hydration shell of water (H₂O). If H₂O is replaced by D₂O, the increase in (hydrogen) binding strength translates into a tighter packing of the components during the settlement process while the higher viscosity of D₂O modulates the diffusion of the components during the setting time. Both processes give rise to a higher mechanical stability of the settled GIC. Since hydrogen bonds are essential in the

setting reaction of GIC's and their curing, the addition of any water soluble polymers to the GIC composition which are able to form hydrogen bonds can further improve the mechanical strength of a GIC under conditions where the H₂O is replaced by D₂O. Examples for such polymers are all nonionic, cationic or anionic polymers, in particular acrylic acid based polymers (carbomers), polyvinyl alcohol based polymers, poly-ethylene oxide polymers and sugar based polymers.

According to the present invention, deuterium oxide or deuterium is used at a concentration of 3 % vol. to 99,9 % vol., preferred at a concentration of 15 % vol. to 99 % vol., more preferred at a concentration of 50 % vol. to 99 % vol., even more preferred at a concentration of 70 % vol. to 99 % vol., most preferred at a concentration of 80 % vol. to 99 % vol., in terms of the fluid phase (in solution).

According to the present invention, the water-soluble polymer is used at a concentration of 0.0001 % w/w to 50 % w/w, more preferred 0.001 % w/w to 25 % w/w and most preferred 0.05 % w/w to 5.0 % w/w.

In a third aspect, the present invention further provides for a dual presence of graphene platelets (with –OH, –NH₂ or –COOH groups or any other, hydrogen bond formation enabled functional groups bound to its edges) and of D₂O replacing partially or totally the H₂O in a dental cement or filler material, preferably a GIC. During curing, this dual presence has an even stronger effect regarding the improvement of the resulting mechanical properties of the GIC. This is because the deuterons replace the hydrogen atoms at the hydrophilic functional groups attached to the graphene edges. The higher (hydrogen) binding strength of the deuterons causes a tighter packing of the solid components including the graphene and its tighter binding to the GIC matrix, resulting in improved mechanical strength of the graphene-GIC composite beyond that of just graphene GIC composites or D₂O enriched GIC's alone.

In RMGIC's and RBC's the effect of graphene on the mechanical properties of the composite dental cement/filler material is different. Here the embedding of the pristine or flouride –modified (hydrophobic) graphene patelets provides essentially a reinforcement of the composite structure over distances which correlates with the spatial extension of the graphene platelets. The resin matrix will bind in the setting process to the graphene via the functional groups or directly to the pristine graphene, because the graphene surface can attract free radicals (induced by an initiator molecule (dual cure) or by shining UV light on the mixture (light-cure) which in turn are essential for a high degree of polymerization of the resin matrix. As a result, the resin matrix in

the vicinity of the graphene platelets exhibits a particularly high polymerization density reinforced by the underlying graphene platelet and surrounded by the cured resin matrix. This composite structure inside the RMGIC's and RBC, respectively, significantly improves the overall mechanical stability of the RMGIC's and RBC, respectively, and helps to prevent the formation of fissures during the curing of the RMGIC's and RBC owing to shrinking of the material.

In general, the reinforcement of dental cements and filling materials by graphene has important ramifications for the prevention of shrinking of the materials. The mechanical properties of graphene bound to the matrix of any dental cement or filling material stabilizes against volume loss during the curing of the material, thus preventing the formation of fractures and fissures. This enables one-step bulk filling techniques for all self curing dental filling materials.

The application of all dental cements or filler materials in patients requires that the color of the surrounding tooth is matched by the dental cements or filler materials to find acceptance with the users (dentists) and their patients. The materials/substances used in the present invention (graphene and D₂O) are both optically transparent and thus do not interfere with the coloring scheme of the the dental cements/filler materials.

The invention relates in its first subject to a dental cement or filler material composition comprising graphene and/or deuterium oxide or deuterium.

A preferred embodiment relates to an inventive dental cement or filler material, whereby the dental cement or filler material is a glass ionomer cement or filler material or resin based cement or filler material or resin modified glass ionomer cement or filler material.

A further preferred embodiment relates to a dental cement or filler material, whereby the graphene is in its pristine form or with its reactive groups chemically modified, preferably modified with hydroxyl, carboxyl, amide or silane functional groups or with the graphene reactive groups bound to flouride atoms.

Another preferred embodiment relates to a dental cement of filler material composition, whereby graphene is comprised at a concentration of 0.00001 % w/w to 90 % w/w, preferred 0.01 % w/w to 10 % w/w, more preferred 0.01 % w/w to 5 % w/w, most preferred 0.05 % w/w to 3 % w/w in terms of the dry phase.

Another preferred embodiment relates to a dental cement or filler material composition, whereby deuterium oxide or deuterium is comprised at a concentration of 3 % vol. to 99,9 % vol., preferred at a concentration of 15 % vol. to 99 % vol., more preferred at a concentration of 50 % vol. to 99 % vol., even more preferred at a concentration of 70 % vol. to 99 % vol., most preferred at a concentration of 80 % vol. to 99 % vol., in terms of the fluid phase (in solution).

A further preferred embodiment, the dental cement or filler material, whereby the glass ionomer cement composition comprises additionally a water soluble polymer, which can form hydrogen bonds, selected from the group consisting of synthetic or natural polymers being either nonionic, cationic or anionic, preferably at neutral pH value, preferably acrylic acid based polymers (carbomers), polyvinyl alcohol based polymers, poly-ethylene oxide polymers and sugar based polymers.

A preferred embodiment relates to a dental cement or filler material for direct or indirect dental restoration and/or prevention. Another preferred embodiment relates to a dental cement or filler material for the use in direct or indirect dental restoration and/or prevention.

All conventional state of the art dental cements and filler materials can be improved according to the invention regarding their mechanical strength and parameters by the inclusion of graphene and/or deuterium oxide or deuterium into their matrix prior to the setting/curing process. All conventional state of the art dental cements and filler materials in well-known compositions and concentrations can be used to carry out the invention

A preferred embodiment of the invention relates to the inclusion of graphene into dental cements or filler materials. The preferred properties of the graphene platelets used for inclusion are single layer graphene with the carbons at the edges of the platelets functionalized with chemical groups which provide for a optimum binding of the graphene platelets with the matrix. For GIC's the most preferred functional groups bound to graphene are hydrophilic and can form hydrogen bonds. For RMGIC's and RBC's the graphene preferably used is either pristine or functionalized with groups which render it hydrophobic like flouride atoms (giving partially or perfluorinated graphene) or silane groups/chains. The invention is not restricted to the above functional modifications, in general all modifications which improve further the binding between the graphene and the matrix and/or the spatial distribution of the graphene within the matrix are preferred.

Multi-layer graphene platelets or graphene obtained from processing carbon nanotubes (both single walled and multi walled) as well as mixtures of all types of graphene represent further preferred embodiments of the invention regarding their inclusion in the matrix.

The amount of graphene used for the inclusion into the matrix is 0.00001 % to 90 %, preferred 0.01 % to 10 % , more preferred 0.01 % to 5 %, most preferred 0.05 % to 3 %. All percentages given for the graphene inclusion are by weight.

The use of deuterium oxide (D₂O) in glass ionomer dental cements/filler materials, either with graphene included or without graphene, is a further especially preferred embodiment of the invention. The deuterium oxide can be partially deuterium enriched or highly deuterium enriched with a degree of enrichment of up to 90 % and even 99.999 %. The D₂O is preferably added to the fluid phase of a GIC prior to the mixing with the solid phase in order to allow for a replacement of part or all of the hydrogen atoms in the fluid phase by deuterium atoms. All known physical and chemical methods to replace the hydrogen atoms in the fluid phase by deuterium atoms represent embodiments of the invention. The preferred final deuterium enrichment in the fluid phase (i.e. the percentage of hydrogen atoms in the fluid phase exchanged by deuterium atoms) prior to the mixing with the solid phase of a GIC is 3 % to 99,9 %, more preferred 50 % to 99%, most preferred 80 % to 99%. The solid phase of a GIC can also contain hydrogen atoms which can be exchanged by deuterium atoms prior to the mixture with the fluid phase. All known physical or chemical methods to achieve such an exchange in said solid phase prior to the mixing with the said fluid phase represent embodiments of the invention. The preferred final deuterium enrichment in the solid phase (i.e. the percentage of hydrogen atoms in the solid phase exchanged by deuterium atoms) prior to the mixing with the fluid phase of a GIC is 1 % to 99,9 %, more preferred 50 % to 99 %, most preferred 80 % to 99 %. All percentages given for the deuterium oxide or deuterium inclusion are by volume.

The dual use of deuterium oxide and graphene in a glass ionomer dental cement or filler material represents another especially preferred embodiment of the invention. This embodiment improves the reinforcement and mechanical parameters of dental cements or filler materials even further. Preferably, the graphene is added to either the solid or the fluid phase prior to the mixing of the two phases but the addition of graphene during the mixing is possible as well. Generally, the graphene addition happens before the commencement of the setting/curing of the GIC. The deuterium oxide addition to the GIC phases with the aim of the exchange of part or all of the hydrogen atoms or the use of other hydrogen-deuterium exchange techniques to achieve

such an exchange are analogous to the case described above for the sole use of deuterium oxide in GIC's. The preferred introduction of deuterium oxide to the GIC is prior to the mixing of the two phases, preferred is the partial replacement of the water component of the fluid phase of the GIC by deuterium oxide, most preferred is the replacement of the complete water component of the fluid phase of the GIC by deuterium oxide.

The additional introduction of water soluble polymers which provides additional hydrogen bonding sites to the glass ionomer dental cement/filler material and with the H₂O replaced partially or in full by D₂O, either with or without graphene, represents a further embodiment of the invention. Preferably, the at least one polymer is added to a dental cement or filler material, preferably a GIC composition, either to its solid or liquid phase prior to mixing but the addition of said polymer during the mixing of the two phases is equally possible, provided the addition is concluded before the conclusion of the setting of the GIC. The amount of polymers added to the GIC composition is in the range 0.0001 % w/w to 50 % w/w, more preferred 0.001 % w/w to 25 % w/w and most preferred 0.05 % w/w to 5.0 % w/w. The at least one polymer is preferably added to an inventive dental cement or filler material comprising deuterium oxide or a combination of deuterium oxide and graphene.

Further embodiments of the invention relate to a dental cement or filler material composition for direct or indirect dental restoration and/or prevention, where the dental cement or filler material comprises graphene. In a preferred embodiment, the dental cement/filler material is a glass ionomer cement/filler material or resin based cement or filler material or resin modified glass ionomer cement/filler material. Preferably, the graphene comprised by the dental cement or filler material is in its pristine form or with its reactive groups chemically modified, preferably modified with hydroxyl, carboxyl, amide or silane functional groups or with the graphene reactive groups bound to fluoride atoms.

Another embodiment relates to a dental cement or filler material composition for direct or indirect dental restoration and/or prevention, where the dental cement or filler material comprises deuterium oxide or deuterium. In a preferred embodiment, the dental cement or filler material is a glass ionomer cement/filler material or resin based cement/filler material or resin modified glass ionomer cement/filler material. Preferably, the glass ionomer cement composition comprises additionally a water soluble polymer which can form hydrogen bonds, whereby the water soluble polymer is selected from the group consisting of synthetic or natural polymers

being either nonionic, cationic or anionic, preferably acrylic acid based polymers (carbomers), polyvinyl alcohol based polymers, poly-ethylene oxide polymers and sugar based polymers.

A yet another embodiment relates to a dental cement or filler material composition for direct or indirect dental restoration and/or prevention, where the dental cement comprises graphene and deuterium oxide or deuterium. Preferably, the cement is a glass ionomer cement/filler material or resin based cement/filler material or resin modified glass ionomer cement/filler material. Preferably, the glass ionomer cement composition comprises additionally a water soluble polymer which can form hydrogen bonds, selected from the groups synthetic or natural polymers being either nonionic, cationic or anionic at neutral pH value, in particular acrylic acid based polymers (carbomers), polyvinyl alcohol based polymers, poly-ethylene oxide polymers and sugar based polymers.

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Examples

Example 1: Graphene preparation

Hydrophobic pristine graphene in organic solution was prepared by liquid-phase exfoliation of graphite (Hernandez, Y et al., High-yield production of graphene by liquid phase exfoliation of graphite. *Nature Nanotechnol.* 3, 563-568, 2008). A Branson W-250 titanium-rod sonifier was employed in pulsed mode (2s pulses with 1 s interval) at highest power (200W) for 20 min. for the sonication of the graphite solution in a thermostated water bath (40°C). The graphite powder was from Sigma-Aldrich (Cat. No. 332461) and the solvent used was N-methylpyrrolidone (NMP). The final solution (after concentration of the centrifugation pellets) contained 1.5% (wt.) of non-oxidized graphene monolayer platelets with a size distribution of less than 30 nm – 5 μ m as determined by electron microscopy (TEM). The solution was freeze-dried in a freeze-drying flask after rotating the flask in liquid nitrogen until all liquid was homogeneously frozen at the wall of the flask. After this, the freeze drying was performed for 2 h using a benchtop manifold freeze dryer (BT85 from Millrock Technology, Kingston, NY, USA) operating at a pressure of 10^{-3} mbar. The freeze-dried graphene flakes were mechanically removed from the flask and stored under vacuum.

Hydrophilic (water soluble) graphene monolayer platelets in aqueous solution were prepared from graphite oxide according to procedures of liquid phase exfoliation and and were further concentrated by centrifugation (Dreyer, D R et al., From conception to realization: an historical account of graphene and some perspectives for its future. *Angew. Chem. Int. Ed.* 49, 9336-9344 , 2010). A Branson W-250 titanium-rod sonifier was employed in pulsed mode (2s pulses with 1 s interval) at 100W output power for 15 min. for the sonication of the graphite oxide solution in a thermostated water bath (60°C). The final aqueous solution comprised a liquid:graphene (exfoliated graphene nano platelets) ratio of 3:1 (wt.) where the individual platelets ranged in size from less than 20 nm up to 50 μ m with a thickness of less than 5 nm to 50nm, as determined by electron microscopy. The graphene platelets obtained this way are covered at their edges by hydroxide groups and hydrogen caps, rendering them water soluble. Further analysis showed that less than 50% of the graphene platelets were thicker than one monolayer. In order to obtain modified graphene platelets with the hydrogen atoms covering the edges replaced by deuterium atoms, the above preparation procedure was performed in deuterium oxide (D₂O) of 98% enrichment (CU Chemie Uetikon AG, CH) under a nitrogen atmosphere. The final D₂O –graphene solution was adjusted to contain graphene nano platelets at the ratio of

3:1 (wt) and electron microscopic analysis showed a similar size distribution of the platelets.

Example 2: Glass-Ionomer cement (GIC) specimen preparation

The following commercially available dental cements were used: A GIC dental filling material (Ketac Molar Easymix (KME), 3M ESPE, Seefeld, Germany) and and GIC dental cement (Ketac Cem (KC), 3M ESPE, Seefeld, Germany), both comprising of a solid and a liquid phase to be mixed on application with a setting time of less than 5 min. The cement specimens KME and KC were prepared according to the user manual instructions by mixing the two phases at a solid : liquid phase ratio of 3.5 (wt.) , and filling the mixtures into cylindrical cast moulds, capped on each open side by a foil of cellulose acetate supported by a glass slide. The size of the cylindrical cast moulds was 4 mm diameter and 6 mm height (for compression and flexural strength measurements) and 18.7 mm diameter and 3.0 mm height (for surface hardness measurements). The specimens were then stored at 37 ° C and 30% rel. humidity for 60 min. , after this the cast moulds were removed and the specimen samples were cured in distilled water for at least 48 h at 37°C.

Example 3: Deuterium oxide-substituted glass-ionomer cement (D-GIC) specimen preparation

The liquid phase of the commercial GIC's from example 1 was freeze dried (lyophilized) in a freeze-drying flask after rotating the flask in liquid nitrogen until all GIC liquid phase was homogeneously frozen at the wall of the flask. After this, the freeze drying was performed for 3 h using a benchtop manifold freeze dryer (BT85 from Millrock Technology, Kingston, NY, USA) operating at a pressure of 10^{-3} mbar. The freeze dried flakes in the flask were resuspended with Deuterium oxide (D₂O) of 98% enrichment (CU Chemie Uetikon AG, CH) at the original volume of the liquid phase. The resulting deuterated liquid phase was –like the original liquid phase– optically clear and featured a 6% higher viscosity owing to the higher density of deuterium oxide compared to water. The D-GIC specimen preparation was analogous to example 2 except that the liquid phase was replaced by the deuterated liquid phase, giving D-KME and D-KC.

Example 4: Composite GIC-graphene oxide cement (GIC-G) specimen preparation

Specimen were prepared analogous to example 2) but with the liquid phase of the said commercial dental cements now containing either 0.1% or 0.3% (wt.) graphene platelets

prepared according to example 1, giving KME-G0.1 , KC-G0.1 and KME-G0.3 , KC-G0.3 specimens, respectively . The volume of the liquid phase was preserved during the addition of the graphene platelet aqueous solution by slow evaporation (at 40°C and ambient pressure under an nitrogen atmosphere) of an equal amount of water from the liquid GIC phase prior to the addition of the platelet solution at the same volume as previously extracted.

Example 5: Composite D-GIC-graphene oxide cement (D-GIC-G) specimen preparation

Specimen were prepared analogous to example 4 but with the liquid phase now replaced by the deuterated liquid phase (example 3) now containing either 0.1% or 0.3% (wt.) graphene platelets prepared according to example 1, giving D-KME-G0.1 , D-KC-G0.1 and D-KME-G0.3 , D-KC-G0.3 specimens, respectively . The addition of the graphene platelets to the liquid phase was performed as described in example 4 with the exception that the deuterium substituted graphene solution prepared according to example 1 has been used.

Example 6: Resin modified glass ionomer cement (RMGIC) specimen preparation

Ketac Cem Plus (3M ESPE, Seefeld, Germany) is a self-curing resin modified glass ionomer luting cement. The control specimens (KCP) were prepared by filling the cement from the mixing dispenser directly into the cylindrical cast moulds and further processed as described below (self-cure only) . The graphene modified specimens were prepared by adding to the cement from the mix-dispenser oxidized graphene platelets (obtained as solid flakes after freeze-drying the aqueous solution as described in example 1) . The platelets were mechanically mixed into the paste (final graphene concentration 0.08 wt%) for 1 min. using a rotating mixing plate (rotation speed 50 rev./min). After this the specimens were produced by filling the graphene-Ketac Cem Plus mixture (KCP-G) mixture into cylindrical cast moulds, capped on each open side by a foil of cellulose acetate supported by a glass slide. The size of the cylindrical cast moulds was 4 mm diameter and 6 mm height (for compression and flexural strength measurements) and 18.7 mm diameter and 3.0 mm height (for surface hardness measurements). The mixture was allowed to self-cure in the casting moulds for 1 h and after this the specimens were removed from the moulds and stored until further use at 37°C at 30% rel. humidity.

Example 7: Resin based cement (RBC) specimen preparation

NX3 dual cure resin cement (Kerr Corp. CA, USA) was used right from the self-mix dispenser unit to obtain the control specimen (NX3) by filling them into cast moulds and allowing self-cure as in example 6. The graphene modified specimen (NX3-G) were obtained by adding pristine graphene platelets prepared according to example 1 to the original NX3 cement from the self-mix dispenser unit and mixing both for 1 min as described in example 6. The final pristine graphene platelets content was 0.08% (wt.). The further specimen preparation (self-cure only) was analogous to example 6.

Example 8: Carbomer modified GIC specimen preparation (CMGIC)

CMGIC specimen were prepared from Ketac Molar Easymix (3M ESPE, Seefeld, Germany) according to Example 2 with the exception that 0,5 % (wt.) of Carbopol®980 (Lubrizol, Cleveland, USA) was added to the solid phase prior to the mixing with the liquid phase.

Example 9: Deuterium oxide-substituted Carbomer modified GIC specimen preparation (D-CMGIC)

D-CMGIC specimen were prepared according to Example 3 with the exception that 0,5 % (wt.) of Carbopol®980 (Lubrizol, Cleveland, USA) was added to the solid phase prior to the mixing with the (deuterium oxide-substituted) liquid phase.

Example 10: Surface hardness measurements

Measurements were performed according to DIN EN ISO 14577 using a ZHU 2.5 universal hardness measurement device from Zwick/Roell (Ulm, Germany) and the Martens-hardness (MH) was calculated from the penetration depth of a sphere into the specimen surface at a maximum load of 10N and a forward speed of 0.1 mm/s. For each data point four measurements using four different specimen were performed. The results for all specimens are listed in **Figure 1** as Marten-hardness (MH).

Example 11: Bending stiffness measurements

Bending stiffness measurements were performed according to DIN EN ISO 4049:2009(E) using a Zwick materials testing machine Z050/THA3 (Zwick/Roell, Ulm, Germany). The test speed of the normal force (i.e. the force applied perpendicular to the specimen long axis halfway between the two holding points of the specimen) was 0.75mm/min.

The bending stiffness σ [MPa] was calculated as

$$\sigma = 3f_{\max} L / (2BH^2)$$

where $f_{\max}$ is the maximum normal force (break down force in [N]) , L is the distance between the holding points [mm], B and H are specimen width and length [mm], respectively. Each value σ is the mean average over four measurements using four different specimen of identical composition.

The results for all specimens are presented in **Figure 2**

Example 12: Compressive strength measurements

Compressive strength measurements were performed according to DIN EN ISO 9917 using a Zwick materials testing machine Z050/THA3 (Zwick/Roell, Ulm, Germany). The test speed of the force applied along the long axis of the test specimen was 0.75mm/min. Compressive strength C was calculated as

$$C = 4f_{\max} / (\pi d^2)$$

where $f_{\max}$ [N] is the force at which the specimen collapses and d [mm] is the diameter of the test specimen. Each value C is the mean average over four measurements using four different specimen of identical composition.

The results for all specimens are presented in **Figure 3**.

Claims

1. A dental cement or filler material composition comprising graphene and/or deuterium oxide or deuterium.
2. A dental cement or filler material according to claim 1, whereby the dental cement or filler material is a glass ionomer cement or filler material or resin based cement or filler material or resin modified glass ionomer cement or filler material.
3. A dental cement or filler material according to claim 1 or 2, whereby the graphene is in its pristine form or with its reactive groups chemically modified, preferably modified with hydroxyl, carboxyl, amide or silane functional groups or with the graphene reactive groups bound to flouride atoms.
4. A dental cement of filler material composition according to any one of claims 1 to 3, whereby graphene is comprised at a concentration of 0.00001 % w/w to 90 % w/w, preferred 0.01 % w/w to 10 % w/w, more preferred 0.01 % w/w to 5 % w/w, most preferred 0.05 % w/w to 3 % w/w in terms of the dry phase.
5. A dental cement of filler material composition according to any one of claims 1 to 4, whereby deuterium oxide or deuterium is comprised at a concentration of 3 % vol. to 99,9 % vol., preferred at a concentration of 15 % vol. to 99 % vol., more preferred at a concentration of 50 % vol. to 99 % vol., even more preferred at a concentration of 70 % vol. to 99 % vol., most preferred at a concentration of 80 % vol. to 99 % vol., in terms of the fluid phase.
6. A dental cement or filler material according to any one of claims 1 to 5, whereby the glass ionomer cement composition comprises additionally a water soluble polymer, which can form hydrogen bonds, selected from the group consisting of synthetic or natural polymers being either nonionic, cationic or anionic, preferably at neutral pH value, preferably acrylic acid based polymers (carbomers), polyvinyl alcohol based polymers, poly-ethylene oxide polymers and sugar based polymers.

7. A dental cement or filler material according to any one of claims 1 to 6 for direct or indirect dental restoration and/or prevention.
8. A dental cement or filler material according to any one of claims 1 to 6 for the use in direct or indirect dental restoration and/or prevention.

FIGURE 1

Table A below shows the results of Example 10:

Sample	Cement type	surface hardness MH [N/mm²]
KME	GIC	215 ± 20
D-KME	GIC	240 ± 17
KME-G 0.1	GIC	265 ± 22
KME-G 0.3	GIC	270 ± 20
D-KME-G 0.1	GIC	255 ± 25
D-KME-G 0.3	GIC	280 ± 23
KC	GIC	208 ± 15
D-KC	GIC	235 ± 10
KC-G 0.1	GIC	240 ± 16
KC-G 0.3	GIC	245 ± 12
D-KC-G 0.1	GIC	270 ± 20
D-KC-G 0.3	GIC	285 ± 15
KCP	RMGIC	135 ± 20
KCP-G	RMGIC	170 ± 15
NX3	RBC	175 ± 20
NX3-G	RBC	210 ± 20
CMGIC	GIC	175 ± 15
D-CMGIC	GIC	205 ± 15

FIGURE 2

Table B below shows the results of Example 11:

Sample	Cement type	Bending stiffness [MPa]
KME	GIC	51 ± 5
D-KME	GIC	93 ± 4
KME-G 0.1	GIC	78 ± 5
KME-G 0.3	GIC	96 ± 7
D-KME-G 0.1	GIC	112 ± 5
D-KME-G 0.3	GIC	129 ± 6
KC	GIC	21 ± 4
D-KC	GIC	45 ± 4
KC-G 0.1	GIC	41 ± 5
KC-G 0.3	GIC	49 ± 5
D-KC-G 0.1	GIC	62 ± 7
D-KC-G 0.3	GIC	65 ± 5
KCP	RMGIC	31 ± 3
KCP-G	RMGIC	68 ± 7
NX3	RBC	112 ± 10
NX3-G	RBC	138 ± 10
CMGIC	GIC	48 ± 5
D-CMGIC	GIC	122 ± 10

FIGURE 3

Table C below shows the results of Example 12:

Sample	Cement type	Compressive strength [MPa]
KME	GIC	225±25
D-KME	GIC	251 ± 15
KME-G 0.1	GIC	270 ± 12
KME-G 0.3	GIC	311 ± 20
D-KME-G 0.1	GIC	296 ± 15
D-KME-G 0.3	GIC	336 ± 15
KC	GIC	131 ± 10
D-KC	GIC	186 ± 15
KC-G 0.1	GIC	204 ± 15
KC-G 0.3	GIC	228 ± 20
D-KC-G 0.1	GIC	243 ± 15
D-KC-G 0.3	GIC	270 ± 25
KCP	RMGIC	201 ± 10
KCP-G	RMGIC	310 ± 15
NX3	RBC	320 ± 20
NX3-G	RBC	355 ± 15
CMGIC	GIC	240 ± 25
D-CMGIC	GIC	260 ± 25

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2014/054846

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K6/083

ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, COMPENDEX, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2009/176891 A1 (CHOGLE SAMI [US] ET AL) 9 July 2009 (2009-07-09) page 7; claims 1-6; example 8 page 1, paragraphs [0001],[0007] -----	1,2,6-8
Y	US 6 599 961 B1 (PIENKOWSKI DAVID A [US] ET AL) 29 July 2003 (2003-07-29) column 1, line 13 - line 16 column 3, line 3 - line 45 column 6, line 9 - line 12 claims ----- -/-	1,2,4, 6-8



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

20 May 2014

Date of mailing of the international search report

19/08/2014

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INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2014/054846

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE MEDLINE [Online] US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US; 28 April 2012 (2012-04-28), GONÇALVES GIL ET AL: "Graphene oxide versus functionalized carbon nanotubes as a reinforcing agent in a PMMA/HA bone cement.", XP002724549, Database accession no. NLM22499394 abstract	1
X	- & NANOSCALE 28 APR 2012, vol. 4, no. 9, 28 April 2012 (2012-04-28), pages 2937-2945, XP002724550, ISSN: 2040-3372 the whole document	1
Y	----- DATABASE WPI Week 201259 Thomson Scientific, London, GB; AN 2012-J01776 XP002724551, & CN 102 492 082 A (UNIV GUILIN TECHNOLOGY) 13 June 2012 (2012-06-13) abstract	1,2,4, 6-8
Y	----- ROBERT J YOUNG ET AL: "The mechanics of graphene nanocomposites: A review", COMPOSITES SCIENCE AND TECHNOLOGY, vol. 72, no. 12, 6 May 2012 (2012-05-06), pages 1459-1476, XP028504225, ELSEVIER, UK ISSN: 0266-3538, DOI: 10.1016/J.COMPSCITECH.2012.05.005 [retrieved on 2012-05-18] page 1469, paragraph 5 - paragraph 5.1 -----	1,6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2014/054846

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

3, 4(completely); 1, 2, 6-8(partially)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 3, 4(completely); 1, 2, 6-8(partially)

A dental cement or filler material composition comprising graphene

2. claims: 5(completely); 1, 2, 6-8(partially)

A dental cement or filler material composition comprising deuterium compounds

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2014/054846

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2009176891	A1	09-07-2009	NONE
US 6599961	B1	29-07-2003	NONE
CN 102492082	A	13-06-2012	NONE

Bibliographic data

Title: STABILISED PHARMACEUTICAL COMPOSITIONS AND METHODS FOR PREPARING SAME

Pub/Pat no: WO9421298A1

Pub/Issue Date: 1994-09-29

Inventor(s): SIMPSON KARL |CRAINIC RADU

Applicant(s): PASTEUR INSTITUT[FR] | SIMPSON KARL[FR] | CRAINIC RADU[FR]

Classification: A61K9/00AI; A61K39/39AI; A61K47/02AI; A61K47/04AI; A61K47/48AI

Application number: WO1994FR00296 1994-03-17

Priority number: FR19930003060 1993-03-17;

Abstract of WO9421298A1

A stabilised pharmaceutical composition containing one or more macromolecules selected from nucleic acids, lipids and carbohydrates, and/or one or more macromolecular complexes. The composition is characterized in that it includes one or more pharmaceutically acceptable carriers of which at least one contains heavy water (D₂O). The composition is suitable for preparing vaccines.



DEMANDE INTERNATIONALE PUBLIÉE EN VERTU DU TRAITE DE COOPERATION EN MATIÈRE DE BREVETS (PCT)

(51) Classification internationale des brevets ⁵ : A61K 47/02	A1	(11) Numéro de publication internationale: WO 94/21298 (43) Date de publication internationale: 29 septembre 1994 (29.09.94)
(21) Numéro de la demande internationale: PCT/FR94/00296 (22) Date de dépôt international: 17 mars 1994 (17.03.94) (30) Données relatives à la priorité: 93/03060 17 mars 1993 (17.03.93) FR (71) Déposant (pour tous les Etats désignés sauf US): INSTITUT PASTEUR [FR/FR]; 25-28, rue du Docteur-Roux, F-75724 Paris Cédex 15 (FR). (71)(72) Déposant et inventeur: SIMPSON, Karl [FR/FR]; Hameau de Bobon, F-07610 Vion (FR). (72) Inventeur; et (75) Inventeur/Déposant (US seulement): CRAINIC, Radu [FR/FR]; 16, parc de Diane, F-78350 Jouy-en-Josas (FR). (74) Mandataires: GROSSET-FOURNIER, Chantal etc.; Grosset-Fournier & Demachy SARL, 103, rue La Fayette, F-75010 Paris (FR).		(81) Etats désignés: AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, GE, HU, JP, KG, KP, KR, KZ, LK, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ, VN, brevet européen (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), brevet OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Publiée <i>Avec rapport de recherche internationale.</i>
(54) Title: STABILISED PHARMACEUTICAL COMPOSITIONS AND METHODS FOR PREPARING SAME (54) Titre: COMPOSITIONS PHARMACEUTIQUES STABILISEES ET LEURS PROCEDES DE PREPARATION (57) Abstract A stabilised pharmaceutical composition containing one or more macromolecules selected from nucleic acids, lipids and carbohydrates, and/or one or more macromolecular complexes. The composition is characterized in that it includes one or more pharmaceutically acceptable carriers of which at least one contains heavy water (D₂O). The composition is suitable for preparing vaccines. (57) Abrégé L'invention concerne une composition pharmaceutique stabilisée comprenant une ou plusieurs macromolécule(s) choisie(s) parmi les acides nucléiques, les lipides ou les glucides, et/ou un ou plusieurs complexe(s) macromoléculaire(s), caractérisée en ce qu'elle comprend un ou plusieurs véhicule(s) pharmaceutiquement acceptable(s), l'un au moins de ces véhicules contenant de l'eau lourde (D₂O). Application à la préparation de vaccins.		

UNIQUEMENT A TITRE D'INFORMATION

Codes utilisés pour identifier les Etats parties au PCT, sur les pages de couverture des brochures publiant des demandes internationales en vertu du PCT.

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COMPOSITIONS PHARMACEUTIQUES STABILISEES ET LEURS PROCEDES DE PREPARATION

La présente invention concerne des compositions pharmaceutiques stabilisées et leurs procédés de préparation.

La présente invention concerne plus particulièrement la stabilisation d'agents prophylactiques, ou thérapeutiques ou de diagnostic constitués ou contenant des macromolécules ou des complexes macromoléculaires.

L'importance sociale et commerciale des macromolécules biologiques augmente.

La plupart des agents thérapeutiques ou protecteurs agissent dans le milieu aqueux des espaces inter- ou intracellulaires, ou les surfaces muqueuses.

Certains agissent dans le milieu hydrophobique constituant les phases membranaires, lipidiques ou dans l'environnement des acides aminés hydrophobes.

Au cours de ces dernières années, les biotechnologies ont eu des influences considérables dans les industries du secteur de la santé. Les nouveaux procédés pour la fabrication et l'isolement de matériaux macromoléculaires sont capables de réaliser, en quantités commerciales, des produits naturels ou issus du génie génétique, dotés de spécificités précédemment irréalisables. (Virus, ADN, ARN, ribozymes, agents anti-sens, etc...)

Les anciens composés thérapeutiques de poids moléculaires relativement faibles (aspirine, paracétamol, catécholamines, pénicilline) ont été caractérisés par une certaine facilité de formulation dans les comprimés, les émulsions, suspensions ou solutions.

Certaines macromolécules - telles que les acides nucléiques, et leurs complexes avec les protéines (et leurs cofacteurs), les glucides complexes, les lipides - et leurs complexes, deviennent d'un intérêt de plus en plus important en tant que vaccins, ou thérapeutiques ou de diagnostic.

Dans les milieux aqueux, toutes les macromolécules et les complexes macromoléculaires indiqués ci-dessus ont une tendance à la dégradation, ce qui limite leur utilité d'agent thérapeutique, vaccinant ou de diagnostic.

Pour beaucoup d'agents, la dégradation n'est pas un problème sérieux (par exemple, en suivant les indications du fabricant, on évite les problèmes associés éventuellement avec l'utilisation d'un vaccin périmé). Néanmoins, même dans le meilleur des cas, l'augmentation de la durée de vie de l'ordre de 50% à 100% (à

la température de 20 °C) d'un produit thérapeutique, sera la bienvenue (par exemple: le vaccin contre la poliomyélite).

Pour d'autres agents, le stockage sous forme de poudre lyophilisée est tout à fait satisfaisant, la dégradation commençant simplement au moment de l'incorporation dans un milieu aqueux.

Pour plusieurs autres agents, les problèmes de stockage sous forme sèche ne peuvent pas être résolus. Pour certains, stables sous forme sèche, la dégradation en milieu aqueux est tellement rapide que la stabilité présente un problème, même dans la courte période entre l'incorporation en milieu aqueux et l'utilisation.

Il y a plusieurs facteurs causant la dégradation : les procédés de fabrication, les enzymes de dégradation (les enzymes protéolytiques, les nucléases, les lipases, les enzymes glycolytiques), les agents d'oxydation ou de réduction (comme l'oxygène, ou les produits chimiques organiques ou inorganiques), la contamination microbienne.

Les liaisons chimiques faibles sont importantes pour les macromolécules biologiques.

La dénaturation thermique ne peut pas être évitée, en raison des nombreuses liaisons chimiques faibles qui caractérisent les macromolécules biologiques. Néanmoins, cela peut-être limité, par exemple, en gardant les températures relativement basses (réfrigération ou congélation). Parfois, de faibles augmentations de température peuvent produire des changements spectaculaires dans l'activité des agents biologiques. De tels changements impliquent la rupture d'une organisation moléculaire qui dépendent des effets coopératifs non-associés avec la dénaturation spécifique d'une protéine ou d'un acide nucléique spécifique.

Afin de remédier au phénomène de déstabilisation, on peut utiliser des adjuvants tel que $MgCl_2$, (pour le vaccin contre la poliomyélite) le polyéthylène glycol, etc; cependant ceux-ci peuvent présenter l'inconvénient de ne pas toujours (en fonction des concentrations) être physiologiquement appropriés.

Pour chaque température ambiante, il y a un spectre d'énergie disponible pour les changements de structure dans une macromolécule ou dans un complexe macromoléculaire (REF: Watson, James et al. pages 126 à 162, *Molecular Biology of the Gene*, 4th Edition (1987) Publishers Benjamin Cummings, ISBN 0-8053-9612-8). Dans le pire des cas, ces changements peuvent être associés à la rupture ou à la création de liaisons chimiques covalentes. Les énergies moins importantes seront peut-être suffisantes pour la création ou la rupture des liaisons chimiques secondaires (les liaisons

hydrogène, les interactions hydrophobes, les interactions de Van-der-Waals) qui jouent un rôle important dans les processus biologiques et les activités des macromolécules biologiques. L'importance des liaisons discutées ci-dessous, est la plus prononcée entre les températures de 0°C et 45°C, correspondant à la gamme des températures associées aux processus vivants dans les organismes supérieurs.

Ces interactions faibles sont toutes favorisées par une complémentarité de structure physique. Par exemple, une hormone et son site d'attachement sont liés par plusieurs liaisons faibles. La complémentarité de structure augmente le nombre des interactions du type Van-der-Waals, hydrophobes ou hydrogène, et ainsi évite l'effet disruptif de l'énergie ambiante.

L'eau elle-même joue un rôle important dans la stabilisation des macromolécules en milieu aqueux.

Les liaisons hydrogène entre les molécules d'eau ordonnent la structure. La structure peut-être mise en évidence avec les techniques de diffraction des rayons-X ou des neutrons.

A la périphérie des macromolécules, des molécules d'eau forment une sorte de couche, d'une profondeur de plusieurs molécules d'eau. Comme une cage, cette couche, renforcée par les liaisons hydrogène - entre des molécules d'eau et entre des molécules d'eau et les macromolécules - a pour effet de préserver l'intégrité du contenu.

La structure électronique de l'eau permet le libre échange des protons (noyaux des atomes d'hydrogène) entre les éléments du treillis d'eau et avec les sites favorables sur les macromolécules en milieu aqueux.

Dans les macromolécules, les molécules d'eau servent souvent de rôle de composant structurel, créant des ponts rigides entre les résidus protéiques, des bases nucléiques, des glucides ou des composants polaires des lipides. Les études cristallographiques montrent clairement l'inclusion des molécules d'eau dans plusieurs cristaux macromoléculaires. L'effet cumulatif coopératif de ces ponts moléculaires dans les structures comme les virus picorna est très sensible aux augmentations de température.

L'eau lourde (ou l'oxyde de deutérium, D₂O ou ²H₂O) a été découverte par Urey en 1932. Cette découverte a précipité un intérêt immédiat, surtout concernant les éventuelles répercussions sur la chimie de la vie. Pendant des années 1932-1939, plus de 200 publications ont montré les effets de l'eau lourde sur les organismes vivants et leurs physiologie et biochimie. Dans tous les cas, D₂O a été utilisée pour remplacer (entre 5% et 100%) H₂O dans les milieux

aqueux, mais les résultats obtenus n'ont pas permis de considérer l'eau lourde comme un agent stabilisateur.

Pendant les années 1970-80, l'eau lourde est devenu un outil important, dans le domaine des études biophysiques, tel que la RMN (résistance magnétique nucléaire) ou la diffusion des neutrons monochromatiques.

Pour la RMN, le noyau de deutérium possède l'atout de ne pas avoir de spin nucléaire. En milieu H_2O , dans un champs magnétique, les signaux de fréquence radio émis par les noyaux excités ne peuvent pas être détectés, parce que tous les hydrogènes de l'eau créent un bruit de fond énorme. Dans le milieu D_2O , l'eau ne contribue pas à un bruit de fond, et la technique de résonance magnétique des protons est devenue très puissante- surtout pour les molécules qui contiennent des protons (^1H) et qui sont solubilisées par D_2O .

Les propriétés nucléaires des noyaux H et D sont bien différentes. Pour un faisceau de neutrons, le deutérium est relativement transparent, tandis que l'eau ordinaire est relativement opaque (Stuhrman H. (1974) J. Appl. Cryst. 7, 173-187). Les diverses techniques permettent la différenciation des informations structurales venant des différentes macromolécules. La cristallographie des neutrons est devenu un outil puissant.

Ceci étant, bien qu'il soit connu que l'eau lourde apporte une augmentation d'énergie des liaisons hydrogène par un facteur de 0,24 kilocalorie par mole, rien ne permettait de prévoir l'influence de l'eau lourde sur la stabilisation de molécules solubilisées par l'eau, car interviennent des phénomènes autres que la nature de la liaison entre le solvant et la molécule, ces phénomènes tels que l'effet cage, étant à ce jour mal connus. Les effets coopératifs eau/ADN/protéines à l'intérieur des virus restent également à ce jour mal connus. Les confirmations peuvent éventuellement être données par les études cristallographiques (neutrons et rayons X).

L'un des buts de l'invention est de proposer des compositions pharmaceutiques stabilisées, physiologiquement compatibles.

L'un des buts de l'invention est de fournir un ensemble de compositions pharmaceutiques stabilisées, dans lesquelles les doses d'agents pharmaceutiques peuvent être réduites par rapport aux mêmes compositions thérapeutiques non stabilisées.

L'un des autres aspects de l'invention est de proposer un procédé de préparation simple d'un ensemble de compositions pharmaceutiques, notamment thérapeutiques, vaccinales ou de diagnostic, stabilisées.

Ces différents aspects sont obtenus par les compositions de l'invention.

A cet égard, la composition pharmaceutique stabilisée de l'invention, comprenant une ou plusieurs macromolécule(s) choisie(s) parmi les acides nucléiques, les lipides ou les glucides et/ou un ou plusieurs complexe(s) macromoléculaire(s), est caractérisée en ce qu'elle comporte un ou plusieurs véhicule(s) pharmaceutiquement acceptable(s), l'un au moins de ces véhicules contenant de l'eau lourde (D_2O).

On a trouvé que la substitution de la totalité ou d'une partie des noyaux d'hydrogène par des noyaux de deutérium, dans les molécules d'eau qui servent de solvant à une molécule ou à un complexe macromoléculaire qui constitue la substance active d'une composition pharmaceutique, permettait de stabiliser ladite composition pharmaceutique.

Dans la nature, l'hydrogène est composé de 0,0156% de deutérium. Celui-ci peut-être purifié par la distillation ou l'électrolyse de l'eau, pour obtenir de l'eau lourde pure, qui peut-être alors électrolysée pour obtenir du deutérium pur.

L'invention concerne l'utilisation de D_2O , ou des mélanges de D_2O et de H_2O , comme agents de stabilisation des macromolécules choisies parmi les acides nucléiques, les lipides ou des glucides ou des complexes macromoléculaires en phase aqueuse (solutions, suspensions ou émulsions).

Dans le contexte de cette invention, ce sont les propriétés de l'oxyde de deutérium, ou eau lourde, qui sont particulièrement intéressantes. Les noyaux H ou D qui s'échangent librement entre les molécules d'eau et entre les nombreux sites des macromolécules biologiques dissoutes, suspendues ou émulsifiées. D et H sont librement échangeables entre les sites d'hydrogène labiles, par exemple tous les hydrogènes dans la masse d'eau, ou beaucoup d'hydrogènes dans les macromolécules biologiques. D'autres hydrogènes, parfois cachés dans le milieu hydrophobe, sont moins accessibles, mais à l'issue d'une période variable, peuvent eux aussi être échangés.

Dans H_2O , D_2O ou des mélanges des deux, les propriétés physiques des solutions montrent une graduation linéaire des propriétés physiques (telles que la densité ou le point d'ébullition ou les valeurs molaires pour l'énergie des liaisons hydrogènes).

Les macromolécules et complexes macromoléculaires visés dans l'invention sont tels qu'ils ont un poids moléculaire d'au moins 1500 Da, et avantageusement supérieur à 5000 Da.

Pour fixer les idées, la stabilisation des macromolécules est telle que pour une température donnée, par exemple aux environs de 40°C, on peut observer une augmentation de stabilité des macromolécules ou complexes

macromoléculaires, conservés dans une solution contenant D₂O, par rapport à ces mêmes macromolécules ou complexes macromoléculaires conservés dans une solution contenant H₂O.

Par exemple, pour les virus de la poliomyélite (voir résultats), cette stabilisation se traduit par une augmentation du nombre de particules virales actives d'un facteur d'environ 10³ à environ 10⁴.

Comme indiqué ci-dessus, les macromolécules visées dans l'invention sont des acides nucléiques, des glucides ou des lipides.

Quant aux complexes macromoléculaires, ils sont avantageusement choisis parmi les complexes protéines-glucides, protéines-lipides, acides nucléiques-glucides, acides nucléiques-lipides, acides nucléiques-protéines, éventuellement complexés avec des glucides et/ou des lipides, et parmi les virus.

A titre d'exemples de macromolécules biologiques et de complexes macromoléculaires, susceptibles d'entrer dans les compositions de l'invention figurent:

1) les acides nucléiques:

l'ADN, pour les applications *in vivo* (par exemple: thérapie génique) et *in vitro* (par exemple: applications diagnostiques);

l'ARN, des ribozymes pour les applications *in vivo* (modifications des acides nucléiques ou des complexes acides nucléiques/protéines);

l'ADN et l'ARN, font partie des génomes des virus utilisés comme vecteurs dans la thérapie ou la vaccinologie;

des complexes d'ADN/ARN naturels ou artificiels;

les acides nucléiques utilisés peuvent être ceux existant sous des formes naturelles ou artificielles (créées par le génie génétique);

2) les glucides:

ces macromolécules n'ont pas encore trouvé d'applications comme thérapeutiques indépendantes, mais dans leurs complexes avec les protéines, elles sont reconnues comme facteur majeur dans la modification des activités de ces protéines; les différentes formes glucidiques peuvent invoquer des activités physiologiques significatives (comme c'est le cas pour la tPA, où les différentes "glycoformes" ont des activités bien différentes);

3) les lipides:

les macromolécules lipidiques jouent des rôles importants dans les membranes cellulaires, mais hormis les molécules relativement petites comme les prostaglandines et les vitamines de groupe A, ces agents n'ont pas trouvé beaucoup d'applications thérapeutiques; leur intérêt se trouve dans leurs complexes macromoléculaires et en particulier dans leurs utilisations dans les

liposomes utilisés comme systèmes de livraisons pour d'autres agents thérapeutiques;

4) les complexes macromoléculaires:

les plus typiques sont les complexes des acides nucléiques et les protéines, ou les protéines et les glucides;

un virus est un complexe macromoléculaire composé éventuellement d'acides nucléiques, de protéines, de glucides et de lipides (par exemple, le virus de la grippe ou le virus HIV);

on peut également citer les complexes artificiels, telle qu'une couche lipidique enrobant un agent thérapeutique, ciblé par des activités localisées dans la couche lipidique, ayant une spécificité de liaison avec des composants de la cible visée.

Les compositions pharmaceutiques impliquées dans la présente invention peuvent être définies par:

A - soit une ou plusieurs macromolécule(s) et/ou un ou plusieurs complexe(s) macromoléculaire(s), maintenus dans un ou plusieurs véhicule(s) pharmaceutiquement acceptable(s),

B - soit une ou plusieurs macromolécule(s), et un ou plusieurs complexe(s) macromoléculaire(s), maintenus séparément par rapport à un ou plusieurs véhicule(s) pharmaceutiquement acceptable(s), la (les) macromolécule(s), le (les) complexe(s) macromoléculaire(s) et le (les) véhicules pharmaceutiquement acceptable(s) étant destinés à être rassemblés en vue de l'administration à un patient,

C - soit au moins deux éléments, maintenus séparément les uns par rapport aux autres, et comportant chacun une ou plusieurs macromolécule(s) et/ou un ou plusieurs complexe(s) macromoléculaire(s), l'un au moins de ces éléments étant en présence d'un véhicule pharmaceutiquement acceptable contenant de l'eau lourde, ces différents éléments étant destinés à être rassemblés en vue de l'administration à un patient,

D - soit au moins deux des compositions pharmaceutiques selon l'une des variantes A, B, ou C, maintenues séparément l'une par rapport à l'autre et destinées à être rassemblées en vue de l'administration à un patient.

A titre d'exemple, une composition correspondant au rassemblement en vue de l'administration à un patient des variantes selon B et C comprend les caractéristiques suivantes:

- d'une part une ou plusieurs macromolécule(s) et/ou un ou plusieurs complexe(s) macromoléculaire(s) sont maintenus séparément par rapport à un ou plusieurs véhicule(s) pharmaceutiquement acceptable(s), et d'autre part au moins

deux éléments sont maintenus séparément les uns par rapport aux autres, et comportent chacun une ou plusieurs macromolécule(s) et/ou un ou plusieurs complexe(s) macromoléculaire(s), l'un au moins de ces éléments étant en présence d'un véhicule pharmaceutiquement acceptable contenant de l'eau lourde, la (les) macromolécule(s), le (les) complexe(s) macromoléculaire(s), le (les) véhicule(s) pharmaceutiquement acceptable(s) et les susdits éléments étant destinés à être rassemblés en vue de l'administration à un patient.

Les macromolécules et/ou les complexes macromoléculaires peuvent être conservés ou non avec le véhicule pharmaceutiquement acceptable contenant de l'eau lourde.

Selon un mode de réalisation avantageux de l'invention, les compositions sont telles que les macromolécules ou les complexes macromoléculaires sont soit en suspension, soit en solution, soit en émulsion dans le véhicule pharmaceutique.

Selon un autre mode de réalisation avantageux de l'invention, les compositions sont telles que la ou les macromolécule(s) et/ou le ou les complexe(s) macromoléculaire(s) et le véhicule pharmaceutiquement acceptable sont maintenus séparément et sont destinés à être rassemblés en vue de l'administration à un patient.

A titre d'exemple, les macromolécules et/ou les complexes macromoléculaires peuvent être lyophilisés et mélangés avec le véhicule pharmaceutiquement acceptable peu de temps avant l'administration à un patient, ou au moment de cette administration.

Selon un autre mode de réalisation avantageux, dans les compositions de l'invention le volume du véhicule pharmaceutique varie d'environ 0,1 ml à environ 20 ml, notamment d'environ 0,1 ml à environ 5 ml, et la quantité de macromolécule ou de complexe macromoléculaire varie d'environ 1 μ g à environ 3g.

Selon un mode de réalisation avantageux pour une dose unitaire, la quantité de macromolécule ou de complexe macromoléculaire est d'environ 10 μ g pour un volume d'environ 1 ml de véhicule pharmaceutique.

Selon encore un autre mode de réalisation, dans les compositions de l'invention, le véhicule pharmaceutique est un mélange H₂O-D₂O et le rapport en poids entre D₂O et H₂O varie d'environ 10% à environ 100%, avantageusement d'environ 40% à environ 95%, et de préférence d'environ 80% à environ 95%.

En général, les mélanges D₂O/H₂O, contenant des concentrations élevées en D₂O (90-100%), donnent une stabilisation équivalente à une réduction de

température ambiante d'un facteur de 4-5°C (c'est à dire dans 95% de D₂O à 25°C - par exemple - une macromolécule subit un effet de dégradation qui équivaut à celui observé normalement dans H₂O à 20°C).

De plus, les concentrations élevées en D₂O offrent une protection importante contre les suites de la contamination microbienne. Peu de microbes tolèrent les concentrations de D₂O dépassant les 70%. Ainsi l'eau lourde est effectivement un milieu auto-stérilisant (à noter, que certaines microbes, généralement non-pathogéniques, peuvent être adaptés à la vie en milieu D₂O).

Les compositions de l'invention sont non toxiques. En effet, il est connu que D₂O à une concentration inférieure à 10% de l'eau corporelle n'a pas d'effet toxique significatif. (Voir A. Barbour, H.G., (1937), Yale J. Biol. Med. 9, 551 - B: Barbour, H.G., (1938), Am. J. Cancer, 32, 440- C: Barbour, H.G. (1938), J. Pharm. Exp. Therap., 62, 158 - D: Barbour, H.G., (1936), J. Pharm. Exp. Rherap., 58, 460).

Les agents pharmaceutiques susceptibles de bénéficier de ces effets stabilisateurs sont souvent chers et contenus dans des petits volumes (vaccin oral, par exemple 0,15 ml); ainsi le prix relativement élevé de D₂O a moins d'importance.

Comme D₂O et H₂O sont entièrement miscibles, et comme les atomes d'hydrogène (¹H et ²H) labiles s'échangent rapidement, on peut constater qu'une dose de D₂O contenant un agent protecteur ou thérapeutique sera rapidement dispersée dans la masse d'eau corporelle. Avec une abondance naturelle de 0,0156% des atomes d'hydrogène, la masse de deutérium dans une personne de poids de 75kg sera d'environ 1g, équivalent à 10g d'eau lourde. L'administration des petits doses (par exemple: 0,1 à 5,0 ml) ne dépassera pas la quantité d'eau lourde déjà présente dan le corps d'un adulte. Même les doses très élevées (10 ml à 50 ml) ne contribuent pas à un niveau de deutérium (environ 0,1%) approchant les concentrations toxiques (soit 10% - 20%) ou mortelles (25% et plus).

L'eau lourde ne s'accumule pas dans l'organisme et elle est rejetée dans l'urine, la transpiration et la respiration, au même titre que l'eau.

En résumé, les expériences et les observations discutées démontrent qu'il y a un effet stabilisateur pour les macromolécules et leurs complexes, d'environ 5°C, associé à l'eau lourde. Malgré les changements de température relativement faibles et qui sont de l'ordre de 2°C à 5°C, les effets coopératifs mentionnés au-dessus peuvent contribuer à une augmentation importante de stabilité. Les macromolécules et même les organismes vivants (certains microbes, généralement non-pathogéniques, pouvant être adaptés à la vie en

milieu D₂O) ont montré une résistance aux températures normalement associées (en milieu H₂O) à la dénaturation thermique. Certains complexes ont montré une résistance réversible à la désagrégation induite par les solutions de faible force ionique (en H₂O, par exemple inférieure à KCl 1mM).

Dans les compositions de l'invention, le véhicule pharmaceutique peut contenir un adjuvant de stabilisation, avantageusement MgCl₂, de la carboxyméthyl cellulose, du polyéthylène glycol, des molécules glucidiques, tels que glycérol, glucose, sucrose, (et autres sucres C6), les sucres pentoses et leurs polymères (les amidons par exemple), ces molécules glucidiques pouvant être complexées avec le polyéthylène glycol, le polyéthylène glycol présentant avantageusement un poids moléculaire d'environ 500 à environ 5000 daltons, et notamment d'environ 1000 à environ 5000 daltons.

En application clinique, D₂O (et ses mélanges) peut également être utilisé avec les régulateurs de tonicité, de pH, de viscosité et d'autres agents stabilisateurs tels que ceux indiqués ci-dessus.

Dans les compositions de l'invention, le rapport en poids entre l'adjuvant de stabilisation et le véhicule pharmaceutique est tel que le véhicule pharmaceutique ne présente pas une viscosité supérieure à environ 0,2 Pascal-secondes (20 centipoises).

Dans les compositions de l'invention le rapport en poids entre l'adjuvant de stabilisation et le véhicule pharmaceutique est inférieur à environ 30%, et est avantageusement d'environ 1% à environ 30%, et de préférence encore d'environ 10% à environ 30%.

Dans les compositions de l'invention les complexes macromoléculaires et les macromolécules sont ceux utilisés:

- dans des compositions vaccinales, telles que celles contre la poliomyélite, contre la rougeole, contre l'hépatite, notamment l'hépatite B ou C, contre la grippe, contre le virus du syndrome immuno déficitaire acquis (virus du SIDA également désigné par HIV = human immunodeficiency virus), contre la diphtérie, contre le tétanos, contre la coqueluche ou contre une combinaison d'au moins deux des susdites pathologies, (par exemple le vaccin contre la diphtérie, le tétanos et la poliomyélite [DTP], ou le vaccin contre la diphtérie, le tétanos, la poliomyélite et la coqueluche, commercialisé sous la marque TETRACOQ,
- en tant qu'agent de diagnostic, notamment pour le diagnostic de la rougeole, de la méningite ou de HIV ou,
- en tant qu'agent thérapeutique, tels que des acides nucléiques ou des ribozymes.

L'invention concerne également un procédé de préparation d'une composition pharmaceutique de l'invention, caractérisé en ce qu'il comprend les étapes suivantes :

- soit la dilution à l'aide de D₂O en quantité appropriée, de la susdite composition pharmaceutique se présentant sous forme de solution aqueuse, préalablement concentrée, par exemple à raison de 20 mg/ml,
- et/ou la dialyse, contre D₂O en quantité appropriée, de la susdite composition pharmaceutique se présentant sous forme de solution aqueuse,
- soit la resuspension de la susdite composition pharmaceutique, se présentant sous forme de solide, à l'aide de D₂O, en quantité suffisante pour obtenir la concentration appropriée de la composition pharmaceutique.

On utilise avantageusement la dialyse dans le cas où la composition pharmaceutique contient une substance active fragile, résistant mal aux autres traitements physiques.

L'invention concerne également un procédé de fabrication d'une préparation stabilisée d'origine virale, contenant des complexes macromoléculaires, caractérisé en ce que lesdits complexes macromoléculaires sont mis en solution, suspension ou émulsion dans un milieu contenant de l'eau lourde.

Par préparation d'origine virale, on entend notamment

- les virus complets, inactivés ou atténués,
- les protéines glycoprotéines virales, qu'elles soient de surface ou internes et qu'elles soient obtenues à partir de virus ou par recombinaison génétique connue, par exemple la protéine de surface du virus de l'hépatite B,
- les vecteurs viraux, tels que les adénovirus, les poxvirus comme le canary pox, ou le virus de la vaccine exprimant les protéines d'origine virale ou bactérienne (voir par exemple le brevet européen: EP 083 286),
- les acides nucléiques (ARN ou ADN) encapsulés ou non, connus par exemple sous le nom de "naked DNA" par la demande de brevet européen EP 465 529 (WO 90/11092), ou le brevet américain USP 4 394 448, qui décrit l'encapsulation de l'ADN ou de fragments d'ADN dans des vésicules lipidiques.

L'invention concerne également un procédé de stabilisation d'une préparation vaccinnante d'origine virale, notamment d'une suspension virale, caractérisé en ce qu'il comprend une étape d'incorporation d'eau lourde:

- soit au virus ou aux cellules à infecter, avant la mise en contact entre les cellules à infecter et le virus,
- soit dans le milieu de culture des cellules infectées par le virus,

- soit dans la préparation vaccinnante obtenue à l'issue de l'étape précédente.

L'eau lourde peut être ajoutée à n'importe quelle étape impliquée dans le procédé de préparation de la suspension virale, de préférence après la récupération des particules virales, à l'issue de l'étape de culture des cellules infectées par le virus.

Dans le cas d'un vaccin préparé sous forme lyophilisée, l'eau lourde peut être utilisée pour la préparation de la suspension virale, avant la lyophilisation et/ou pour la réhydratation du susdit vaccin lyophilisé. On utilise de préférence l'eau lourde au stade de la réhydratation.

Les vaccins auxquels s'appliquent avantageusement l'invention sont les suivants: grippe, varicelle, rougeole, oreillons, rubéole, dengue, HIV, herpès.

Les exemples cités ci-dessous ont purement une valeur illustratrice, et ne doivent pas être interprétés comme limitant la portée de cette invention.

Ils démontrent:

1:- la thermostabilisation d'un vaccin important;

2:- la stabilisation d'un complexe macromoléculaire modèle, illustrant la réversibilité des effets de thermostabilisation dus à D₂O et ses effets isotopes associés.

Ces exemples peuvent être appliqués aux cas suivants:

à un ribozyme,

au vaccin recombinant contre l'hépatite B et C,

au vaccin contre la grippe,

au vaccin contre la rougeole,

au vaccin DTP (diphtérie, tétanos, poliomyélite),

au vaccin contre le tétanos,

au kit pour le diagnostic d'HIV.

Il convient de noter que dans ce qui précède et ce qui suit, l'ensemble des documents de la littérature cités est incorporé par référence.

Descriptions des figures:

La figure 1 représente la thermostabilité, à 37°C, d'une composition pharmaceutique selon l'invention.

L'axe des abscisses correspond au temps (exprimé en jours) et l'axe des ordonnées correspond au logarithme de la concentration des particules virales (exprimé en unité DICC₅₀/ml, DICC₅₀ étant la dose infectieuse cytotoxique dans la cellule).

La courbe représentée par des losanges correspond à une composition pharmaceutique en solution aqueuse; la courbe représentée par des carrés correspond à une composition pharmaceutique en solution aqueuse, contenant MgCl_2 (à raison de 1M) comme adjuvant; la courbe représentée par des triangles correspond à une composition pharmaceutique de l'invention, solubilisée dans de l'eau lourde à 95%; la courbe représentée par des croix correspond à une composition pharmaceutique de l'invention, solubilisée dans D_2O et contenant MgCl_2 (à raison de 1M) comme adjuvant.

La figure 2 représente la thermostabilité à 42°C d'une composition pharmaceutique selon l'invention.

L'axe des abscisses correspond au temps (exprimé en jours) et l'axe des ordonnées correspond au logarithme de la concentration des particules virales (exprimé en unité $\text{DICC}_{50}/\text{ml}$).

La courbe représentée par des losanges correspond à une composition pharmaceutique en solution aqueuse; la courbe représentée par des carrés correspond à une composition pharmaceutique en solution aqueuse, contenant MgCl_2 (à raison de 1M) comme adjuvant; la courbe représentée par des triangles correspond à une composition pharmaceutique de l'invention, solubilisée dans de l'eau lourde à 95%; la courbe représentée par des croix correspond à une composition pharmaceutique de l'invention, solubilisée dans D_2O et contenant MgCl_2 (à raison de 1M) comme adjuvant.

La figure 3 représente la thermostabilité à 45°C d'une composition pharmaceutique selon l'invention.

L'axe des abscisses correspond au temps (exprimé en jours) et l'axe des ordonnées correspond au logarithme de la concentration des particules virales (exprimé en unité $\text{DICC}_{50}/\text{ml}$).

La courbe représentée par des losanges correspond à une composition pharmaceutique en solution aqueuse; la courbe représentée par des carrés correspond à une composition pharmaceutique en solution aqueuse, contenant MgCl_2 (à raison de 1M) comme adjuvant; la courbe représentée par des triangles correspond à une composition pharmaceutique de l'invention, solubilisée dans de l'eau lourde à 95%; la courbe représentée par des croix correspond à une composition pharmaceutique de l'invention, solubilisée dans D_2O et contenant MgCl_2 (à raison de 1M) comme adjuvant.

La figure 4 représente l'inactivation thermique du poliovirus Sabin de type 3. La concentration initiale du virus est fixée à 5×10^6 DICC₅₀/50 μ l. (DICC₅₀ = dose infectieuse cytotoxique dans la cellule).

Des suspensions du virus de Sabin de type 3 de titres égaux (5×10^6 DICC₅₀/50 μ l) sont préparés dans les milieux suivants:

a) Dulbecco-MEM dans l'eau normale (correspondant à l'abréviation "milieu H" sur la figure);

b) Dulbecco-MEM dans D₂O de concentration finale 87% (correspondant à l'abréviation "milieu D" sur la figure);

c) MgCl₂ 1M dans H₂O, Tris tamponné à pH 7.2 (correspondant à l'abréviation "milieu H-Mg" sur la figure); et

d) MgCl₂ 1M dans D₂O de concentration finale 87%, Tris tamponné à pH 7.2 (correspondant à l'abréviation "milieu D-Mg" sur la figure).

Après 3 jours d'incubation (partie A de la figure 4) et après 7 jours d'incubation (partie B de la figure 4) à 4°C, 37°C, 42°C et 45°C, le type du virus est déterminé. Les résultats sont exprimés comme différence de log de DICC₅₀/50 μ l entre le titre initial du virus maintenu à -30°C et le titre de chaque échantillon. La moyenne et l'erreur standard sont indiqués pour chaque résultat.

La figure 5 représente l'inactivation thermique du poliovirus Sabin de type 1 en présence de D₂O et/ou MgCl₂ (voir détails figure 4). La partie A de la figure 5 correspond à 3 jours d'incubation, et la partie B de la figure 5 correspond à 7 jours d'incubation.

La figure 6 représente l'inactivation thermique du poliovirus Sabin de type 2 en présence de D₂O et/ou MgCl₂ (voir détails Figure 4). La partie A de la figure 6 correspond à 3 jours d'incubation, et la partie B de la figure 6 correspond à 7 jours d'incubation.

La figure 7 représente la stabilisation du poliovirus Sabin de type 3, avec diverses concentrations d'eau lourde et de MgCl₂.

On réunit les mêmes conditions expérimentales que celles décrites dans la figure 4, sauf pour la concentration de D₂O (partie A de la figure 7) ou MgCl₂ (partie B de la figure 7).

Les résultats sont exprimés comme différence entre le titre initial de la suspension virale conservée à -30°C (log de DICC₅₀/50μl) et le titre de chacun des échantillons après 3 jours d'incubation à 45°C. Le titre initial du virus est de 5x10⁶ DICC₅₀/50μl.

La figure 8 représente la présence ou l'absence de sédimentation de PNN (noyau de nucléosome), selon la force ionique de l'eau, et selon la présence ou non d'eau lourde.

La sédimentation des PNN a été faite à pH 7,75 (en milieu de D₂O, pH 8,15 selon l'électrode de verre) 0,2mM EDTA, à une force ionique selon le Tableau 1 ci-après. Toutes les sédimentations sont faites dans le MSE CENTRISCAN, ultracentrifugeuse analytique, dans le rotor comportant 6 cellules de mesure à 50.000 t/mn. Les parties A à C correspondent à un intervalle de mesure de 6 minutes, les parties D à G correspondent à un intervalle de mesure de 8 minutes, longueur d'onde: 254nm; concentration des échantillons: 0,5 à 1,0 mg/ml.

L'axe des abscisses correspond au rayon de la mesure et l'axe des ordonnées à la concentration en ADN. Cette figure démontre que l'eau lourde consolide les particules de PNN autant que la force ionique.

EXEMPLE 1-

Thermostabilisation du poliovirus avec l'eau lourde

L'immunogénicité des virus atténués dans le vaccin de Sabin contre la polio, dépend de leur degré infectieux au moment de l'administration. Les niveaux de prises du vaccin oral contre la polio (OPV) sont liés à la dose de chacune des trois souches virales 1, 2 et 3, constituant le vaccin. Une explication pour les niveaux de prises assez bas dans certains pays du tiers monde, peut être les ruptures dans la chaîne du froid avant l'administration du vaccin. La souche 3 du vaccin de Sabin est très critique, à cause de son immunogénicité et de sa thermostabilité qui sont relativement médiocres.

L'Organisation Mondiale de la Santé (OMS) demande à ce jour que l'OPV soit stabilisé par l'addition de MgCl₂ (concentration 1 M) dans le vaccin. Loin d'être la solution idéale, (perte de moins de 0,5 log d'activité après sept jours à 45°C), cette méthode est actuellement le meilleur compromis.

Il est démontré ici que selon l'invention, l'eau lourde à 95 % offre, même sans aucun autre adjuvant, une stabilisation équivalente ou supérieure à celle offerte par MgCl_2 , en suivant les procédés entièrement standard (à part, bien sûr, le fait d'utiliser l'eau lourde).

Objectifs expérimentaux

Toutes les références à l'eau lourde (D_2O), dans les exemples ci-après, font référence à l'eau lourde à 95 %.

1 Détermination et quantification des effets de D_2O sur la stabilisation du vaccin de Sabin.

2 Comparaison des effets de thermostabilisation de: (a) D_2O et (b) D_2O additionné de MgCl_2 .

La souche type 3 est responsable de la plupart des problèmes associés au vaccin de Sabin. Pour cette raison, ce virus a été la cible des études préliminaires.

Contexte globale de l'étude sur le vaccin de polio.

Dans l'homme, l'immunogénicité de l'OPV est associée au caractère infectieux de la suspension virale. La possibilité que présente l'OPV de se dégrader dans les régions ayant des températures ambiantes élevées est un problème (voir ci-dessus). Pour l'OMS, le but d'éliminer la polio avant l'année 2000 dépend en partie de l'amélioration des procédés de stabilisation des vaccins actuels (surtout pour les pays en voie de développement).

Les résultats suivants montrent que la souche 3 du vaccin de Sabin, bénéficie d'une stabilisation très marquée dans l'eau lourde à 95 % (les autres conditions étant semblables à celles utilisées avec l'eau), sans autre agent de stabilisation. L'effet de D_2O est légèrement supérieure à la meilleure des solutions actuelles (MgCl_2 , 1M)

Méthodes expérimentales

La souche Sabin 3 est choisie comme modèle. La culture des virus est faite selon les procédés classiques.

L'addition d'eau lourde est effectuée en resuspendant le culot de virus (sédimenté dans une ultracentrifugeuse selon les conditions standard) dans D_2O (19 volumes) et un tampon PBS (tampon salin phosphaté) 20 fois concentré (1 volume). L'ultracentrifugation est sans gêne significative pour les virus. Les

études comparatives sont faites avec les milieux standard (H_2O , $H_2O/MgCl_2$ et $MgCl_2 + D_2O$).

La concentration du virus est ajustée à approximativement 10^{10} unités/ml dans D_2O à 95%.

Les virus sont soumis à différentes conditions thermiques selon les courbes montrées ci-après.

Virus - titration

Pour tous les échantillons, des dilutions en série sont effectuées (MEM Eagles avec sels d'Earle, 4%SVF, 3% bicarbonate de sodium, pénicilline, streptomycine). Une suspension de cellules Hep2C est ajoutée et l'ensemble est incubé à $35^{\circ}C$ pendant 5 à 7 jours. Les plaques sont colorées avec du "noir de naphthalène" pour évaluer la viabilité des cellules. Les titres sont ensuite calculés. Chaque expérience comporte des contrôles.

Les résultats ci-après concernent la mesure de la stabilité des compositions de l'invention aux trois températures suivantes:

$37^{\circ}C$,

$42^{\circ}C$,

$45^{\circ}C$.

RESULTATS, Jours à $37^{\circ}C$ Log des unités virales, moyenne de trois échantillons

Jours	H_2O	$MgCl_2$	D_2O	$MgCl_2/D_2O$
0	10	10	10	10
2	8.6	9.5	9.6	9.6
5	7.8	8.1	8.1	8.2
7	6.8	7.5	7.7	7.8

RESULTATS, Jours à $42^{\circ}C$ Log des unités virales, moyenne de trois échantillons

Jours	H_2O	$MgCl_2$	D_2O	$MgCl_2/D_2O$
0	10	10	10	10
2	5.4	8.8	9.4	9.3
5	3.4	5.4	6.0	6.2
7	2.8	5.2	5.5	5.4

RESULTATS, Jours à 45°C Log des unités virales, moyenne de trois échantillons

Jours	H ₂ O	MgCl ₂	D ₂ O	MgCl ₂ /D ₂ O
0	10	10	10	10
2	4.6	7.8	8.5	8.7
5	1.8	4.8	5.8	6.5
7	0.4	4.7	4.7	4.9

EXEMPLE 2

Stabilisation de l'architecture de la nucléosome par D₂O

Le noyau de la nucléosome (PNN) est composé de quatre paires de molécules protéiques, les histones H2a H2b, H3 et H4. Chaque octamère de protéine est entouré de 144 paires de base d'ADN, complétant 1,75 tours superhélicale (Noll M. (1974) Nature 251, 249-251; Kornberg R.D. (1974) Science 184, 868-871; Finch J. et al. (1977) Nature 269, 29-35). Gordon a étudié l'effet de la force ionique (en milieu H₂O) sur les propriétés de sédimentation de la PNN (Gordon V.C. et al. (1978) Proc. Nat. Acad. Sci (USA). 75, 660-663). L'exemple ci-après illustre les différences importantes entre les propriétés de stabilisation de l'eau ordinaire et de l'eau lourde envers la PNN. La PNN peut être considérée comme une sorte de système modèle de la famille des complexes acide nucléique/protéine.

Méthodes expérimentales

La sédimentation des PNN préparées à partir d'érythrocytes de poulet (Suau P. et al (1977) Nuc. Acids. Res. 4, 3769-3786) est étudiée avec l'ultracentrifugeuse analytique (MSE CENTRISCAN) dans plusieurs conditions de force ionique et de concentrations en eau lourde. Plusieurs échantillons en H₂O sont après ré-équilibrés dans l'eau lourde en dialysant contre un volume important d'eau lourde, à diverses concentrations et compositions ioniques. Toutes les solutions sont tamponnées avec 0,2mM EDTA, pH 7,75 (en D₂O ajusté à pH 8,15 selon l'électrode de verre). La force ionique a été apportée par KCl.

Résultats

La sédimentation des PNN est dépendante de la composition ionique de l'eau. Très évidente est la désagrégation des PNN en milieu H₂O à faible

puissance ionique (Fig 4A). Dans l'eau lourde, cette dégradation des PNN ne se manifeste pas (Fig 4D). Même la dépendance ionique de la valeur de sédimentation (en unités Svedburg - s) devient très limitée (Voir Tableau 2 et Fig 4). La PNN désagrégée (4A), après dialyse contre l'eau lourde, redevient une particule discrète avec une sédimentation caractéristique pour une PNN.

Dans toutes les études, les PNN ont adopté - en milieu D₂O - la forme la plus compacte observée dans les milieux H₂O (après normalisation des valeurs de sédimentation pour corriger les différences de densité et de viscosité de l'eau lourde et de ses mélanges).

Dans les données complémentaires, non présentées ici, il est observé que la stabilisation à faible force ionique est manifeste à partir d'environ 40% de D₂O.

TABLEAU 1

Echantillon	[KCl] mM	Solvant	Valeur s	rayon de la première mesure (voir indice) mm
A	0	H ₂ O	-	63.15
B	5	"	10.2	63.15
C	25	"	11.2	65.43
D	0	D ₂ O	11.0*	63.29
E	25	"	11.2*	63.44
F- A redialysé	0	"	11.0*	63.00
G- C redialysé	0	"	11.1*	63.22

* valeurs corrigées selon viscosité et densité de D₂O.

Exemple 3: Stabilisation du vaccin poliovirus

Cette stabilisation est effectuée dans les conditions décrites dans les figures 4 à 7.

Les résultats représentés sur les figures 4 à 7 démontrent l'effet stabilisant de l'eau lourde. Quand les poliovirus (Sabin type 1, 2 et 3) sont préparés selon les procédures industrielles (dilution d'une suspension virale primaire: fluide surnageant clarifié de cultures de cellules infectées) en remplaçant les solutions

d'eau par des solutions contenant D₂O, dont la concentration finale est d'environ 90%, les résultats obtenus sont plus marquants que ceux obtenus avec les virus remis en suspension après la centrifugation (voir figs. 1-3).

Exemple 4: Stabilisation du vaccin poliovirus dans différentes solutions contenant de l'eau lourde.

L'addition de D₂O se fait au moment de la préparation du mélange de trois vaccins monovalents (Sabin de type 1, type 2, type 3) préparés en solution aqueuse. La concentration finale en D₂O est de 80%.

L'essai porte sur les formules suivantes:

1. Mélange albumine-MgCl₂ en solution aqueuse
2. Mélange albumine-MgCl₂ en solution contenant D₂O
3. Mélange acides aminés-MgCl₂ en solution contenant D₂O
4. Mélange acides aminés-saccharose en solution contenant D₂O

Titre global (DICC50/dose)							
Essais	70°C	7j à 37°C	perte (en log)	5j à 45°C	perte (en log)	7j à 45°C	perte (en log)
1 témoin alb.	6,63	5,48	1,15	3,47	3,16	3,20	3,43
2 alb. D ₂ O	6,57	5,90	0,67	4,67	1,90	4,13	2,44
3 A.A. DO	6,70	5,95	0,75	4,53	2,17	4,10	2,60
4 Sacch. D ₂ O	6,41	5,34	1,07	4,04	2,37	3,44	2,97

Exemple 5: Stabilisation du vaccin varicelle

Le virus Varicelle est un virus fragile.

La solution stabilisante habituelle du vaccin Varicelle a été préparée en solution aqueuse et en solution contenant de l'eau lourde (D₂O), à une concentration de 80%.

Une récolte de virus a été diluée dans les mêmes proportions avec chacun des stabilisants et lyophilisée. Les produits lyophilisés ont été réhydratés dans leur solvant respectif.

	Titres (PFU/dose) après réhydratation 2h à 37°C
H ₂ O	Perte = 0,69 log
D ₂ O	Perte = 0,37 log

PFU = unité conduisant à la formation de plaque.

REVENDICATIONS

1. Composition pharmaceutique stabilisée comprenant une ou plusieurs macromolécule(s) choisie(s) parmi les acides nucléiques, les lipides ou les glucides, et/ou un ou plusieurs complexe(s) macromoléculaire(s), caractérisée en ce qu'elle comprend un ou plusieurs véhicule(s) pharmaceutiquement acceptable(s), l'un au moins de ces véhicules contenant de l'eau lourde (D₂O).

2. Composition selon la revendication 1, dans laquelle les complexes macromoléculaires sont choisis parmi les complexes protéines-glucides, protéines-lipides, acides nucléiques-glucides, acides nucléiques-lipides, acides nucléiques-protéines, éventuellement complexés avec des glucides et/ou des lipides, et parmi les virus.

3. Composition selon l'une quelconque des revendications précédentes, caractérisée en ce que les macromolécules ou les complexes macromoléculaires sont soit en suspension, soit en solution, soit en émulsion dans le véhicule pharmaceutiquement acceptable.

4. Composition selon la revendication 1 ou 2, caractérisée en ce que la ou les macromolécule(s) et/ou le ou les complexe(s) macromoléculaire(s) et le véhicule pharmaceutiquement acceptable sont maintenus séparément et sont destinés à être rassemblés en vue de l'administration.

5. Composition selon l'une quelconque des revendications 1 à 4, caractérisée en ce que le volume du véhicule pharmaceutique varie d'environ 0,1 ml à environ 20 ml, notamment d'environ 0,1 ml à environ 5 ml, et la quantité de macromolécule ou de complexe macromoléculaire varie d'environ 1 µg à environ 3g.

6. Composition selon l'une quelconque des revendications précédentes, caractérisé en ce que le véhicule pharmaceutique est un mélange H₂O-D₂O et en ce que le rapport en poids entre D₂O et H₂O varie d'environ 10% à environ 100%, avantageusement d'environ 40% à environ 95%, et de préférence d'environ 80% à environ 95%.

7. Composition selon l'une quelconque des revendications précédentes, caractérisée en ce que le véhicule pharmaceutique contient un adjuvant de stabilisation, avantageusement $MgCl_2$, de la carboxyméthyl cellulose, du polyéthylène glycol, des molécules glucidiques, tels que glycérol, glucose, sucrose (et autres sucres en C6), pentoses et leurs polymères, ces molécules glucidiques pouvant être complexées avec le polyéthylène glycol, le polyéthylène glycol présentant avantageusement un poids moléculaire d'environ 500 à environ 5000 daltons, et notamment d'environ 1000 à environ 5000 daltons.

8. Composition selon la revendication 7, caractérisée en ce que le rapport en poids entre l'adjuvant de stabilisation et le véhicule pharmaceutique est tel que le véhicule pharmaceutique ne présente pas une viscosité supérieure à environ 0,2 Pascal-secondes (20 centipoises).

9. Composition selon la revendication 8, caractérisée en ce que le rapport en poids entre l'adjuvant de stabilisation et le véhicule pharmaceutique est inférieur à environ 30%, et est avantageusement d'environ 10% à environ 30%.

10. Composition selon l'une quelconque des revendications 1 à 9, caractérisée en ce que les complexes macromoléculaires et les macromolécules sont ceux utilisés :

- dans des compositions vaccinales, telles que celles contre la grippe, contre la poliomyélite, contre la rougeole, contre la varicelle, contre les oreillons, contre la rubéole, contre la dengue, contre l'hépatite, notamment l'hépatite B ou C, contre le virus du syndrome immuno déficitaire acquis (virus du SIDA), contre la diphtérie, contre le tétanos, contre la coqueluche ou contre une combinaison d'au moins deux des susdites pathologies, ou
- en tant qu'agent de diagnostic, tel que pour le diagnostic de la rougeole, de la méningite ou de HIV ou,
- en tant qu'agent thérapeutique, tels que des protéines, le facteur VIII, des anticorps polyclonaux ou monoclonaux, des hormones, des acides nucléiques ou des ribozymes.

11. Procédé de préparation d'une composition pharmaceutique selon l'une quelconque des revendications 1 à 10, caractérisé en ce qu'il comprend les étapes suivantes:

- soit la dilution à l'aide de D₂O de la susdite composition pharmaceutique se présentant sous forme de solution aqueuse préalablement concentrée, par exemple à raison de 20 mg/ml,
- et/ou la dialyse contre D₂O de la susdite composition pharmaceutique se présentant sous forme de solution aqueuse,
- soit la resuspension de la susdite composition pharmaceutique se présentant sous forme de solide, à l'aide de D₂O en quantité suffisante pour obtenir la concentration appropriée de la composition pharmaceutique.

12. Procédé de préparation d'une composition stabilisée contenant des complexes macromoléculaires d'origine virale, caractérisé en ce que lesdits complexes macromoléculaires sont mis en solution, suspension ou émulsion dans un milieu contenant de l'eau lourde.

13. Procédé de stabilisation d'une préparation d'origine virale, éventuellement vaccinante, notamment d'une suspension virale, caractérisé en ce qu'il comprend une étape d'incorporation d'eau lourde:

- soit au virus ou aux cellules à infecter avant la mise en contact entre les cellules à infecter et le virus,
- soit dans le milieu de culture des cellules infectées par le virus,
- soit dans la préparation vaccinante obtenue à l'issue de l'étape précédente.

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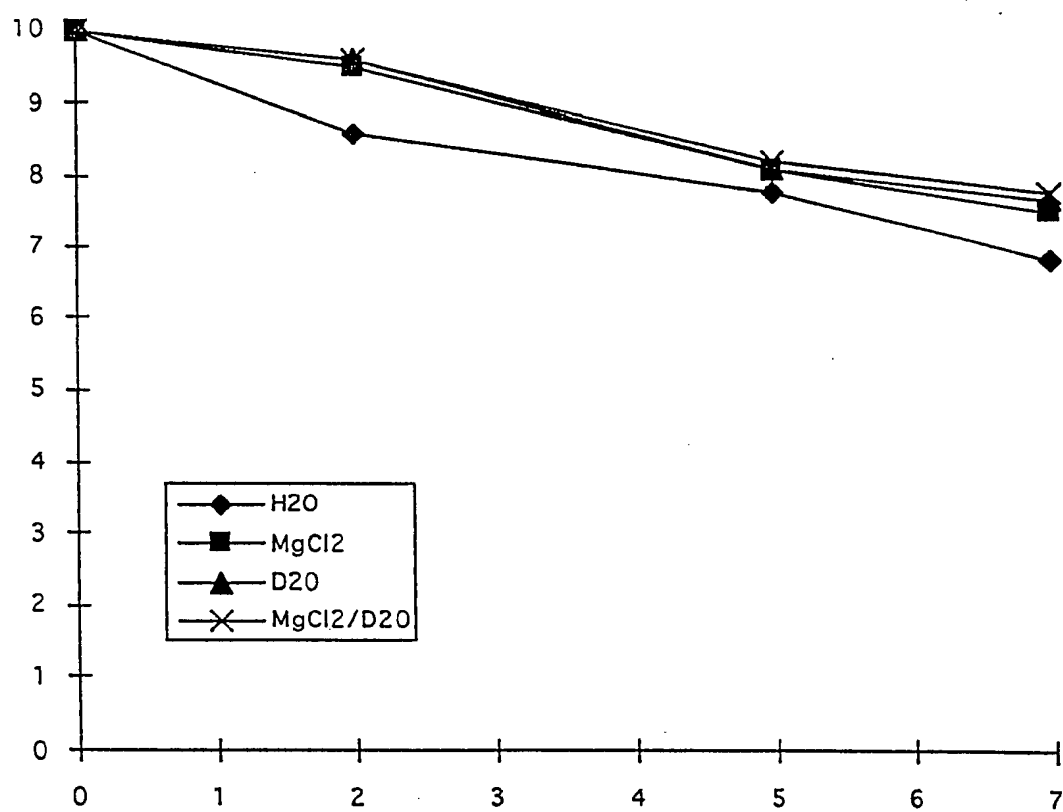


FIGURE 1

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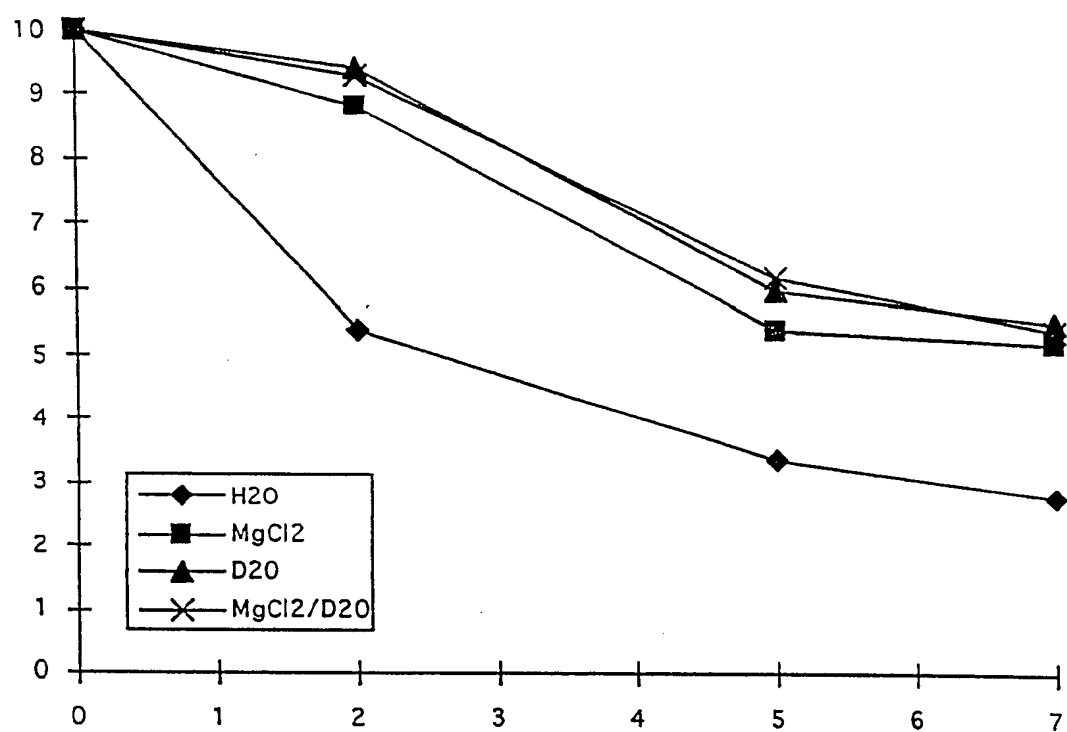


FIGURE 2

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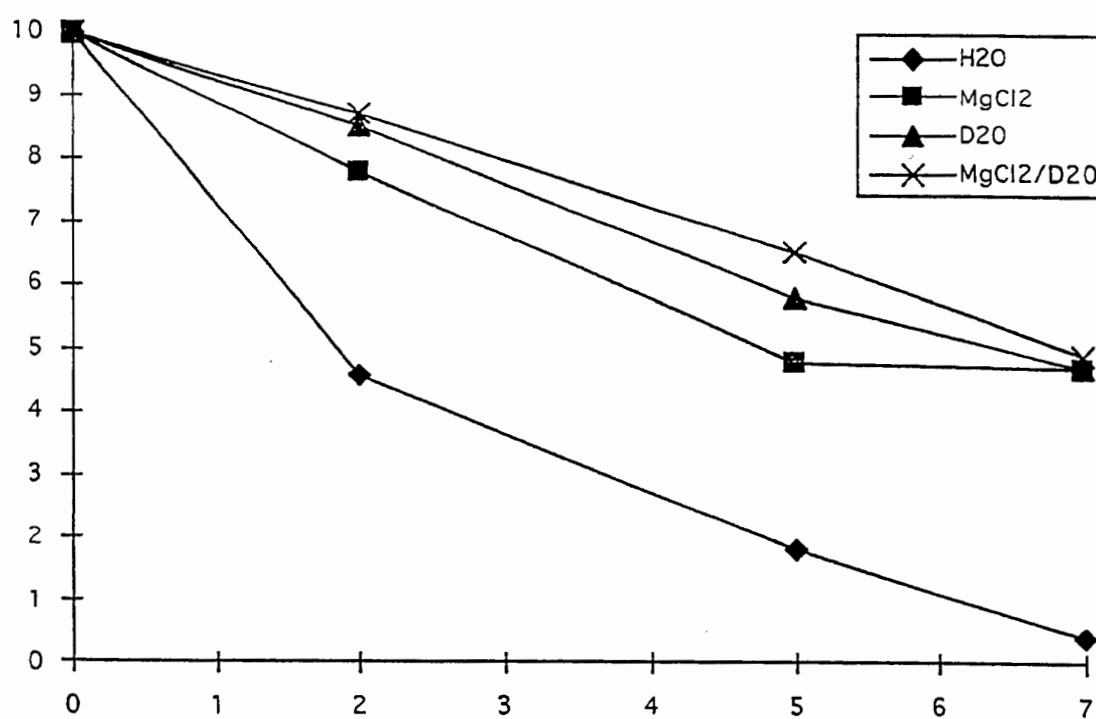
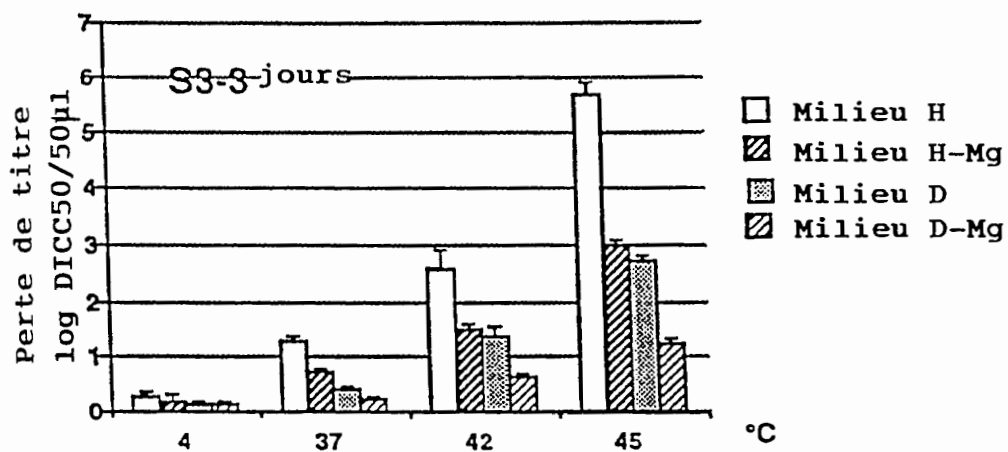


FIGURE 3

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4A



4B

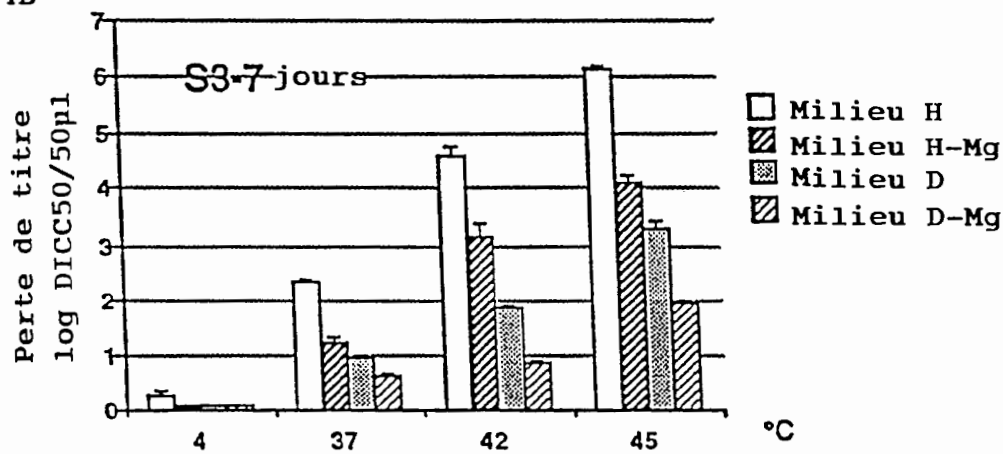
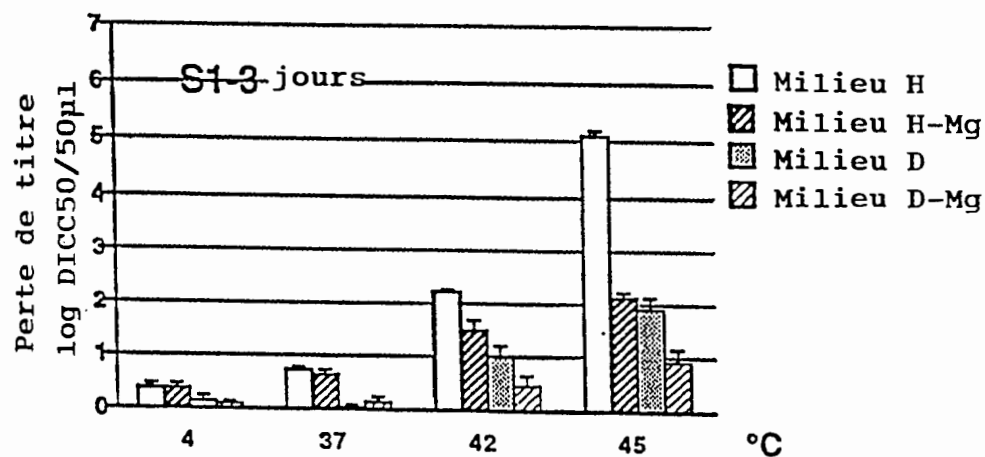


FIGURE 4

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5A



5B

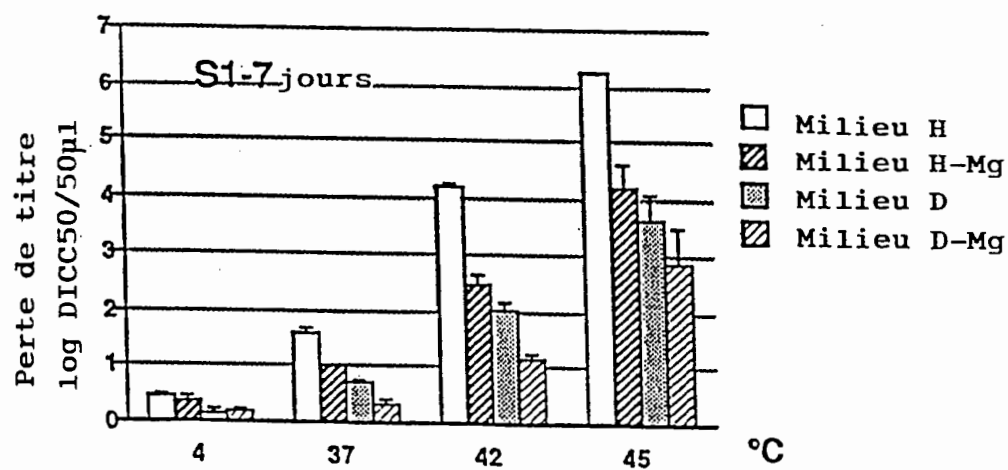
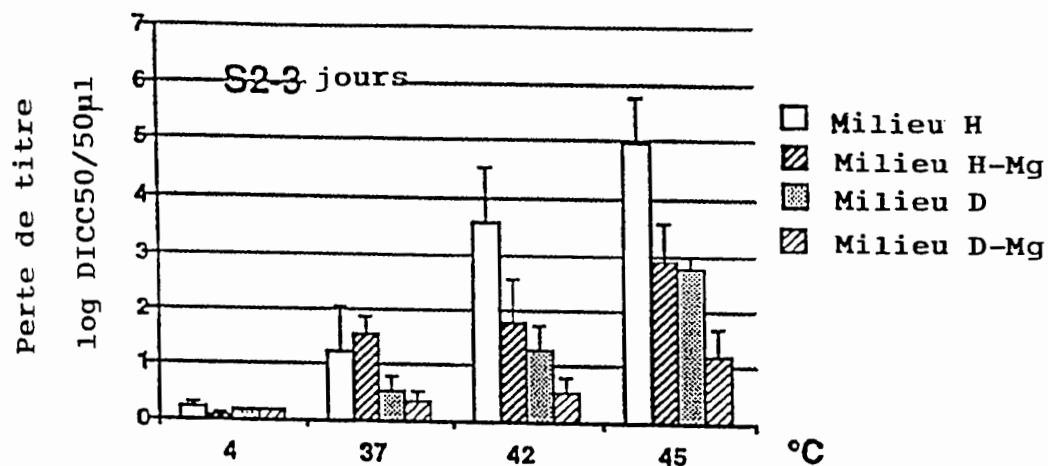


FIGURE 5

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6A



6B

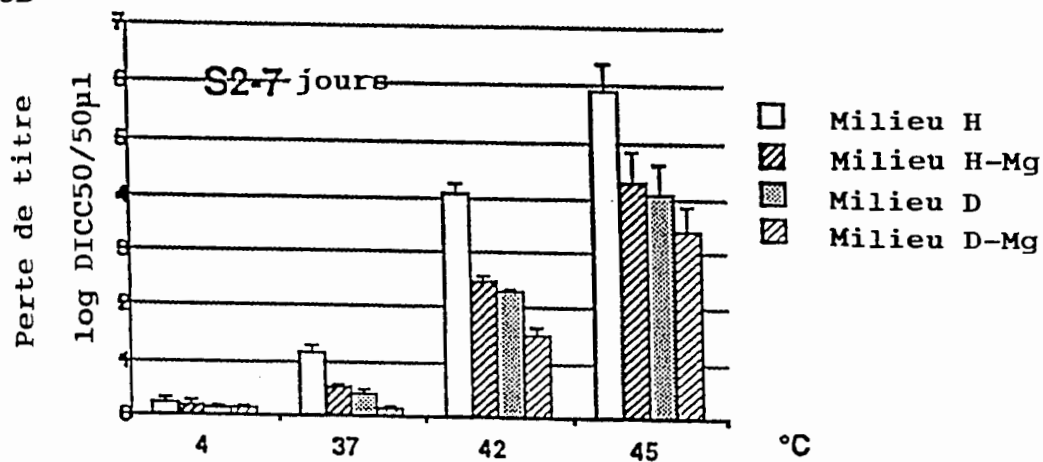
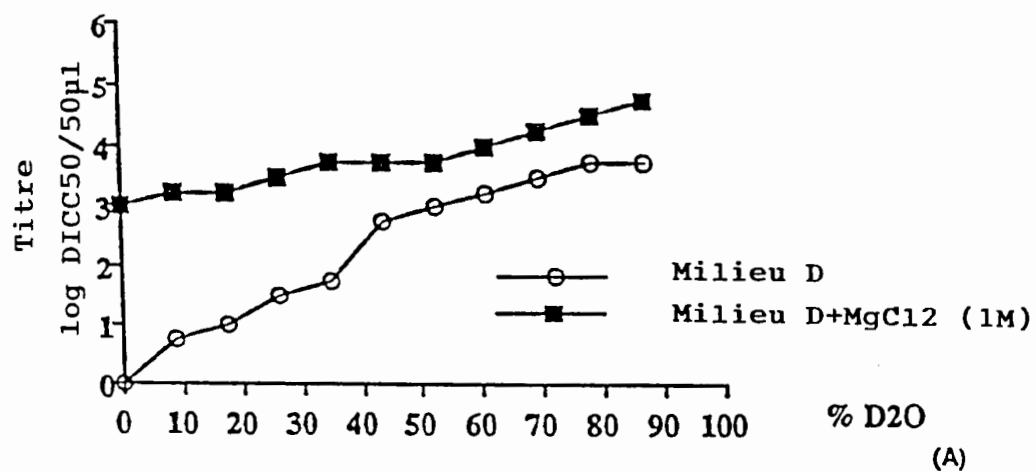


FIGURE 6

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7A



7B

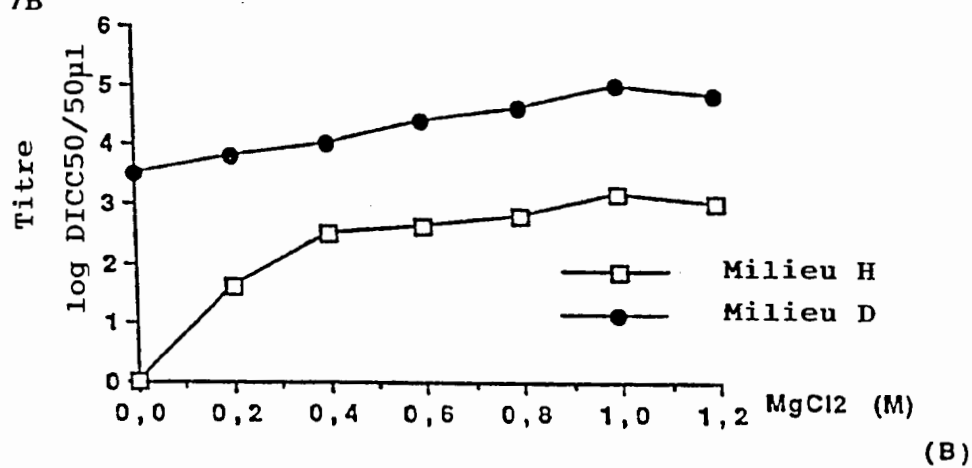


FIGURE 7

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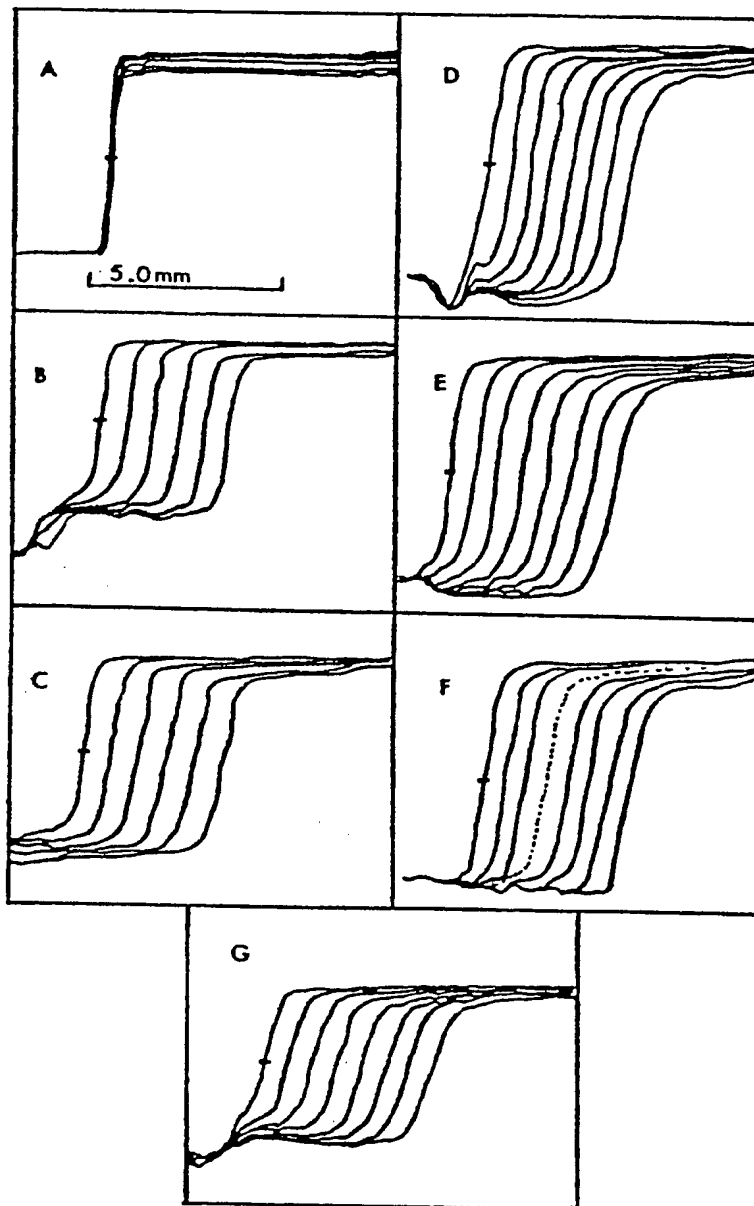


FIGURE 8

INTERNATIONAL SEARCH REPORT

Int ional Application No
PCT/FR 94/00296

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 A61K47/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP,A,0 332 826 (TEVA) 20 September 1989 see the whole document ---	1-12
Y	CHEMICAL ABSTRACTS, vol. 116, no. 4, 27 January 1992, Columbus, Ohio, US; abstract no. 28099b, see abstract & Z.NATURFORSCH. vol. 46, no. 9-10 , 1991 pages 789 - 793 C.HEYDE ET AL. 'HEAVY WATER (D2O) PROTECTIVE EFFECT ON PROTEINS IN PHARMACEUTICALS.FOR EXAMPLE:HUMAN CHOLINESTERASE' --- -/--	1-12

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

30 May 1994

Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CHEMICAL ABSTRACTS, vol. 105, no. 17, 27 October 1986, Columbus, Ohio, US; abstract no. 148271w, see abstract & TSITOLOGIYA vol. 28, no. 8 , 1986 pages 790 - 795 V.YA.ALEKSANDROV 'STABILIZING EFFECT OF HEAVY WATER (D2O) ON THE CELL' ---	1-12
Y	CHEMICAL ABSTRACTS, vol. 95, no. 19, 9 November 1981, Columbus, Ohio, US; abstract no. 164216n, see abstract & STUD.BIOPHYS. vol. 84, no. 1 , 1981 pages 69 - 70 U.LEMM ET AL. 'STABILIZATION OF BIOCHEMICALLY ACTIVE PROTEINS BY HEAVY WATER' ---	1-12
Y	CHEMICAL ABSTRACTS, vol. 95, no. 5, 3 August 1981, Columbus, Ohio, US; abstract no. 37552u, see abstract & EUR.J.BIOCHEM. vol. 116, no. 3 , 1981 pages 441 - 445 U.LEMM ET AL. 'STABILIZATION OF ENZYMES AND ANTISERUMS BY HEAVY WATER' ---	1-12
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INTERNATIONAL SEARCH REPORT

Int. onal Application No

PCT/FR 94/00296

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CHEMICAL ABSTRACTS, vol. 71, no. 23, 8 December 1969, Columbus, Ohio, US; abstract no. 110159g, see abstract & NUCL.SCI.ABSTR. vol. 23, no. 12 , 1969 page 22242 K.JUNG 'EFFECT OF HEAVY WATER ON THE THERMAL RESISTANCE OF MICROORGANISMS' ---	1-12
Y	CHEMICAL ABSTRACTS, vol. 72, no. 15, 13 April 1970, Columbus, Ohio, US; abstract no. 76039b, see abstract & J. HYG. vol. 67, no. 4 , 1969 pages 679 - 690 P.R.M.STEELE ET AL. 'FACTORS AFFECTING THE VIABILITY OF FREEZE THAWED T4 BACTERIOPHAGE.II.INFLUENCE OF CERTAIN ELECTROLYTES ON THE DEGREE OF INACTIVATION.' ---	1-12
Y	CHEMICAL ABSTRACTS, vol. 70, no. 21, 26 May 1969, Columbus, Ohio, US; abstract no. 94238d, see abstract & BIOFIZIKA vol. 14, no. 1 , 1969 pages 186 - 187 Y.B.POPOV ET AL. 'EFFECT OF DEUTERIUM OXIDE ON THE HEAT RESISTANCE OF STAPHYLOPHAGE.' -----	1-12

Information on patent family members

PCT/FR 94/00296

Form PCT/ISA/210 (patent family annex) (July 1992)

A. CLASSEMENT DE L'OBJET DE LA DEMANDE

CIB 5 A61K47/02

Selon la classification internationale des brevets (CIB) ou à la fois selon la classification nationale et la CIB

B. DOMAINES SUR LESQUELS LA RECHERCHE A PORTE

Documentation minimale consultée (système de classification suivi des symboles de classement)

CIB 5 A61K

Documentation consultée autre que la documentation minimale dans la mesure où ces documents relèvent des domaines sur lesquels a porté la recherche

Base de données électronique consultée au cours de la recherche internationale (nom de la base de données, et si cela est réalisable, termes de recherche utilisés)

C. DOCUMENTS CONSIDERES COMME PERTINENTS

Catégorie *	Identification des documents cités, avec, le cas échéant, l'indication des passages pertinents	no. des revendications visées
Y	EP,A,0 332 826 (TEVA) 20 Septembre 1989 voir le document en entier ---	1-12
Y	CHEMICAL ABSTRACTS, vol. 116, no. 4, 27 Janvier 1992, Columbus, Ohio, US; abstract no. 28099b, voir abrégé & Z.NATURFORSCH. vol. 46, no. 9-10 , 1991 pages 789 - 793 C.HEYDE ET AL. 'HEAVY WATER (D2O) PROTECTIVE EFFECT ON PROTEINS IN PHARMACEUTICALS.FOR EXAMPLE:HUMAN CHOLINESTERASE' --- -/-	1-12

☒ Voir la suite du cadre C pour la fin de la liste des documents☒ Les documents de familles de brevets sont indiqués en annexe

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C.(suite) DOCUMENTS CONSIDERES COMME PERTINENTS		
Catégorie *	Identification des documents cités, avec, le cas échéant, l'indication des passages pertinents	no. des revendications visées
Y	CHEMICAL ABSTRACTS, vol. 105, no. 17, 27 Octobre 1986, Columbus, Ohio, US; abstract no. 148271w, voir abrégé & TSITOLOGIYA vol. 28, no. 8 , 1986 pages 790 - 795 V.YA.ALEKSANDROV 'STABILIZING EFFECT OF HEAVY WATER (D2O) ON THE CELL' ---	1-12
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Renseignements relatifs aux membres de familles de brevets

De Internationale No

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Formulaire PCT/ISA/210 (annexe familles de brevets) (juillet 1992)

Bibliographic data

Title: NEW MEDICAL FORMS WITH PROLONGED EFFECT APPLIED IN EYE MEDICINE AND THE METHOD FOR PREPARATION THEREOF

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Abstract of WO9624331A1

The invention pertains to new medical forms of pharmaceuticals with prolonged effect applied in eye medicine, which are formed by a pregel or gel based on the copolymers of 2-(2-carboxybenzoyloxy)ethyl methacrylate with methacrylate comonomers selected from the group comprising methyl methacrylate and butyl methacrylate; the carboxyl groups of which are at least in part modified and/or neutralized with at least one pharmaceutically active substance and/or neutralized with Na⁺ and/or K⁺ ions and/or organic bases and which contains, if it is desired, encapsulated another pharmaceutically active substances. One of the methods for the preparation of these new medical forms consists in at least partial neutralization of functional carboxyl groups of the latex, based on the copolymers of 2-(2-carboxybenzoyloxy)ethyl methacrylate with methacrylate comonomers selected from the group comprising methyl methacrylate and butyl methacrylate, with a basic pharmaceutically active substance and/or sodium hydroxide and/or potassium hydroxide and/or organic bases. The scope of the invented solution comprises also further methods for the preparation of the medical forms according to the invention.



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(21) International Application Number: PCT/CZ96/00006 (22) International Filing Date: 6 February 1996 (06.02.96) (30) Priority Data: PV 315-95 7 February 1995 (07.02.95) CZ (71) Applicants (for all designated States except US): ÚSTAV MAKROMOLEKULÁRNÍ CHEMIE AKADEMIE VĚD ČESKÉ REPUBLIKY [CZ/CZ]; Heyrovského nám. 2, 162 06 Praha 6 (CZ). VÚFB A.S. [CZ/CZ]; Kouřimská 17, 130 60 Praha 3 (CZ). FAKULTNÍ THOMAYEROVA NEMOCNICE [CZ/CZ]; Vídeňská 800, 140 59 Praha 4 (CZ). (72) Inventors; and (75) Inventors/Applicants (for US only): BLEHA, Miroslav [CZ/CZ]; Lublaňská 46, 120 00 Praha 2 (CZ). PRCHLÍK, Jaroslav [CZ/CZ]; nám. Před Bateriemi 1, 162 00 Praha 6 (CZ). SEDLÁKOVÁ, Zdeňka [CZ/CZ]; Batličková 253/1, 182 00 Praha 8 (CZ). BOUCHAL, Karel [CZ/CZ]; Nikose Belojannise 6, 150 00 Praha 5 (CZ). SEDLÁČEK, Karel [CZ/CZ]; Noutonická 47, 152 00 Praha 5 (CZ).		(74) Agent: GABRIELOVÁ, Marta; Ústav makromolekulární chemie AV ČR, Heyrovského nám. 2, 162 06 Praha 6 (CZ). (81) Designated States: CA, CN, JP, KR, SG, SK, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: NEW MEDICAL FORMS WITH PROLONGED EFFECT APPLIED IN EYE MEDICINE AND THE METHOD FOR PREPARATION THEREOF		
(57) Abstract The invention pertains to new medical forms of pharmaceuticals with prolonged effect applied in eye medicine, which are formed by a pregel or gel based on the copolymers of 2-(2-carboxybenzoyloxy)ethyl methacrylate with methacrylate comonomers selected from the group comprising methyl methacrylate and butyl methacrylate, the carboxyl groups of which are at least in part modified and/or neutralized with at least one pharmaceutically active substance and/or neutralized with Na⁺ and/or K⁺ ions and/or organic bases and which contains, if it is desired, encapsulated another pharmaceutically active substances. One of the methods for the preparation of these new medical forms consists in at least partial neutralization of functional carboxyl groups of the latex, based on the copolymers of 2-(2-carboxybenzoyloxy)ethyl methacrylate with methacrylate comonomers selected from the group comprising methyl methacrylate and butyl methacrylate, with a basic pharmaceutically active substance and/or sodium hydroxide and/or potassium hydroxide and/or organic bases. The scope of the invented solution comprises also further methods for the preparation of the medical forms according to the invention.		

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Description

New medical forms with prolonged effect applied in eye medicine and the method for preparation thereof

5

Technical Field

The invention pertains to new medical forms of pharmaceuticals with the prolonged effect applied in ophthalmology and to the method of their preparation.

10

Background Art

One of the fundamental prerequisites of successful treatment is the optimal pharmacotherapeutical regime which depends, among others, on attaining the required therapeutic level of medical substance in the organism and its stabilization during therapy.

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For this reason and because of efforts to extent the time interval between drug administration, the medical forms with prolonged effect acquire still larger importance. These medical forms are intensively developed over the whole world, particularly in eye medicine, unfortunately, so far without completely satisfactory results.

20

In dosing eye drops as the most frequent medical form of ophthalmic drugs, the solution of active substance runs on the cornea and is eliminated from this area practically within 30 seconds (Nor M. Acta Ophtal. 42,727 (1964)). In the usual case of active substance passing through the cornea interface, the time of action of the active substance is insufficient for the above reason.

25

This problem of short residence time of the administered ophthalmic drug on the eye surface is solved in part by increasing the viscosity of drops by additives based mostly on the derivatives of cellulose and poly(vinylalcohol) (Sieg J.W., Robinson J.R., J.Pharm.Sci. 66, 1222 (1977)). A considerable disadvantage of the modified forms of collyrium is, besides the difficult dosing, the deteriorated filtrability of the prepared solutions in the drops production. With

30

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respect to the necessary sterility of eye drops, the filtration through a bacteriological filter with a small pore diameter (approx. 0.1-0.2 μm) is prescribed in the production of this medical form. Also the application of gel plates
5 under the eye lid is accompanied with unfavourable side effects and irritation.

The present method represents a qualitatively more efficient solution of the problem of prolonged action of the active substance on eye. It is based on the preparation of
10 polymer materials with the affectable capacity of their transition from a dispersion form into polymeric transparent gel either "in situ" in the eye or outside the eye.

Disclosure of Invention

15 The subject of the invention are new medical forms with prolonged effect for pharmaceuticals applied in ophthalmology consisting of a pregel or gel based on the copolymers of 2-(2-carboxybenzoyloxy)ethyl methacrylate with methacrylate comonomers selected from the group comprising methyl methacrylate and butyl methacrylate, carboxy groups of which are,
20 at least in part, modified and/or neutralized with at least one pharmaceutically active substance and/or neutralized with Na^+ and/or K^+ ions and/or organic bases, and which pregel or gel contains, if it is desirable, another pharmaceutically
25 active substance in an encapsulated form.

The active substances, selected from the group comprising pilocarpine, atropine, lysine base, lysine salicylate, physostigmine salicylate, sodium salt of diclofenac, timolol, carbachol, ciprofloxacin, Zn^{++} or their combinations, are
30 suitable as pharmaceutically active substances according to the invention.

New medical forms according to the invention can further contain, besides the pharmaceutically active substance, also another encapsulated pharmaceutically active compound.

35 The method for the preparation of the new medical forms according to the invention consists in, at least partial, neutralization of functional carboxy groups of a latex based

on the copolymers of 2-(2-carboxybenzoyloxy)ethyl methacrylate with the methacrylate comonomers, selected from the group comprising methyl methacrylate and butyl methacrylate, with the basic pharmaceutically active substance and/or sodium hydroxide and/or potassium hydroxide and/or organic bases.

Another possible procedure in the preparation of the medical forms according to the invention consists in an ion-exchange reaction of the at least partially neutralized latex, based on the copolymers of 2-(2-carboxybenzoyloxy)ethyl methacrylate with methacrylate comonomers selected from the group comprising methyl methacrylate and butyl methacrylate, with the cations of pharmaceutically active substance and/or with Zn^{++} ions and neutralization of the residual carboxy groups.

The new medical forms according to the invention may be also prepared by the method which consists in subjecting the latex, or the at least partially neutralized latex, based on the copolymers of 2-(2-carboxybenzoyloxy)ethyl methacrylate with methacrylate comonomers selected from the group comprising methyl methacrylate and butyl methacrylate, to the reaction with at least one pharmaceutically active substance of the cationic and/or anionic character and in neutralization of the residual carboxy groups.

The new medical forms for pharmaceuticals applied in ophthalmology according to the present invention are based on our investigations which showed that the synthesized copolymer of 2-(2-carboxybenzoyloxy)ethyl methacrylate (CBM) with appropriately selected comonomers exhibits, in the neutralization of carboxyl groups with bases, a high degree of swelling and that the latex particles are transformed to gel during these changes. The transport properties of CBM materials are strongly affected by the presence of carboxyl groups, above all by the degree of neutralization which is decisive for the water content in the swelled structure of copolymers. These copolymers proved suitable polymeric carriers of ophthalmological preparations with prolonged

action. The prepared emulsion types of copolymers are modified with the selected drugs during the neutralization process and then either applied in the form of dispersion, which is transformed into the gel form in eye by a small change of pH, or applied directly as a gel.

The active substances, such as pilocarpine, atropine, sodium diclofenac [sodium 2-(2,6-dichloroanilino)phenylacetate], timolol [1-(1,1-dimethylethylamino)-3-(4-(4-morpholinyl)-1,2,5-thiadiazol-3-yl)oxypropanol-2], carbachol [2-N,N,N-trimethyl-ammonium ethyl urethane], ciprofloxacin [1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid], tobramycin [O-3-amino-3-deoxy- α ,D-glucopyranosyl-(1,6)-O-(2,6-diamino-2,3,6-trideoxy- α ,D-ribohexo-pyranosyl-1,4)-2-deoxy-D-streptamine] or their suitable combinations, are ionically bonded to latex particles of a catex type; the remaining carboxyl groups are neutralized to Na⁺ or K⁺ forms and the swelling degree of these particles in water increases in this way; in the region of pH above 6.8, a strong physical interaction between these particles occurs and, after their application on the eye cornea, the gel is formed due to the increase of pH to about 7.3 (pH of eye fluid).

The formation of gel can be also utilized for encapsulation of further types of pharmaceuticals, which are not chemically bonded to the functional groups of latex particles but are fixed in the gel matrix from which they are gradually released by diffusion. The combination of both ways, i.e. of the ionic bond and encapsulation, may be advantageously used for the drug types containing the pharmaceutically active components of both cationic and anionic character, e.g. Ketozolac tromethamine, where tromethamine [tris(hydroxymethyl)aminomethane] is ionically bonded to the latex particles and the released 5-benzoyl-2,3-dihydroxy-1,4-pyrrolizine-1 carboxylic acid (Ketozolac) is encapsulated in the gel formed. Lysine salicylate may be analogously fixed in the gel; lysine is bound to the latex particles and the released salicylic acid is encapsulated in the formed gel. This gel

releases the drug with retardation due to the ion-exchange reaction between the drug cations and Na^+ or K^+ present in the eye fluid or by diffusion of the encapsulated drug component from the gel.

The initial latexes of copolymers of 2-(2-carboxybenzoyloxy)-ethyl methacrylate (CBM) with the methacrylate comonomers - methyl methacrylate (MMA), butyl methacrylate (BuMA) - contain particles of these copolymers of the size characterized by diameter $d = 100\text{--}150$ nm. The content of carboxyl groups in the case of CBM homopolymer attains as much as 3.6 mmol per g of latex dry substance (or 0.36 mmol per ml of 10% latex); low contents of carboxyl groups may be obtained by copolymerization of CBM with the above mentioned methacrylate comonomers.

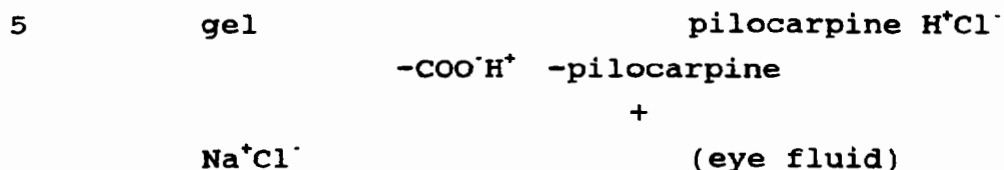
During the neutralization of COOH groups (H^+ -forms) with bases, a considerable swelling of particles with water occurs depending on the neutralization degree and the volume increases about hundredtimes. If these particles contain more than 2.4 mmol COOH per g of dry substance, the formation of gel occurs at high degrees of their neutralization (Z. Sedláková et col., Angew.Makromol.Chemie 201(1992) 33-48). This gel is the potential carrier of drugs which are able to create ionic bonds with COO^- groups or which can be encapsulated in the formed gel.

Bonding of drugs on the functional carboxyl groups (COOH) of the latex particles is carried out either by neutralization of the COOH groups with the basic types of pharmaceuticals, e.g. ciprofloxacin, or by ion-exchange reactions of the Na^+ -forms of latex with the cations of pharmaceuticals, e.g. pilocarpine or Zn^{++} , according to the following scheme:



An advantage of such a form of pharmaceuticals application

on eye cornea consists in extension the time of drug action. The prolongation of drugs is caused by diffusion course of the ion exchanging reaction



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The gel slows down flooding up the pharmaceuticals from the eye caused by weeping. Considerable transparency of the gel and the necessary compatibility with the eye cornea secure that the patient has not substantially worse vision during application of this medical form.

15

With respect to the high content of COOH groups in the latex particle (0.25-0.35 mmol per 1 ml latex), these latexes may bind as much as 70 mg drug (MW 200) in 1 ml, whereas the common medical forms are administered in concentrations 0.1-1% (10 mg per ml).

20

As follows from the above facts, about 20% of functional groups can be utilized for the ionic binding of drug and the remaining groups may serve for the bond of another type of drug. In the case of a single-component system, the remaining COOH groups can be transferred to the Na^+ form or K^+ form or to their combination by partial neutralization with NaOH (sodium hydroxide) or KOH (potassium hydroxide). The gels prepared in this way then contain both the desired pharmaceuticals and the suitable ratio of Na^+ and K^+ ions which can adjust the ionic conditions in the eye.

25

30

All gels prepared from the latexes of copolymers of 2-(2-carboxybenzoyloxy)ethyl methacrylate (CBM) exhibit strong antibacterial effects and are biocompatible.

35

The invention is further illustrated in the examples of performance without, however, limiting the scope to them.

Examples

Example 1

The latex of copolymer 2-(2-carboxybenzoyloxy)ethyl methacrylate with butyl methacrylate (CBM - BuMA) was prepared by mixing 18.6 g CBM (6.7×10^{-2} mol) and 6.3 g BuMA (4.47×10^{-2} mol) and dosing this monomer mixture into 100 ml aqueous phase; this phase contained 1 g lauryl sulfate and 0.1 g $K_2S_2O_8$ as one of the components of redox system. Emulgat-
ion of the reaction mixture required vigorous stirring (350 r.p.m.) for 15 minutes. At the same time, the reactor was inertized (flushed with nitrogen N_2). After the emulgation stage, the solution of 0.1 g sodium disulfite in 5 ml H_2O was added and the subsequent radical emulsion copolymerization proceeded at 25°C for 24 hours. The conversion degree attained was 96%. Latex CBM - BuMA prepared in this way had the following characteristics:

content of dry substance 0.2 g/ml, particle size d_n (diameter 70 nm, $[COOH] = 5.37 \times 10^{-4}$ mol/ml.

Low-molecular weight electrolytes (residues of the initiation system and the reaction products of initiation reaction) were removed by pressure filtration of the latex with deionization water through the membrane DIAFLOW XM 300 (product of AMICON, USA); the process was checked by measuring conductivity of the filtrate and stopped when conductivity $\lambda = 600 \mu S$ was attained. The latex processed in the described way was partially neutralized with 0.1 M solution of lysine base and then with 0.5 M NaOH (sodium hydroxide) until a pregel state was achieved. The procedure consisted in the stepwise neutralization of 10 ml processed latex CBM - BuMA with 27 ml 0.1 M lysine base, which corresponds to 50% conversion of COOH functional groups of the latex to lysine monophthalate. The gradual addition of 4.5 ml 0.5 M NaOH ionized further COOH groups (the content of COO⁻ groups corresponded to about 90% conversion of COOH groups) forming the pregel with particle size $d_n = 500-700$ nm. The pregel contained 10 mg lysine/ml (1 wt.%).

Example 2

The latex of CBM - BuMA copolymer prepared according to Example 1 with the distinction that the aqueous phase contained 0.5 g lauryl sulfate/100 ml H₂O, contained 0.2 g dry copolymer/ml, had particle size $d_n = 100$ nm and $[\text{COOH}] = 5.3 \times 10^{-4}$ mol/ml. After purification by pressure filtration, 10 ml of this latex was neutralized with 2.7 ml 0.1 M lysine base (2.7×10^{-4} mol) and further neutralization of COOH groups was carried out with a mixture of 0.25 M NaOH (sodium hydroxide) and 0.25 M KOH (potassium hydroxide) in such a way, that about 90% total COOH groups was neutralized and the latex formed a pregel of pH about 6.8-6.9. This modified pregel contained 0.2 wt.% lysine (2 mg/ml) and the ratio Na^+/K^+ (of sodium to potassium ions) corresponded to about 1.

Example 3

The latex of copolymer CBM - BuMA, prepared according to Example 2, was pressure filtered, mixed with lysine salicylate and then transformed to a pregel, which contained encapsulated lysine salicylate, by neutralization of functional groups with 0.5 M NaOH. The procedure consisted in stirring 10 ml latex with 700 mg lysine salicylate in 8 ml H₂O for 48 hours. The gradual addition of 8.5 ml 0.5 M NaOH gave rise to the pregel containing 3 wt.% lysine salicylate (30 mg lysine salicylate/ml).

Example 4

The latex of copolymer CBM - BuMA, prepared according to Example 1 with the distinction that the aqueous phase contained 0.25 g lauryl sulfate, and characterized by parameters: content of dry substance 0.18 g/ml, $d_n = 150$ nm, $[\text{COOH}] = 5.25 \times 10^{-4}$ mol cm⁻³, was refined and then modified with Zn⁺⁺ ions by the following procedure: 10 ml latex was stirred with 20 mg Zn(CH₃COO)₂ · 2 H₂O (zinc acetate) dissolved in 5 ml H₂O ($[\text{Zn}^{++}] = 0.9 \times 10^{-4}$ mol Zn) at 25°C for 2 hours and then the residual COOH groups were neutralized with 0.5 M NaOH until the pregel was formed (neutralization degree about 85%, pH

6.7 to 6.8); the pregel contained approx. 0.27 wt.% $\text{Zn}^{++} = 2.7$ mg Zn^{++}/ml .

Example 5

5 The latex CBM - BuMA modified with Zn^{++} ions according to Example 4 was neutralized, after the reaction with the aqueous solution containing 20 mg $\text{Zn}(\text{CH}_3\text{COO})_2$, first with 0.1 M solution of lysine base (10 ml) and then with approx. 4 ml of the mixture of 0.25 M NaOH and 0.25 M KOH to the degree of
10 neutralization of COOH groups about 90% when the latex created the pregel state with particle size $d_n = 600-800$ nm; pH 6.8. The pregel contained 0.46 wt.% lysine (4.6 mg/ml) and 0.17 wt.% Zn^{++} (1.7 mg/ml); the Na^+/K^+ ratio was about 1.

15 Example 6

 The latex CBM - BuMA prepared according to Example 4 was mixed with lysine salicylate and then neutralized to create the pregel containing encapsulated lysine salicylate and ionically bonded Zn^{++} ions. The procedure consisted in mixing
20 10 ml latex with 20 mg $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2 \text{H}_2\text{O}$ dissolved in 5 ml H_2O [$\text{Zn}^{++} = 0.9 \times 10^{-4}$ mol] at 25°C for 2 hours, followed by addition of 700 mg lysine salicylate dissolved in 8 ml H_2O . The mixture was then stirred for 24 hours at 25°C and then the pregel was
25 created by neutralization with 0.5 M NaOH, which contained 23 mg/ml (2.3 wt.%) of encapsulated lysine salicylate and 2.3 mg Zn^{++}/ml (0.23 wt.%).

Example 7

 The latex CBM - BuMA prepared according to Example 1 was
30 pressure filtered and then partially neutralized (about 10% COOH groups) with 0.5 M NaOH and subjected to the ion-exchanging reaction with physostigminium salicylate. The following neutralization with 0.5 M NaOH created the pregel containing ionically bonded physostigminium cation and encapsulated
35 sodium salicylate. The procedure consisted in mixing 10 ml latex with 60 mg physostigminium salicylate in 6 ml H_2O for 24 hours at 25°C and the following neutralization with 0.5 M

NaOH (about 9 ml) which created the pregel containing physostigminium cations in the amount corresponding to 2.4 mg physostigminium salicylate per ml (0.24 wt.%).

5 **Example 8**

The latex CBM - BuMA prepared according to Example 1 was pressure filtered, partially neutralized with 0.5 M NaOH (about 10% COOH groups), and then modified with Zn^{++} ions and physostigminium salicylate. The following neutralization of
10 remaining COOH groups with 0.5 M NaOH converted the latex into the pregel containing Zn^{++} and the ionically bonded physostigminium cation. For example, 10 ml latex was neutralized with 1 ml 0.5 M NaOH, 20 mg $Zn(CH_3COO)_2 \cdot 2 H_2O$ in 5 ml H_2O was added and the mixture was stirred for 2 hours at 25°C. The
15 solution containing 60 mg physostigminium salicylate in 6 ml H_2O was then dropwise added and stirring was continued for further 24 hours. The following neutralization with 0.5 M NaOH (about 8 ml) formed the pregel containing 2 mg physostigminium cation/ml (0.2 wt.%) and 2 mg Zn^{++} /ml (0.2 wt.%).

20

Example 9

The latexes CBM - BuMA prepared according to Example 2 may be partially neutralized after pressure filtration with 1-(1,1-dimethylethylamino)-3-(4-(4-morpholinyl)-1,2,5-thiadiazol-3-yl)oxypropanol-2 (Timolol) and then another part of
25 the latex functional COOH groups can be neutralized with 0.5 M NaOH. In the subsequent ion-exchanging reaction, N-(2-(aminocarbonyl)oxy)ethyl-N,N,N-trimethylammonium cation (Carbachol) may be ionically bonded on latex particles and may form
30 the gel containing the combination of both pharmaceuticals by addition further portion of 0.5 M NaOH at pH 6.8. The following procedure was used: 10 ml latex was partly neutralized with 31.6 mg Timolol (10^{-4} mol - 0.34 wt.%), 5 ml 0.5M NaOH was added, the latex was mixed with 90 mg Carbachol
35 (5×10^{-4} mol - 0.9 wt.%) and, after 3 hours of stirring at 25°C, pH was adjusted to 6.8 by addition of 0.5 M NaOH to create the pregel from latex particles.

Example 10

The latex CBM - BuMA prepared according to Example 1 was pressure filtered, modified with 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid (Ciprofloxacin) and the pregel containing Ciprofloxacin was formed by the following neutralization with 0.5 M NaOH up to the pH value of 6.8. The procedure consisted in mixing 10 ml latex with 33 mg antibiotics for 3 hours at 20°C followed by neutralization with 9 ml 0.5 M NaOH under vigorous stirring. Temperature 20°C was kept by cooling and the pregel with pH 6.8 was formed after metering the NaOH solution.

Example 11

The latex CBM - BuMA prepared according to Example 4 was modified with Zn^{++} ions and then mixed with an aqueous solution of sodium (2-(2,6-dichloroanilino)phenyl)acetate (sodium Diclophenac). The following neutralization with 0.5 M NaOH (KOH or KOH-NaOH mixture) created the pregel which contained ionically bonded Zn^{++} cations and encapsulated sodium Diclophenac. The following procedure was used: 10 ml latex was mixed with 20 mg $Zn(CH_3COO)_2 \cdot 2 H_2O$ dissolved in 8 ml H_2O at 25°C for 2 hours, then the solution of 30 mg Na^+ Diclophenac in 5 ml H_2O was dropwise added into the latex and the subsequent neutralization with 0.5 M NaOH created the pregel which contained 2.3 mg Zn^{++}/ml and about 1 mg Diclophenac/ml.

Claims

1. New medical forms of pharmaceuticals with a prolonged effect, applied in eye medicine, formed by a pregel or gel based on the copolymers of 2-(2-carboxybenzoyloxy)ethyl methacrylate with methacrylate comonomers selected from the group comprising methyl methacrylate and butyl methacrylate, carboxyl groups of which are at least in part modified and/or neutralized by at least one pharmaceutically active substance and/or neutralized with Na⁺ and/or K⁺ ions and/or organic bases and which, if it is desired, contains encapsulated another pharmaceutically active substance.
2. New medical forms of pharmaceuticals with a prolonged effect, applied in eye medicine, according to Claim 1, wherein the substances selected from the group comprising pilocarpine, atropine, lysine base, lysine salicylate, physostigminium salicylate, sodium diclophenac, timolol, carbachol, ciprofloxacin, Zn⁺⁺ ions or their combinations are used as the pharmaceutically active substances.
3. The new medical forms for pharmaceuticals applied in eye medicine according to Claims 1 and 2, wherein the said forms contain encapsulated pharmaceutically active substance.
4. The method for preparation the new medical forms according to Claims 1 to 3, wherein functional carboxyl groups of the latex based on copolymers of 2-(2-carboxybenzoyloxy)ethyl methacrylate with methacrylate comonomers selected from the group comprising methyl methacrylate and butyl methacrylate are neutralized, at least in part, with a basic pharmaceutically active substance and/or sodium hydroxide and/or potassium hydroxide and/or organic bases.

5. The method for preparation the new medical forms according to Claims 1 to 3, wherein the at least partially neutralized latex, based on the copolymers of 2-(2-carboxybenzoyloxy)-ethyl methacrylate with methacrylate comonomers selected from the group comprising methyl methacrylate and butyl methacrylate, is subjected to the ion-exchanging reaction with cations of the pharmaceutically active substance and/or Zn^{++} ions and the remaining carboxylic groups are neutralized.
6. The method for preparation new medical forms according to Claims 1 to 3, wherein the latex and/or at least partially neutralized latex, based on the copolymers of 2-(2-carboxybenzoyloxy)ethyl methacrylate with methacrylate comonomers selected from the group comprising methyl methacrylate and butyl methacrylate, is subjected to the reaction with at least one pharmaceutically active substance of the cationic and/or anionic character and the remaining carboxyl groups are neutralized.

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/CZ 96/00006

A. CLASSIFICATION OF SUBJECT MATTER

A 61 K 9/06, A 61 K 9/08, A 61 K 9/22, A 61 K 47/32

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A 61 K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	EP, A, 0 424 042 (LABORATOIRES MERCK, SHARP & DOHME-CHIBRET) 24 April 1991 (24.04.91), abstract; claims 1-3, 9-12, 14, 15; example 1, page 2, lines 23-27; page 3, lines 3-21; page 3, line 53 - page 4, line 8.	1, 2, 6
Y	--- CHEMICAL ABSTRACTS, vol. 118, no. 12, issued 1993, March 22, (Columbus, Ohio, USA), SEDLAKOVA Z. et al. "Synthesis of 2-(2-carboxy- benzoyloxy)ethyl methacrylate and its radical poly- merization and copoly-	1, 2, 6

☒ Further documents are listed in the continuation of box C.

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Date of the actual completion of the international search
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INTERNATIONAL SEARCH REPORT

International Application No

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	DE, A, 3 722 837 (RÜTGERSWERKE AG) 19 January 1989 (19.01.89), abstract; claims 1,2,5; column 1, line 67 - column 2, line 60. --	1-3
A	GB, A, 2 007 091 (TOKO YAKUHI KOGYO KABUSHIKI KAISHA) 16 May 1979 (16.05.79), abstract; claims 1-4,8,9; page 1, lines 39-54. --	1,2,4
A	US, A, 3 962 414 (MICHAELS A.S.) 08 June 1976 (08.06.76), abstract; claims 1,5-9; column 7, line 67 - column 8, line 6; column 19, line 43 - column 20, line 26. ----	1-3,5

ANHANG

zum internationalen Recherchen-
bericht über die internationale
Patentanmeldung Nr.

ANNEX

to the International Search
Report to the International Patent
Application No.

ANNEXE

au rapport de recherche inter-
national relatif à la demande de brevet
international n°

PCT/CZ 96/00006 SAE 127704

In diesem Anhang sind die Mitglieder
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/US96/17446 (22) International Filing Date: 28 October 1996 (28.10.96) (30) Priority Data: 60/007,098 31 October 1995 (31.10.95) US 9603904.5 23 February 1996 (23.02.96) GB (71) Applicant (for all designated States except US): MERCK & CO., INC. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): THOMPSON, Wayne, J. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). RANSOM, Richard, W. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). MALLORGA, Pierre [FR/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). SUGRUE, Michael, F. [GB/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). (74) Common Representative: MERCK & CO., INC.; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).		(81) Designated States: AL, AM, AU, AZ, BA, BB, BG, BR, BY, CA, CN, CU, CZ, EE, GE, HU, IL, IS, JP, KG, KR, KZ, LC, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TR, TT, UA, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>	
(54) Title: MUSCARINE ANTAGONISTS			
(57) Abstract This invention is concerned with novel 1,3-dihydro-1-[1-(1-heteroaryl)piperidin-4-yl]piperidin-4-yl]-2H-benzimidazolones, derivatives thereof, their preparation, method of use and pharmaceutical compositions. These compounds are endowed with antimuscarinic activity and are useful in the treatment and/or prevention of myopia (commonly known as nearsightedness).			

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TITLE OF THE INVENTION
MUSCARINE ANTAGONISTS

BACKGROUND OF THE INVENTION

This invention relates to control of ocular development in general and, more particularly, to the treatment of the eye to prevent and/or arrest the development of myopia (nearsightedness). Approximately one of every four persons suffer from myopia, i.e., an elongation of the eye along the visual axis. In particular, myopia afflicts 10% to 75% of the youth of the world, depending upon race, geographic distribution and level of education. Myopia is not a trivial maldevelopment of the eye. In its pathologic form, the sclera continues to grow and as result the retina stretches and degenerates resulting in permanent blindness.

Inheritance, environmental forces such as diet, sun intake, and substantial eye use, etc., are but a few theories that have been postulated to explain the on-set of myopia. In that regard, preventive measures such as eye rest, eye exercise, eye glasses, contact lens and drug and surgical therapies have been proposed. However, these measures are neither ideal nor risk-free. The surgical therapies (e.g. corneal surgery using excimer lasers or conventional knives) attempted for this condition are drastic and often unsuccessful. Moreover, neither of the therapies (excimer lasers or conventional knives) are easily reversed or sufficiently predictable in their results. Complications from contact lens wear range from allergic reactions to permanent loss of vision due to corneal ulceration. Even with the complications associated with contact lens wear, there are roughly 24 million wearers in the United States, with the number expected to double in the next 3 years. While eyeglasses eliminate most of the medical risks listed above, they are not an acceptable option as evidenced by the contact lens wearers who tolerate the frustration of contact lens wear.

One particular drug therapy utilized in the treatment of myopia involves the use of cycloplegics. Cycloplegics are topically

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administered drugs that relax the ciliary muscle of the eye, which is the muscle that focuses the eye by controlling lens dimensions. The classic cycloplegic drug is the belladonna alkaloid atropine, available for over a century. Atropine is a long-acting non-specific antimuscarinic agent that antagonizes the action of the neurotransmitter acetylcholine (ACh) at autonomic effector cells innervated by postganglionic cholinergic nerves of the parasympathetic nervous system. However, use of atropine, is impractical in that it causes mydriasis (increase of pupil size) and its action on the ciliary muscle to inhibit ocular focusing impairs near visual work like reading. There is strong evidence that the receptors in the iris and ciliary muscle responsible for the side effects of atropine are of the m3 subtype. Additionally, studies have shown that muscarinic receptors in the retina of a variety of non-human species are comprised of m1, m2 and m4 subtypes. Accordingly, a muscarinic antagonist with low m3 activity would be efficacious in prevention of the development of myopia without the undesirable side effects associated with the use of atropine.

There is now substantial evidence to link the posterior part of the eye, specifically image quality at the retina and hence an extension of the nervous system, to the postnatal regulation of ocular growth. There is significant evidence of myopia in an eye that is subjected to retinal image impairment. It has been shown that axial myopia can be experimentally induced, in either birds or primates, in an eye in which the retina is deprived of formed images, e.g., by suturing the eyelids or wearing an image diffusing goggle. The experimental myopia induced in birds or primates such as monkeys mimics, in many respects, the axial myopia of humans.

Thus, the phenomenon of an animal's vision process apparently contributes to the feedback mechanism by which postnatal ocular growth is normally regulated and refractive error is determined. This indicates that this mechanism is neural and likely originates in the retina. R. A. Stone, et al. have found a method of controlling the abnormal postnatal growth of the eye of a maturing

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animal. The method comprises controlling the presence of a neurochemical, its agonist or antagonist, which neurochemical is found to be changed under conditions during maturation leading to abnormal axial length. See U.S. Pat. No. 4,066,772 and 5,284,843. Therein it is disclosed that retinal concentrations of dopamine were found to be reduced during such image deprivation and the ocular administration of a dopamine-related agent, e.g., apomorphine, a dopamine agonist, was found to inhibit or actually prevent the axial enlargement of the eye under conditions ordinarily leading to such enlargement.

There have also been recent advances made in the understanding of the cholinergic nervous system and the receptors thereto. Cholinergic receptors are proteins embedded in the wall of a cell that respond to the chemical acetylcholine. Particularly, it is now known that the cholinergic receptors are subdivided into nicotinic and muscarinic receptors and that the muscarinic receptors are not all of the same type. Recent literature indicates that there are at least five types of cholinergic muscarinic receptors (types m1 through m5). Receptors of type m1 are those present in abundance and thought to be enriched in the brain neural tissue and neural ganglia. The other receptors are concentrated in other tissues such as the heart, smooth muscle tissue or glands. While many pharmacological agents interacting with muscarinic receptors influence several types of receptors, some agents are known to have a major effect on a single type of receptor with relative selectivity. Still other agents may have a significant effect on more than one or even all types of receptors.

It is known, for example, that pirenzepine, (Gastrozepin, LS 519) 5, 11-Dihydro-11-[4-methyl-1-piperazinyl]acetyl]-6H-pyrido[2,3-b] benzodiazepin-6-one, and its dihydrochloride are anticholinergic, antimuscarinic, and relatively selective for m1 receptors. See U.S. Pat. No. 5,122,522 and WO9015604-A. It is also known that 4-DAMP (4-diphenylacetoxy-N-methylpiperadine methiodide) is a relatively selective antagonist

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for smooth muscle (ordinarily called m3 type but variously called type m2 or m3, as the current classification of receptors is in flux). Pirenzepine, being primarily an m1 antagonist, inhibits axial elongation, but is far less effective at pupil dilation than atropine or another cycloplegic agent. This makes it possible to suppress the development of myopia without dilating the pupil and paralyzing the accommodation activity of the ciliary muscle. Additionally, the administration of a drug topically into the eye of a developing child for a long period of time makes it desirable to have a minimal likelihood of sensitization of the eye. Pirenzepine and atropine test positive in sensitization assays and this is an undesirable side effect.

It is therefore an object of this invention to provide a muscarinic antagonist which is effective in the treatment and prevention of myopia without many side effects.

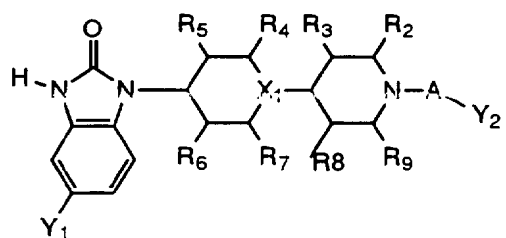
SUMMARY OF THE INVENTION

This invention is concerned with novel 1,3-dihydro-1-[1-(1-heteroaryl)piperidin-4-yl]piperidin-4-yl]-2H-benzimidazolones, their compositions and method of use. The novel compounds are selective muscarinic antagonists of the m1, m2, and m4 subtypes with low activity at the m3 subtype. The compounds have good ocular penetration (bioavailability) when dosed as a 0.1 - 4% aqueous solution, preferably a 0.5-2% solution. The compounds are effective for the treatment and/or prevention of myopia.

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DETAILED DESCRIPTION OF THE INVENTION

The novel compounds of this invention are represented by the structural formula I:



I

or pharmaceutically acceptable salts thereof, or diastereomers, enantiomers or mixtures thereof; wherein:

R₂ - R₉ are independently H, alkyl, halo, alkoxy, OH, HOCH₂-, aryl, 3-pyridyl, 5-pyrimidinyl, alkoxycarbonyl, amino, dialkylamino, alkene, thioalkyl, or alkylamino; alternatively, R₄ and R₇ or R₂ and R₉ may be connected as an ethylene bridge to form a bicyclic heterocycle;

Y₁ is H, alkyl, halo, alkylamino, dialkylamino, alkoxy, alkoxyamino, or amino;

Y₂ is heterocycle, or heterocyclyl;

A is H, CHR₁, C(R₁)₂ or carbonyl;

R₁ is alkyl, alkoxy, aryl, heteroaryl, heterocyclyl or heterocycle; and

X₁ is N or C.

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The term heterocycle or heterocyclic, as used herein except where noted, represents a stable 5- to 7- membered monocyclic heterocyclic ring, which is either saturated or unsaturated, and which consists of carbon atoms and from one to three heteroatoms selected from the group consisting of N, O and S, and including any bicyclic group in which any of the above defined heterocyclic rings is fused to a benzene ring. The heterocyclic ring may be attached at any heteroatom or carbon atom which results in the creation of a stable structure. Examples of such heterocyclic rings include pyridine, pyrazine, pyrimidine, pyridazine, triazine, imidazole, pyrazole, triazole, quinoline, isoquinoline, quinazoline, quinoxaline, phthalazine, oxazole, isoxazole, thiazole, isothiazole, thiadiazole, oxadiazole, pyrrole, furan, thiophene, hydrogenated derivatives of these heterocycles such as piperidine, pyrrolidine, azetidine, tetrahydrofuran, and N-oxide derivatives of heterocycles containing basic nitrogen. Any fused combinations of any of these above-defined heterocyclic rings is also a part of this definition. Attached to the heterocyclic ring can be substituents such as alkyls, amines, alkylamino, or halogens (F, Cl, Br, I).

The term alkyl is intended to include branched, cyclic and straight chain saturated aliphatic hydrocarbon groups having 1 to 15 carbon atoms, unless otherwise defined. Preferred straight or branched alkyl groups include methyl, ethyl, propyl, isopropyl, butyl, t-butyl and the like. Preferred cycloalkyl groups include cyclopentyl and cyclohexyl.

The term alkoxy represents an alkyl group of indicated carbon atoms attached through an oxygen linkage.

The term alkylamino represents an alkyl group of indicated carbon atoms attached through a nitrogen atom linkage.

The term dialkylamino represents two alkyl groups of indicated carbon atoms attached through a nitrogen atom linkage.

The term small alkyl is intended to indicate those alkyls with C1 to C6 carbon atoms, either branched or linear in connection.

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The term halo as used herein, represents fluoro, chloro, bromo or iodo.

The term aryl refers to aromatic rings e.g., phenyl, substituted phenyl and the like groups as well as rings which are fused e.g., naphthyl and the like. Aryl thus contains at least one ring having at least 6 atoms, with up to two such rings being present, containing up to 10 atoms therein, with alternating (resonating) double bonds between adjacent carbon atoms. The preferred aryl groups are phenyl and naphthyl. Aryl groups may likewise be substituted with 1-3 groups such as alkyl, halo, carboxyalkyl, alkylamino, dialkylamino, alkoxy, alkoxyamino and the like.

The term heteroaryl refers to a monocyclic aromatic hydrocarbon group having 5 or 6 ring atoms, or a bicyclic aromatic group having 8 to 10 atoms, containing at least one heteroatom, O, S, or N, in which a carbon or nitrogen atom is the point of attachment, and in which one additional carbon atom is optionally replaced by a heteroatom selected from O or S, and in which from 1 to 3 additional carbon atoms are optionally replaced by nitrogen heteroatoms. The heteroaryl group is optionally substituted with up to three groups. Heteroaryl thus includes aromatic and partially aromatic groups which contain one or more heteroatoms. Examples of this type are pyrrol, pyridine, oxazole, thiazole and oxazine. Additional nitrogen atoms may be present together with the first nitrogen and oxygen or sulfur, giving e.g., thiadiazole.

A preferred embodiment of the novel compounds of this invention is realized when:

R₂ - R₉ are independently H, alkyl, or halo;

Y₁ is H, alkyl, or halo;

Y₂ is 5-pyrimidinyl, 3-pyridyl, or 1-methyl-5-imidazolyl;

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R₁ is alkyl, alkoxy, phenyl, 5-pyrimidinyl, 3-pyridyl, or 1-methyl-5-imidazolyl; and

X₁ is N.

The pharmaceutically acceptable salts of the compounds of formula I include the conventional non-toxic salts or the quarternary ammonium salts of the compounds of formula I formed e.g. from non-toxic inorganic or organic acids. For example, such conventional non-toxic salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric and the like; and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pamoic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isethionic, and the like.

The pharmaceutically acceptable salts of the present invention can be synthesized from the compounds of formula I which contain a basic or acidic moiety by conventional chemical methods. Generally, the salts are prepared by reacting the free base or acid with stoichiometric amounts or with an excess of the desired salt-forming inorganic or organic acid or base in a suitable solvent or various combinations of solvents.

The compounds of the present invention may have asymmetric centers and occur as racemates, racemic mixtures, and as individual diastereomers, with all possible isomers, including optical isomers, being included in the present invention.

Examples of the novel compounds of this invention are as follows:

(3''S,4''R,1'''R and 3''R,4''S,1'''R) 1,3-Dihydro-1-{1'-[1''-(1'''-(5''''-pyrimidinyl)-1'''-ethyl)-3''-methylpiperidin-4''-yl]-piperidin-4'-yl}-2H-benzimidazol-2-one;

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(3'R,4'R,1'''R and 3'S,4'S,1'''R) 1,3-Dihydro-1-{1'-[1''-(1'''-(5'''-pyrimidinyl)-1'''-ethyl)-piperidin-4''-yl]-3'-methylpiperidin-4'-yl} -2H-benzimidazol-2-one;

(3'S,4'R,1'''R and 3'R,4'S,1'''R) 1,3-Dihydro-1-{1'-[1''-(1'''-(5'''-pyrimidinyl)-1'''-ethyl)-piperidin-4''-yl]-3'-methylpiperidin-4'-yl} -2H-benzimidazol-2-one;

(3'R,4'R,1'''R and 3'S,4'S,1'''R) 1,3-Dihydro-1-{1'-[1''-(1'''-(5'''-pyrimidinyl)-1'''-ethyl)-piperidin-4''-yl]-3'-ethoxycarbonylpiperidin-4'-yl} -2H-benzimidazol-2-one;

(3'S,4'R,1'''R and 3'R,4'S,1'''R) 1,3-Dihydro-1-{1'-[1''-(1'''-(5'''-pyrimidinyl)-1'''-ethyl)-piperidin-4''-yl]-3'-ethoxycarbonylpiperidin-4'-yl} -2H-benzimidazol-2-one;

(2''R,4''S,1'''R and 2''R,4''R,1'''R) 1,3-Dihydro-1-{1'-[1''-(1'''-(5'''-pyrimidinyl)-1'''-ethyl)-2''-methyl-piperidin-4''-yl]-piperidin-4'-yl} -2H-benzimidazol-2-one;

(2''S,4''S,1'''R) 1,3-Dihydro-1-{1'-[1''-(1'''-(5'''-pyrimidinyl)-1'''-ethyl)-2''-methyl-piperidin-4''-yl]-piperidin-4'-yl} -2H-benzimidazol-2-one;

(2''S,4''R,1'''R) 1,3-Dihydro-1-{1'-[1''-(1'''-(5'''-pyrimidinyl)-1'''-ethyl)-2''-methyl-piperidin-4''-yl]-piperidin-4'-yl} -2H-benzimidazol-2-one;

(3'S,4'R,1'''S and 3'R,4'S,1'''S) 1,3-Dihydro-1-{1'-[1''-(1'''-phenylethyl)-piperidin-4''-yl]-3'-ethoxycarbonylpiperidin-4'-yl} -2H-benzimidazol-2-one;

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(3''S,4''R,1'''R) 1,3-Dihydro-1-{1'-[1''-(1'''-(5''''-pyrimidinyl)-1'''-ethyl)-3''-methylpiperidin-4''-yl]-piperidin-4'-yl} -2H-benzimidazol-2-one;

(3''R,4''S,1'''R) 1,3-Dihydro-1-{1'-[1''-(1'''-(5''''-pyrimidinyl)-1'''-ethyl)-3''-methylpiperidin-4''-yl]-piperidin-4'-yl} -2H-benzimidazol-2-one;

1,3-Dihydro-1-{1'-[1''-(3'''-pyridinecarbonyl)-2''-methyl-piperidin-4''-yl]-piperidin-4'-yl} -2H-benzimidazol-2-one;

(1'''R) 1,3-dihydro-1-{1'-[8''-(1'''-(5''''-pyrimidinyl)-1'''-ethyl)piperidin-4''-yl]-8-azabicyclo[3.2.1]octan-3 α -yl} -2H-benzimidazol-2-one;

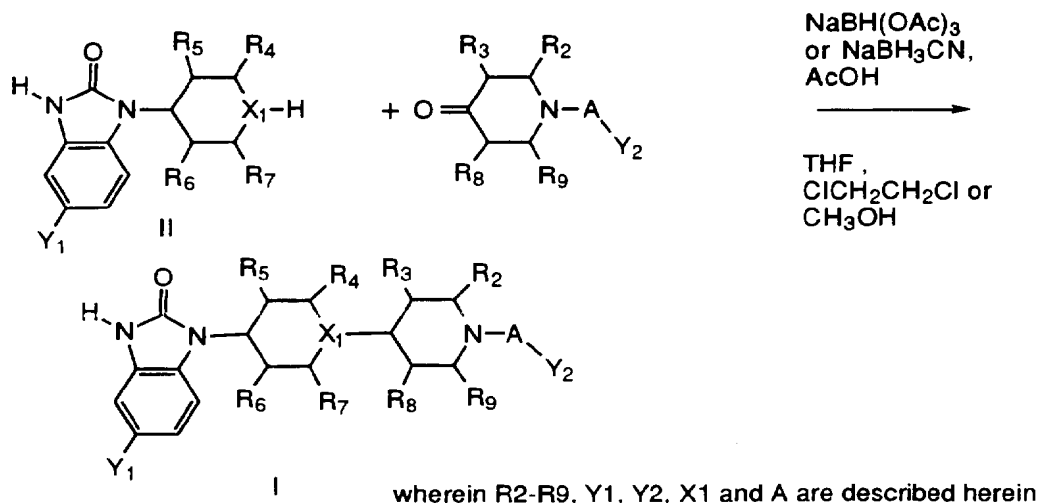
(1'''R) 1,3-dihydro-1-{1'-[8''-(1'''-(5''''-pyrimidinyl)-1'''-ethyl)piperidin-4''-yl]-8-azabicyclo[3.2.1]octan-3 β -yl} -2H-benzimidazol-2-one;

1,3-Dihydro-1-{1'-[1''-(1'''-methylimidazol-5'''-carbonyl)-piperidin-4''-yl]-piperidin-4'-yl} -2H-benzimidazol-2-one; and

1,3-Dihydro-1-{1'-[1''-(1'''-methylimidazol-5'''-ylcarbonyl)-2''-methylpiperidin-4''-yl]-piperidin-4'-yl} -2H-benzimidazol-2-one.

The novel compounds of this invention are prepared by the following non-limiting procedures:

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The reaction is preferably carried out at room temperature (20-30°C) at a pH in the range of 2-7 (acidic) by the addition of glacial acetic acid or hydrochloric acid. A suitably protected 4-piperidone such as A-Y₂ = CO₂Et, CO₂CH₂Ph, or CO₂C(CH₃)₃ can be used as an intermediate. Deprotection by the usual methods (hydrogenation or acidic hydrolysis followed by basification) provides the free amine compound which can be acylated or alkylated by standard procedures. By this route the most preferred compounds can be obtained after isolation and purification.

The starting materials are either commercially available or can be obtained by conventional procedures such as those described in the Examples section.

The selectivity of the compounds can be measured by radioligand displacement from m₁-m₅ receptors expressed in chinese hamster ovary cells (CHO) as described in the Examples section. The functional activity of the compounds can be assessed by measuring the agonist induced contractile response on muscle tissue from rabbit vas deferens (M₁), the guinea pig left atria (M₂), or the guinea pig ileum (M₃) as described in the Examples section. The functional activity at the human muscarinic receptors can be assessed by measuring agonist induced phosphoinositide hydrolysis in CHO cells expressing the human m₁ and m₃ receptors or agonist

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inhibition of forskolin-stimulated adenylate cyclase activity in CHO cells expressing the human m2 receptor as described in the Examples section.

The instant compounds of this invention are useful in treating and/or preventing the development of myopia. Therapy to inhibit axial-elongation myopia during maturation can be administered by the use of the agent in eye drops. Indeed, in the vast majority of cases, treatment agents are administered to human eyes by the application of eye drops. Eye drops are typically made up at a concentration of active agent between about 0.1 and 4% in the ophthalmic medium. A 0.5%-2% solution of the instant muscarinic antagonist in water would be a likely concentration for clinical use. A pH of about 4.5 to about 7.5 is expected to be acceptable as an ophthalmic drop and practical in terms of known solubility and stability of piperidines. Phosphate buffering is also common for eye drops and is compatible with the instant muscarinic antagonist. A common regimen for application of eye drops is one to three times a day spaced evenly throughout waking hours. More effective agents may require fewer applications or enable the use of more dilute solutions. Alternatively, ointments and solid inserts are now coming into increased use in clinical practice. These aid the ocular penetration of the drug. It is, of course, also possible to administer the above-described active agents in therapeutically effective amounts and dosages in pills, capsules, or other preparations of systemic administration.

In experiments in animals where axial myopia has been experimentally induced by depriving the retina of formed images, it has been noted that amblyopia was also experimentally and coincidentally induced in primates. Amblyopia is evidenced by poor visual acuity in the eye resulting in poor visual performance. Normally, visual acuity improves during maturation. It is known that amblyopia may occur in humans from unknown causes or as part of strabismus. Accordingly, it is expected that administration of

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therapeutically effective amounts and dosages of the instant muscarinic antagonist might prevent or inhibit the development of permanent or persistent amblyopia in maturing humans with decreased likelihood of sensitization of the eye. It is also expected that humans who have already developed amblyopia from other or even unknown causes might be aided by similar therapeutic treatment with the aforementioned agents.

The following examples are provided in order that this invention might be more fully understood; they are not to be construed as limitative of the invention. The compounds are characterized analytically using techniques such as nuclear magnetic resonance, mass spectrometry, chromatography and the like.

EXAMPLE 1

1-(1S-(5-Pyrimidinyl)-ethyl)-4-oxopiperidine

To a stirred mixture of 2.3 g of (S)-(-)- 5-(1-aminoethyl)pyrimidine (O. Cervinska and P. Malon, *Coll. Czechoslov. Chem. Commun.* **1977**, 42 , 3464-72.), 20 mL of ethanol and 2.0 g of K₂CO₃ heated to reflux was added dropwise over 30 min, a solution of 6.2 g of 1-methyl-1-ethyl-4-oxopiperidinium iodide in 110 mL of water. When the addition was complete, the mixture was heated under reflux for an additional 1 h, cooled, basified to pH 9 with K₂CO₃ and extracted with 5 times with 50 mL portions of chloroform. The combined organic extracts were dried over MgSO₄ and concentrated under reduced pressure. Chromatography over silica gel, eluting with 1:1 tetrahydrofuran:ethyl acetate gave 3.8 g of 1-(1S-(5-pyrimidinyl)-ethyl)-4-oxopiperidine as an oil: ¹H NMR (400 MHz, CDCl₃) 9.14 (s, 1H), 8.78 (s, 2H), 3.8 (q, J = 7 Hz, 1H), 2.7-2.9 (m, 4H), 2.45 (m, 4H), 1.5 (d, J = 7 Hz, 3H).

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EXAMPLE 2

1-(1R-(5-Pyrimidinyl)-ethyl)-4-oxopiperidine

To a stirred mixture of 2.3 g of (R)-(+)- 5-(1-aminoethyl)pyrimidine (O. Cervinska and P. Malon, *Coll. Czechoslov. Chem. Commun.* **1977**, 42 , 3464-72.), 20 mL of ethanol and 2.0 g of K₂CO₃ heated to reflux was added dropwise over 30 min, a solution of 6.2 g of 1-methyl-1-ethyl-4-oxopiperidinium iodide in 110 mL of water. When the addition was complete, the mixture was heated under reflux for an additional 1 h, cooled, basified to pH 9 with K₂CO₃ and extracted 5 times with 50 mL portions of chloroform. The combined organic extracts were dried over MgSO₄ and concentrated under reduced pressure. Chromatography over silica gel, eluting with 1:1 tetrahydrofuran:ethyl acetate gave 3.8 g of 1-(1R-(5-pyrimidinyl)-ethyl)-4-oxopiperidine as an oil: ¹H NMR (400 MHz, CDCl₃) 9.14 (s, 1H), 8.78 (s, 2H), 3.8 (q, J = 7 Hz, 1H), 2.7-2.9 (m, 4H), 2.45 (m, 4H), 1.5 (d, J = 7 Hz, 3H).

EXAMPLE 3

1-(1R-(5-Pyrimidinyl)-ethyl)-3-methyl-4-oxopiperidine

Step 1: A mixture of 10 g of 1-benzyl-3-methyl-4-oxopiperidine and 8 mL of methyl iodide in 100 mL of acetone was stirred at 25 to 30°C for 6 days then concentrated under reduced pressure to dryness. Drying under vacuum overnight gave 17 g of 1-methyl-1-benzyl-3-methyl-4-oxopiperidinium iodide as a beige foam.

Step 2: To a stirred mixture of 0.9 g of (R)-(+)- 5-(1-aminoethyl)pyrimidine (O. Cervinska and P. Malon, *Coll. Czechoslov. Chem. Commun.* **1977**, 42 , 3464-72.), 35 mL of ethanol and 1 g of K₂CO₃ heated to reflux was added dropwise over 30 min, a solution of 4 g of 1-methyl-1-benzyl-3-methyl-4-oxopiperidinium iodide in 60 mL of water. When the addition was complete, the mixture was heated

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under reflux for an additional 1 h, cooled, and extracted 3 times with 100 mL portions of chloroform. The combined organic extracts were dried over MgSO₄ and concentrated under reduced pressure.

Chromatography over silica gel, eluting with 90:10 ethyl acetate:tetrahydrofuran gave 1.45 g of 1-(1R-(5-pyrimidinyl)-ethyl)-3-methyl-4-oxopiperidine as an oil: ¹H NMR (400 MHz, CDCl₃) 9.15 (s, 1H), 8.78 (s, 2H), 3.8 (m, 1H), 3.0-3.2 (m, 3H), 2.5 (m, 1H), 2.4 (m, 2H), 2.2 (m, 1H), 1.65 and 1.45 (2 doublets, J = 7 Hz, 3H), 1.2 and 0.98 (2 doublets, J = 7 Hz, 3H).

EXAMPLE 4

trans -1-(3-Methyl-4-piperidinyl)benzimidazol-2H-one

Step 1: A mixture of 1 g of 1-benzyl-3-methyl-4-oxopiperidine, 1 g of 1,2-phenylenediamine, 10 mL of methanol, 0.32 g of sodium cyanoborohydride and 2 mL of glacial acetic acid was stirred at room temperature for 12 h, basified by addition of 1 mL of 6N sodium hydroxide and concentrated to near dryness under reduced pressure. The residue was partitioned between 100 mL of ethyl acetate and 10 mL of saturated aqueous sodium carbonate and the organic layer dried over MgSO₄ and concentrated under reduced pressure. The residue was dissolved in 25 mL of ethyl acetate and stirred with 10 mL of saturated aqueous sodium carbonate in an ice bath while a solution of 10 mL of 1.93 M phosgene in toluene was added. The resulting mixture was allowed to warm to room temperature and stirred for 4 h, diluted with 250 mL of ethyl acetate and filtered to remove 2-hydroxybenzimidazole byproduct. The layers were separated and the organic layer dried over MgSO₄ and concentrated under reduced pressure. Chromatography over silica gel, eluting with 80:20 ethyl acetate:hexane gave first 0.75 g of *cis* - 1-(1-benzyl-3-methyl-4-piperidinyl) benzimidazol-2H-one as a white solid: ¹H NMR (400 MHz, CDCl₃) 9.65 (s, 1H), 7.4-7.0 (m, 9H), 4.4 (m, 1H), 3.5 (dd, 2H), 3.1 (m, 2H), 2.8 (d, 1H), 2.38 (d, 1H), 2.3 (br s, 1H), 2.18 (br t, 1H), 1.7 (br m, 1H), 1.2 (d, 3H); followed by

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0.25 g of *trans* - 1-(1-benzyl-3-methyl-4-piperidiny) benzimidazol-2H-one as a white solid in the later fractions: ^1H NMR (400 MHz, CDCl_3) 11 (s, 1H), 7.4-7.0 (m, 9H), 4.1 (br m, 2H), 3.6 (s, 2H), 3.1 (m, 2H), 2.6 (br m, 2H), 2.3 (br t, 1H), 1.95 (br t, 1H), 1.85 (br d, 1H), 0.76 (d, 3H).

Step 2: A mixture of 200 mg of *trans* - 1-(1-benzyl-3-methyl-4-piperidiny) benzimidazol-2H-one, 100 mL of absolute methanol, 0.5 mL of 2N ethanolic HCl and 100 mg of 20% palladium hydroxide on carbon was shaken under 55 psi of hydrogen for 3 days, then filtered and concentrated to dryness under reduced pressure. The residue was partitioned between 5 x 50 mL portions of chloroform and 5 mL of 2N sodium hydroxide. The combined organic extracts were dried over MgSO_4 and concentrated under reduced pressure. Trituration with 10 mL of ethyl acetate gave 150 mg of *trans* - 1-(3-methyl-4-piperidiny) benzimidazol-2H-one as a white solid. ^1H NMR (400 MHz, CDCl_3) 9.2 (br s, 1H), 7.3 (m, 1H), 7.1 (m, 3H), 4.1 (br m, 1H), 3.3 (m, 2H), 2.9 (t, 1H), 2.5 (m, 1H), 2.4 (m, 1H), 1.7-1.9 (br m, 2H), 1.8 (d, 3H).

EXAMPLE 5

(3'R,4'R,1'''R and 3'S,4'S,1'''R) 1,3-Dihydro-1-{1'-[1''-(1'''-(5'''-pyrimidinyl)-1'''-ethyl)-piperidin-4''-yl]-3'-methylpiperidin-4''-yl}-2H-benzimidazol-2-one

A mixture of 0.20 g of 1-(1R-(5-pyrimidinyl)-ethyl)-4-oxopiperidine, 0.15 g of *trans*-1-(3-methyl-4-piperidiny)benzimidazol-2H-one, 10 mL of 1,2-dichloroethane, 0.3 mL of glacial acetic acid and 0.5 g of sodium triacetoxyborohydride was stirred at room temperature for 48 h. The reaction mixture was poured into 100 mL chloroform and 25 mL saturated aqueous Na_2CO_3 and the layers separated. The aqueous layer was extracted with 2 X 25 mL of chloroform and the combined organic layers dried over MgSO_4 and concentrated under reduced pressure. Purification of the residue by preparative thin chromatography on silica gel eluting with 430:50:20 CHCl_3 :MeOH:conc. NH_4OH followed by

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trituration with 10 mL of ethyl acetate gave 90 mg of (3'R,4'R,1'''R and 3'S,4'S,1'''R) 1,3-dihydro-1-{1'-[1''-(1'''-(5'''-pyrimidinyl)-1'''-ethyl)-piperidin-4''-yl]-3'-methylpiperidin-4'-yl} -2H-benzimidazol-2-one as a white solid: ¹H NMR (400 MHz, CDCl₃) 10.9 (s, 1H), 9.15 (s, 1H), 8.7 (s, 2H), 7.26 (br m, 1H), 7.0-7.1 (br m, 3H), 4.05 (br m, 1H), 3.6 (q, J = 7 Hz, 1H), 3.0-3.2 (m, 3H), 2.7 (m, 1H), 2.6-2.3 (m, 4H), 2.1-2.0 (m, 3H), 1.9-1.8 (br m, 3H), 1.6 (br m, 2H), 1.4 (d, J = 7 Hz, 3H), 0.76 (d, J = 7 Hz, 3H). The citrate salt crystallized from ethyl acetate/methanol: Analysis calculated for C₂₄H₃₂N₆O•1.0 H₂O•1.5 C₆H₈O₇: C: 54.53, H: 6.38, N: 11.56; found C: 54.85, H: 6.38, N: 11.56.

EXAMPLE 6

(3''S,4''R,1'''R) 1,3-Dihydro-1-{1'-[1''-(1'''-(5'''-pyrimidinyl)-1'''-ethyl)-3''-methylpiperidin-4''-yl]-piperidin-4'-yl} -2H-benzimidazol-2-one and (3''R,4''S,1'''R) 1,3-dihydro-1-{1'-[1''-(1'''-(5'''-pyrimidinyl)-1'''-ethyl)-3''-methylpiperidin-4''-yl]-piperidin-4'-yl} -2H-benzimidazol-2-one

A mixture of 1.2 g of 1-(1R-(5-pyrimidinyl)-ethyl)-3-methyl-4-oxopiperidine, 2.0 g of 1-(4-piperidinyl)benzimidazol-2H-one, 40 mL of 1,2-dichloroethane, 0.6 mL of glacial acetic acid, 2 g of powdered activated 4Å molecular sieves was stirred at 80°C for 30 min, then at 40°C for 1 h, and cool to room temperature over 1 h. To this mixture was added 3.0 g of sodium triacetoxyborohydride. Stirring was continued at room temperature for 48 h. The reaction mixture was poured into 100 mL chloroform and 25 mL saturated aqueous Na₂CO₃ and the layers separated. The aqueous layer was extracted with 2 X 100 mL of chloroform and the combined organic layers dried over MgSO₄ and concentrated under reduced pressure. Purification of the residue by low pressure chromatography eluting with 85:15 CH₂Cl₂:MeOH, followed by preparative thin chromatography on silica gel eluting with 210:40:15 CHCl₃:MeOH: conc. NH₄OH followed by trituration with 10

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mL of ethyl acetate gave 540 mg of (3''S,4''R,1'''R and 3''R,4''S,1'''R) 1,3-dihydro-1-{1'-[1''-(1'''-(5''''-pyrimidinyl)-1'''-ethyl)-3''-methylpiperidin-4''-yl]-piperidin-4'-yl} -2H-benzimidazol-2-one as a gum: ¹H NMR (400 Mhz, CDCl₃) 9.4 (s, 1H), 9.1 (s, 1H), 8.71 (2 singlets, 2H), 7.3 (br m, 1H), 7.05 (br m, 3H), 4.4 (br m, 1H), 3.52 and 3.45 (2 quartets, J = 7 Hz, 1H), 3.3-3.2 (m, 2H), 3.0 (m, 1H), 2.8-2.7 (m, 1H), 2.5-2.3 (m, 3H), 2.2-1.9 (br m, 5H), 1.8 (br m, 3H), 1.8-1.5 (br m, 1H), 1.35 (t, 3H), 1.06 (t, 3H). The citrate salt precipitated from ethyl acetate/methanol: Analysis calculated for C₂₄H₃₂N₆O•0.5 H₂O•1.5 C₆H₈O₇: C: 55.22, H: 6.32, N: 11.71; found C: 55.45, H: 6.44, N: 11.77.

Preparative HPLC on C-18 column (Deltapak, C-18 eluting with a gradient of 0:100 to 100:0 of acetonitrile: 0.1% ammonium bicarbonate gave first 0.20 g of (3''R,4''S,1'''R) 1,3-dihydro-1-{1'-[1''-(1'''-(5''''-pyrimidinyl)-1'''-ethyl)-3''-methylpiperidin-4''-yl]-piperidin-4'-yl} -2H-benzimidazol-2-one: [α]_D = + 23.5° (c = 0.575, MeOH); mp 116-118°C; ¹H NMR (400 Mhz, CDCl₃) 9.15 (s, 1H), 9.05 (br s, 1H), 8.7 (s, 2H), 7.3 (br m, 1H), 7.05 (br m, 3H), 4.4 (br m, 1H), 3.45 (q, 1H), 3.25 (m, 2H), 3.0 (m, 1H), 2.6 (d, 1H), 2.5 (m, 2H), 2.2-1.9 (br m, 5H), 1.85 (br m, 3H), 1.65 (br m, 2H), 1.38 (d, 3H), 1.07 (d, 3H); followed by (3''R,4''S,1'''R) 1,3-dihydro-1-{1'-[1''-(1'''-(5''''-pyrimidinyl)-1'''-ethyl)-3''-methylpiperidin-4''-yl]-piperidin-4'-yl} -2H-benzimidazol-2-one: [α]_D = - 26.0° (c = 0.575, MeOH); mp 113-115°C; ¹H NMR (400 Mhz, CDCl₃) 9.15 (s, 1H), 8.73 (s, 2H), 8.62 (br s, 1H), 7.3 (br m, 1H), 7.05 (br m, 3H), 4.4 (br m, 1H), 3.55 (q, 1H), 3.25 (m, 2H), 2.8 (m, 1H), 2.4 (m, 2H), 2.2-1.9 (br m, 6H), 1.85 (br d, 1H), 1.65 (br m, 4H), 1.36 (d, 3H), 1.08 (d, 3H).

EXAMPLE 7

(3'S,4'R,1'''R and 3'R,4'S,1'''R) 1,3-Dihydro-1-{1'-[1''-(1'''-(5''''-pyrimidinyl)-1'''-ethyl)-piperidin-4''-yl]-3'-methylpiperidin-4'-yl} -2H-benzimidazol-2-one

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Step 1: A mixture of 660 mg of *cis* - 1-(1-benzyl-3-methyl-4-piperidiny) benzimidazol-2H-one (a product of Step 1, Example 4), 100 mL of absolute methanol, 2 mL of 2N ethanolic HCl and 400 mg of 20% palladium hydroxide on carbon was shaken under 55 psi of hydrogen for 3 days, then filtered and concentrated to dryness under reduced pressure. The residue was partitioned between 5 x 50 mL portions of chloroform and 5 mL of 2N sodium hydroxide. The combined organic extracts were dried over MgSO₄ and concentrated under reduced pressure. Trituration with 10 mL of ethyl acetate gave 500 mg of *cis* - 1-(3-methyl-4-piperidiny) benzimidazol-2H-one as a white solid. ¹H NMR (400 MHz, CDCl₃) 9.2 (br s, 1H), 7.2 (m, 1H), 7.05 (m, 3H), 4.48 (dt, 1H), 3.3 (d, 2H), 3.06 (dd, 1H), 3.0-2.85 (complex m, 2H), 2.78 (dt, 1H), 1.8 (br dd, 1H), 1.68 (br, 1H), 1.12 (d, 3H).

Step 2: A mixture of 0.25 g of 1-(1R-(5-pyrimidinyl)-ethyl)-4-oxopiperidine, 0.20 g of *cis*-1-(3-methyl-4-piperidiny)benzimidazol-2H-one, 10 mL of 1,2-dichloroethane, 0.3 mL of glacial acetic acid and 0.5 g of sodium triacetoxyborohydride was stirred at room temperature for 48 h. The reaction mixture was poured into 100 mL chloroform and 25 mL saturated aqueous Na₂CO₃ and the layers separated. The aqueous layer was extracted with 2 X 25 mL of chloroform and the combined organic layers dried over MgSO₄ and concentrated under reduced pressure. Purification of the residue by preparative thin chromatography on silica gel eluting with 430:50:20

CHCl₃:MeOH:conc. NH₄OH followed by trituration with 10 mL of ethyl acetate gave 450 mg of (3'S,4'R,1'''R and 3'R,4'S,1'''R) 1,3-dihydro-1-{1'-[1''-(1'''-(5'''-pyrimidinyl)-1'''-ethyl)-piperidin-4''-yl]-3'-methylpiperidin-4'-yl} -2H-benzimidazol-2-one as a white solid: ¹H NMR (400 MHz, CDCl₃) 10.7 (br s, 1H), 9.15 (s, 1H), 8.7 (s, 2H), 7.2 (m, 1H), 7.1 (m, 2H), 7.0 (m, 2H), 4.45 (dt, 1H), 3.58 (q, J = 7 Hz, 1H), 3.1-2.75 (m, 5H), 2.6 (d, 1H), 2.4-2.2 (m, 4H), 2.0 (m, 2H), 1.85-1.5 (br m, 4H), 1.4 (d, J = 7 Hz, 3H), 1.1 (d, J = 7 Hz, 3H). The citrate salt crystallized from ethyl acetate/methanol: Analysis calculated for

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C₂₄H₃₂N₆O•1.0 CH₃CO₂CH₂CH₃•1.3 C₆H₈O₇: C: 56.69, H: 6.70, N: 11.08; found C: 56.40, H: 6.77, N: 11.35.

EXAMPLE 8

(±)-2-Methyl-4-oxo-1-propyl piperidine

Step 1: A mixture of 6 g of methyl acetoacetate, 5 mL of 1-propylamine, 40 mL of 1,2-dichloroethane, 3 mL of glacial acetic acid and 16 g of sodium triacetoxyborohydride was stirred at room temperature for 4 days. The reaction mixture was poured into 200 mL chloroform and 50 mL saturated aqueous Na₂CO₃ and the layers separated. The aqueous layer was extracted with 200 mL of chloroform and the combined organic layers dried over MgSO₄ and concentrated under reduced pressure. After drying under vacuum, 9 g of (±)-methyl 2-(1-propylamino)butyrate was obtained as an oil: TLC (EtOAc); ¹H NMR (400 MHz, CDCl₃) 6.2 (br s, 1H), 3.65 (s, 3H), 3.2 (complex m, 2H), 2.65 (complex m, 2H), 2.45 (dd, 1H), 1.55 (m, 2H), 1.4 (d, 3H), 0.95 (t, 3H).

Step 2: A mixture of 9 g of (±)-methyl 2-(1-propylamino)butyrate, 100 mL of anhydrous methanol and 10 mL of methyl acrylate was kept at room temperature in a stoppered flask for 3 days. The mixture was then concentrated under reduced pressure and purified by evaporative distillation. The colorless distillate, 10.5 g, obtained at oven temperature 90-95°C, 1mm, was (±)-methyl 2-(2-methoxycarbonylethyl(1-propyl)amino)butyrate: TLC (EtOAc); ¹H NMR (400 MHz, CDCl₃) 3.66 (s, 3H), 3.64 (s, 3H), 2.8-2.2 (complex m, 6H), 1.45 (complex m, 2H), 1.0 (d, 3H), 0.85 (t, 3H).

Step 3: To a stirred solution of 162 mL of 0.5 M potassium bistrimethylsilylamide in toluene and 600 mL of anhydrous tetrahydrofuran cooled to -78°C under nitrogen atmosphere was added dropwise a solution of 7.5 g of (±)-methyl 2-(2-methoxycarbonylethyl(1-propyl)amino)butyrate in 50 mL of dry tetrahydrofuran over 20 min, keeping the internal temperature under

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-70°C. The mixture was allowed to warm to -20°C for 2 h, then quenched with 100 mL of saturated aqueous sodium bicarbonate and extracted into 3 x 200 mL portions of ethyl acetate. The combined extracts were dried over MgSO₄ and concentrated under reduced pressure. There was obtained 6.6 g of predominantly (±)-*trans* -5-carbomethoxy-2-methyl-4-oxo-1-propyl piperidine as an oil: TLC (EtOAc); ¹H NMR (400 MHz, CDCl₃) 3.76 (s, 3H), 3.2 (m, 1H), 2.9 (m, 1H), 2.62-2.0 (complex m, 6H), 1.5 (m, 2H), 1.25 (d, 3H), 0.5 (dd, 3H).

Step 4: A solution of 6.5 g of predominantly (±)-*trans* -5-carbomethoxy-2-methyl-4-oxo-1-propyl piperidine and 30 mL of 20% hydrochloric acid was heated to reflux for 2 h. The solution was cooled in an ice bath, basified by addition of NaOH pellets until the pH was 9-10 and extracted with 5 x 100 mL of chloroform. The combined organic extracts were dried over MgSO₄ and concentrated under reduced pressure. After azeotropic drying with toluene there was obtained 5.6 g of predominantly (±)-2-methyl-4-oxo-1-propyl piperidine as an amber oil: TLC (EtOAc); ¹H NMR (400 MHz, CDCl₃) 3.1 (m, 1H), 2.9 (m, 1H), 2.8-2.6 (complex m, 3H), 2.5 (m, 4H), 1.5 (m, 2H), 1.15 (d, 3H), 0.9 (t, 3H).

EXAMPLE 9

1-(1R-(5-Pyrimidinyl)-ethyl)-2R-methyl-4-oxopiperidine and 1-(1R-(5-Pyrimidinyl)-ethyl)-2S-methyl-4-oxopiperidine

Step 1: A mixture of 5.6 g of predominantly (±)-2-methyl-4-oxo-1-propyl piperidine, 250 mL of acetone and 5 mL of iodomethane was stirred at room temperature for 48 h. The mixture was filtered and concentrated under reduced pressure. After drying under vacuum there was obtained 6 g of (±)-2-methyl-4-oxo-1-methyl-1-propyl-piperidinium iodide as a brown gum (mixture of diastereomers).

Step 2: To a stirred mixture of 0.25 g of (R)-(+)-5-(1-aminoethyl)pyrimidine (O. Cervinska and P. Malon, *Coll. Czechoslov.*

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Chem. Commun. **1977**, 42, 3464-72.), 10 mL of ethanol and 0.3 g of K₂CO₃ heated to reflux was added dropwise over 30 min, a solution of 1.2 g of (±)-2-methyl-4-oxo-1-methyl-1-propyl-piperidinium iodide in 25 mL of water. When the addition was complete, the mixture was heated under reflux for an additional 1 h, cooled, and extracted 3 times with 100 mL portions of chloroform. The combined organic extracts were dried over MgSO₄ and concentrated under reduced pressure. Chromatography over silica gel, eluting with a gradient of 95:5 to 80:20 ethyl acetate:tetrahydrofuran gave first 150 mg of 1-(1R-(5-pyrimidinyl)-ethyl)-2R-methyl-4-oxopiperidine as an oil: ¹H NMR (400 MHz, CDCl₃) 9.18 (s, 1H), 8.8 (s, 2H), 4.2 (dd, 1H), 3.3 (dd, 1H), 3.2 (dd, 2H), 2.7 (m, 1H), 2.6 (m, 1H), 2.3 (m, 1H), 1.4 (d, 3H) and 1.2 (d, 3H); followed by 200 mg of 1-(1R-(5-pyrimidinyl)-ethyl)-2S-methyl-4-oxopiperidine as an oil: ¹H NMR (400 MHz, CDCl₃) 9.18 (s, 1H), 8.78 (s, 2H), 4.2-4.0 (complex m, 3H), 3.08-2.9 (complex m, 3H), 2.6 (m, 1H), 2.4-2.2 (m, 1H), 1.5 (d, 3H) and 1.1 (d, 3H).

EXAMPLE 10

(2''R, 4''S, 1'''R and 2''R, 4''R, 1'''R) 1,3-Dihydro-1-{1'-[1''-(1'''-(5'''-pyrimidinyl)-1'''-ethyl)-2''-methylpiperidin-4''-yl]-piperidin-4'-yl}-2H-benzimidazol-2-one

A mixture of 0.20 g of 1-(1R-(5-pyrimidinyl)-ethyl)-2R-methyl-4-oxopiperidine, 0.450 g of 1-(4-piperidinyl)benzimidazol-2H-one, 8 mL of 1,2-dichloroethane, 0.2 mL of glacial acetic acid and 0.40 g of sodium triacetoxyborohydride was stirred at room temperature for 48 h. The reaction mixture was poured into 100 mL chloroform and 25 mL saturated aqueous Na₂CO₃ and the layers separated. The aqueous layer was extracted with 2 X 25 mL of chloroform and the combined organic layers dried over MgSO₄ and concentrated under reduced pressure. Purification of the residue by preparative thin chromatography on silica gel eluting with 80:20 CH₂Cl₂:MeOH followed by trituration with 10 mL of ethyl acetate gave 120 mg of (2''R

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,4"S,1"R and 2"R,4"R,1"R) 1,3-dihydro-1-{1'-[1"-(1"'-(5""-pyrimidinyl)-1"'-ethyl)-2"-methylpiperidin-4"-yl]-piperidin-4'-yl} -2H-benzimidazol-2-one as a gum: ¹H NMR (400 MHz, CDCl₃) 10.6 (br s, 1H), 9.5 and 9.06 (2 s, 1H), 8.8 and 8.7 (2 s, 2H), 7.3 (br m, 1H), 7.05 (br m, 3H), 4.4 (br m, 1H), 3.6 and 3.5 (2 quartets, 1H), 3.1 (m, 1H), 2.7 (m, 1H), 2.45 (m, 4H), 2.2 (m, 1H), 1.8 (br m, 5H), 1.25 (br m, 3H), 1.8-1.5 (complex m, 7H). The citrate salt precipitated from ethyl acetate/methanol: Analysis calculated for C₂₄H₃₂N₆O•1.0 CH₃CO₂CH₂CH₃•1.2 C₆H₈O₇: C: 57.19, H: 6.76, N: 11.37; found C: 57.21, H: 6.93, N: 11.64.

EXAMPLE 11

(2"S,4"S,1"R) 1,3-Dihydro-1-{1'-[1"-(1"'-(5""-pyrimidinyl)-1"'-ethyl)-2"-methyl-piperidin-4"-yl]-piperidin-4'-yl} -2H-benzimidazol-2-one
and (2"S,4"R,1"R) 1,3-dihydro-1-{1'-[1"-(1"'-(5""-pyrimidinyl)-1"'-ethyl)-2"-methyl-piperidin-4"-yl]-piperidin-4'-yl} -2H-benzimidazol-2-one

A mixture of 0.20 g of 1-(1R-(5-pyrimidinyl)-ethyl)-2R-methyl-4-oxopiperidine, 0.450 g of 1-(4-piperidinyl)benzimidazol-2H-one, 8 mL of 1,2-dichloroethane, 0.2 mL of glacial acetic acid and 0.40 g of sodium triacetoxyborohydride was stirred at room temperature for 48 h. The reaction mixture was poured into 100 mL chloroform and 25 mL saturated aqueous Na₂CO₃ and the layers separated. The aqueous layer was extracted with 2 X 25 mL of chloroform and the combined organic layers dried over MgSO₄ and concentrated under reduced pressure. Purification of the residue by preparative thin chromatography on silica gel eluting with 425:50:25 CHCl₃:MeOH:NH₄OH gave product contaminated with 1-(1R-(5-pyrimidinyl)-ethyl)-2R-methyl-4-hydroxypiperidine formed by reduction of the piperidone. Further purification by preparative thin chromatography on silica gel eluting twice with 375:100:25 tetrahydrofuran: CH₂Cl₂:MeOH gave the pure product diastereomers in two bands. The upper band gave 20 mg of (2"S,4"S,1"R) 1,3-dihydro-

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1-{1'-[1''-(1'''-(5''''-pyrimidinyl)-1'''-ethyl)-2''-methyl-piperidin-4''-yl]-piperidin-4'-yl}-2H-benzimidazol-2-one after trituration with 10 mL of ethyl ether: ^1H NMR (400 MHz, CDCl_3) 10.0 (br s, 1H), 9.1 (s, 1H), 8.75 (s, 2H), 7.3 (br m, 1H), 7.05 (m, 3H), 4.4 (br m, 1H), 3.75 (q, 1H), 3.2-2.9 (m, 3H), 2.8-2.1 (m, 6H), 1.9 (br d, 2H), 1.8-1.4 (complex m, 2H), 1.4 (d, 3H), 1.3-1.1 (complex m, 3H), 1.0 (d, 3H);

The citrate salt precipitated from ethyl acetate/methanol: Analysis calculated for $\text{C}_{24}\text{H}_{32}\text{N}_6\text{O} \cdot 1.0 \text{ CH}_3\text{CO}_2\text{CH}_2\text{CH}_3 \cdot 1.0 \text{ C}_6\text{H}_8\text{O}_7$: C: 58.27, H: 6.90, N: 11.99; found C: 58.09, H: 6.92, N: 11.77.

The lower band gave 40 mg of (2''S,4''R,1'''R) 1,3-dihydro-1-{1'-[1''-(1'''-(5''''-pyrimidinyl)-1'''-ethyl)-2''-methyl-piperidin-4''-yl]-piperidin-4'-yl}-2H-benzimidazol-2-one after trituration with 10 mL of ethyl ether: ^1H NMR (400 MHz, CDCl_3) 10.3 (br s, 1H), 9.15 (s, 1H), 8.7 (s, 2H), 7.3 (m, 1H), 7.1-7.0 (m, 3H), 4.36 (br q, 2H), 3.15 (m, 3H), 2.6-2.0 (complex m, 6H), 1.9 (m, 4H), 1.55 (d, 3H), 1.4 (br m, 2H), 1.2 (d, 3H). The citrate salt precipitated from ethyl acetate/methanol: Analysis calculated for $\text{C}_{24}\text{H}_{32}\text{N}_6\text{O} \cdot 0.45 \text{ CHCl}_3 \cdot 2.0 \text{ C}_6\text{H}_8\text{O}_7$: C: 50.99, H: 5.69, N: 9.79; found C: 50.72, H: 6.07, N: 9.42.

EXAMPLE 12

(±)-*cis*-1-(3-Ethoxycarbonyl-4-piperidinyl)benzimidazol-2H-one

Step 1: A mixture of 15 g of ethyl 4-oxo-3-piperidine carboxylate hydrochloride, 500 mL of methylene chloride, 16 g of di-tert-butylidicarbonate and 15 mL of triethylamine was stirred for 4 days at room temperature. The resulting mixture was washed with 100 mL of sat'd. sodium carbonate, dried over MgSO_4 and concentrated under reduced pressure. Drying under vacuum overnight gave 19 g of ethyl 1-tert-butylloxycarbonyl-4-oxo-3-piperidine carboxylate as a solid: ^1H NMR (400 MHz, CDCl_3) 12 (s, 1H), 4.3 (dd, 2H), 4.1 (s, 2H), 3.6 (m, 2H), 2.4 (m, 2H), 1.5 (s, 9H), 1.3 (t, 3H).

Step 2: A mixture of 4 g of ethyl 1-tert-butylloxycarbonyl-4-oxo-3-piperidine carboxylate, 3 g of 1,2-phenylenediamine, 40 mL of absolute

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methanol, 1 mL of acetic acid and 1 g of sodium cyanoborohydride was stirred at room temperature overnight. The resulting mixture was concentrated to dryness under reduced pressure and partitioned between 3 x 200 mL of chloroform and 100 mL of sat'd. sodium carbonate. The combined extracts were dried over MgSO₄ and concentrated under reduced pressure. The residue was dissolved in 200 mL of ethyl acetate and stirred with 100 mL of saturated aqueous sodium carbonate in an ice bath while a solution of 10 mL of 1.93 M phosgene in toluene was added. The resulting mixture was allowed to warm to room temperature and stirred for 24 h, diluted with 250 mL of ethyl acetate. The layers were separated and the organic layer dried over MgSO₄ and concentrated under reduced pressure. Low pressure chromatography over silica gel, eluting with 90:10 ethyl acetate:tetrahydrofuran gave first 0.50 g of (±)-*trans* --1-(1-tert -butyloxycarbonyl-3-ethoxycarbonyl-4-piperidiny)benzimidazol-2H-one as a white solid: ¹H NMR (400 MHz, CDCl₃) 9.8 (s, 1H), 7.05 (m, 4H), 4.6-4.2 (br m, 3H), 3.9 (dd, 2H), 3.6 (br m, 2H), 3.0 (br m, 1H), 2.5 (br m, 1H), 1.85 (br d, 1H), 1.5 (s, 9H), 0.95 (t, 3H); followed by 4.6 g of (±)-*cis* --1-(1-tert -butyloxycarbonyl-3-ethoxycarbonyl-4-piperidiny) benzimidazol-2H-one as a white solid: ¹H NMR (400 MHz, CDCl₃) 7.05 (m, 4H), 4.6 (br d, 1H), 4.5 (br m, 2H), 3.9 (complex m, 2H), 3.6 (br m, 2H), 3.05 (br m, 1H), 2.95 (br m, 1H), 1.85 (br d, 1H), 1.5 (s, 9H), 0.95(t, 3H). The (±)-*cis* --1-(1-tert -butyloxycarbonyl-3-ethoxycarbonyl-4-piperidiny)benzimidazol-2H-one (0.5 g) could be converted into predominantly the more stable (±)-*trans* --1-(1-tert -butyloxycarbonyl-3-ethoxycarbonyl-4-piperidiny)benzimidazol-2H-one by stirring for 2 days in 5 mL of ethanol with 325 mg of cesium carbonate at room temperature.

Step 3: To a stirred solution of 1.5 g of (±)-*cis* --1-(1-tert -butyloxycarbonyl-3-ethoxycarbonyl-4-piperidiny)benzimidazol-2H-one in 100 mL of ethyl acetate and 25 mL of methylene chloride cooled in an ice bath was added a stream of hydrogen chloride gas for 10 min. The mixture was allowed to warm to room temperature for 5 h, then concentrated to dryness under reduced pressure, and partitioned

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between sat'd. sodium carbonate and 3 x 100 mL of chloroform. The combined extracts were dried over MgSO₄ and concentrated under reduced pressure. Drying under vacuum gave 0.50 g of (±)-*cis*-1-(3-ethoxycarbonyl-4-piperidinyl)benzimidazol-2H-one as a foam.

EXAMPLE 13

(3'R,4'R,1'''R and 3'S,4'S,1'''R) 1,3-Dihydro-1-{1'-[1''-(1'''-(5''''-pyrimidinyl)-1'''-ethyl)-piperidin-4''-yl]-3'-ethoxycarbonylpiperidin-4'-yl} -2H-benzimidazol-2-one

A mixture of 0.450 g of 1-(1R-(5-pyrimidinyl)-ethyl)-2R-methyl-4-oxopiperidine, 0.50 g of (±)-*cis*-1-(3-ethoxycarbonyl-4-piperidinyl)benzimidazol-2H-one, 20 mL of 1,2-dichloroethane, 0.6 mL of glacial acetic acid and 1.8 g of sodium triacetoxyborohydride was stirred at room temperature for 3 days. The reaction mixture was poured into 100 mL chloroform and 25 mL saturated aqueous Na₂CO₃ and the layers separated. The aqueous layer was extracted with 2 X 50 mL of chloroform and the combined organic layers dried over MgSO₄ and concentrated under reduced pressure. Purification of the residue by preparative thin chromatography on silica gel eluting with 400:50:25 CHCl₃:MeOH:NH₄OH gave product contaminated with 1-(1R-(5-pyrimidinyl)-ethyl)-2R-methyl-4-hydroxypiperidine formed by reduction of the piperidone. Further purification by preparative thin chromatography on silica gel eluting with 375:100:25 tetrahydrofuran:CH₂Cl₂:MeOH gave 0.60 g of (3'R,4'R,1'''R and 3'S,4'S,1'''R) 1,3-dihydro-1-{1'-[1''-(1'''-(5''''-pyrimidinyl)-1'''-ethyl)-piperidin-4''-yl]-3'-ethoxycarbonylpiperidin-4'-yl} -2H-benzimidazol-2-one: ¹H NMR (400 MHz, CDCl₃) 9.15 (s, 1H), 8.9 (br s, 1H), 8.7 (s, 2H), 7.5 (br dd, 1H), 7.05 (m, 3H), 4.4 (br m, 1H), 4.05 (m, 1H), 3.9 (m, 1H), 3.56 (br dd, 1H), 3.4 (d, 1H), 3.25-3.0 (complex m, 4H), 2.8 (m, 1H), 2.62 (m, 1H), 2.55 (m, 1H), 2.38 (m, 1H), 2.0 (m, 1H), 1.8-1.4 (complex m, 6H), 1.4 (d, 3H), 1.0 (dt, 3H); The citrate salt precipitated from ethyl acetate/methanol: Analysis calculated

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for C₂₆H₃₄N₆O₃•1.4 C₆H₈O₇: C: 55.26, H: 6.09, N: 11.24; found C: 55.19, H: 6.29, N: 11.29.

EXAMPLE 14

(±)-*trans* -1-(3-Ethoxycarbonyl-4-piperidiny)benzimidazol-2H-one

To a stirred solution of 1.0 g of (±)-*trans* -1-(1-tert -butoxycarbonyl-3-ethoxycarbonyl-4-piperidiny)benzimidazol-2H-one in 150 mL of ethyl acetate cooled to -78°C was added a stream of hydrogen chloride gas for 5 min. The mixture was allowed to warm to room temperature for 2 h, then concentrated to dryness under reduced pressure, and partitioned between sat'd. sodium carbonate and 3 x 100 mL of chloroform. The combined extracts were dried over MgSO₄ and concentrated under reduced pressure. Drying under vacuum gave 0.700 g of (±)-*trans* -1-(3-ethoxycarbonyl-4-piperidiny)benzimidazol-2H-one as a solid: ¹H NMR (400 MHz, CDCl₃): 11 (br s, 1H), 7.1 (m, 4H), 4.5 (br m, 1H), 3.9 (dd, 2H), 3.6 (br m, 1H), 3.5 (d, 1H), 3.3 (d, 1H), 2.85 (m, 2H), 2.5 (br q, 1H), 1.9 (d, 1H), 0.9 (t, 3H).

EXAMPLE 15

(3'S,4'R,1'''R and 3'R,4'S,1'''R) 1,3-Dihydro-1-{ 1'-[1''-(1'''-(5'''-pyrimidinyl)-1'''-ethyl)-piperidin-4''-yl]-3'-ethoxycarbonylpiperidin-4'-yl} -2H-benzimidazol-2-one

A mixture of 0.350 g of 1-(1R-(5-pyrimidinyl)-ethyl)-2R-methyl-4-oxopiperidine, 0.250 g of (±)-*trans* -1-(3-ethoxycarbonyl-4-piperidiny)benzimidazol-2H-one, 8 mL of 1,2-dichloroethane, 0.2 mL of glacial acetic acid and 0.8 g of sodium triacetoxyborohydride was stirred at room temperature for 48 h. The reaction mixture was poured into 100 mL chloroform and 25 mL saturated aqueous Na₂CO₃ and the layers separated. The aqueous layer was extracted with 2 X 50 mL of chloroform and the combined organic layers dried over MgSO₄ and concentrated under reduced pressure. Purification of the residue by

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preparative thin chromatography on silica gel eluting with 20:1 THF:NH₄OH gave product contaminated with 1-(1R-(5-pyrimidinyl)-ethyl)-2R-methyl-4-hydroxypiperidine formed by reduction of the piperidone. Further purification by preparative thin chromatography on silica gel eluting with 90:10 CH₂Cl₂:MeOH gave 0.24 g of (3'S,4'R,1'''R and 3'R,4'S,1'''R) 1,3-dihydro-1-{1'-[1''-(1'''-(5''''-pyrimidinyl)-1'''-ethyl)-piperidin-4''-yl]-3'-ethoxycarbonylpiperidin-4'-yl} -2H-benzimidazol-2-one: ¹H NMR (400 MHz, CDCl₃) 9.15 (s, 1H), 8.7 (s, 2H), 7.5 (br m, 1H), 7.05 (m, 3H), 4.4 (br m, 1H), 3.9 (q, 2H), 3.6 (br m, 2H), 3.2 (d, 1H), 3.05 (br m, 2H), 2.85 (d, 1H), 2.45 (complex m, 4H), 2.55 (t, 1H), 1.8 (m, 1H), 1.6 (m, 6H), 1.4 (2xd, 3H), 0.95 (dt, 3H); The citrate salt precipitated from ethyl acetate/methanol: Analysis calculated for C₂₆H₃₄N₆O₃•1.4 C₆H₈O₇•0.4 CH₃CO₂CH₂CH₃: C: 54.81, H: 6.18, N: 10.48; found C: 55.03, H: 6.30, N: 10.39.

EXAMPLE 16

(±)-1-(2,2-Dimethylethoxycarbonyl)-2-methyl-4-oxopiperidine

Step 1: The methodology of D. L. Comins and J. D. Brown, *Tetrahedron Letters*, **1986**, pp 4549-4552 was used: To a stirred solution of 2.5 g of 4-methoxypyridine in 75 mL of anhydrous tetrahydrofuran cooled to -23°C under a nitrogen atmosphere was added 8 mL of 3M methylmagnesium chloride in tetrahydrofuran followed by 4 g of benzylchloroformate, keeping the internal temperature below -20°C. After stirring for 4 h at -25±5°C, the reaction was quenched with 100 mL of 1N HCl and extracted with 5 x 100 mL portions of ether. The combined extracts were dried over MgSO₄ and concentrated under reduced pressure. Further drying under vacuum gave 5.1 g of 2(R,S)-methyl-1-(benzyloxycarbonyl)-2,3-dihydro-4-pyridone as a greenish oil: TLC (25% EtOAc in hexane); ¹H NMR (400 MHz, CDCl₃) 7.75 (d, 1H), 7.4 (s, 5H), 5.30 (d, 1H), 5.26 (s, 2H), 4.7 (m, 1H), 2.82 (dd, 1H), 2.38 (d, 2H), 1.25 (t, 3H).

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Step 2: A mixture of 1.1 g of the crude 2(R,S)-methyl-1(benzyloxycarbonyl)-2,3-dihydro-4-pyridone, 200 mL of ethyl acetate, 1 g of di-tert-butyldicarbonate and 1 g of 10% palladium on carbon was shaken under 55 psi of hydrogen for 24 h. The mixture was filtered, concentrated under reduced pressure, and purified by low pressure chromatography on silica gel eluting with 40% ethyl acetate in hexane. There was obtained first 0.20 g of (±)-1-(2,2-dimethylethoxycarbonyl)-2-methyl-4-oxopiperidine: ¹H NMR (400 MHz, CDCl₃) 4.65 (m, 1H), 4.2 (m, 1H), 3.3 (m, 1H), 2.65 (dd, 1H), 2.45 (m, 1H), 2.3 (m, 1H), 2.2 (dd, 1H), 1.45 (s, 9H), 1.14 (d, 3H). Later fractions gave 0.50 g of the (±)-1-(2,2-dimethylethoxycarbonyl)-2-methyl-4-hydroxypiperidine: ¹H NMR (400 MHz, CDCl₃) 4.3 (m, 1H), 4.15 (t, 1H), 3.8 (m, 1H), 3.25 (m, 1H), 2.15 (s, 1H), 2.8 (m, 1H), 1.65 (m, 3H), 1.42 (s, 3H), 1.25 (d, 3H).

EXAMPLE 17

1,3-Dihydro-1-{1'-[1''-(2''',2'''-dimethylethoxycarbonyl)-2''-methylpiperidin-4''-yl]piperidin-4'-yl}-2H-benzimidazol-2-one

A mixture 0.20 g of (±)-1-(2,2-dimethylethoxycarbonyl)-2-methyl-4-oxopiperidine, 0.40 g of 4-(2-oxo-1-benzimidazoliny)l)piperidine, 5 mL of 1,2-dichloroethane, 0.1 mL of glacial acetic acid and 0.40 g of sodium triacetoxymethylborohydride was stirred at room temperature for 48 h. The reaction mixture was poured into 200 mL chloroform and 200 mL saturated aqueous Na₂CO₃ and the layers separated. The aqueous layer was extracted with 2 X 25 mL of chloroform and the combined organic layers dried over MgSO₄ and concentrated under reduced pressure. Trituration of the crude product with ether-hexane 0.13 g of 1,3-dihydro-1-{1'-[1''-(2''',2'''-dimethylethoxycarbonyl)-2''-methylpiperidin-4''-yl]piperidin-4'-yl}-2H-benzimidazol-2-one: 7.23 (m, 1H), 7.1 (m, 1H), 7.0 (m, 2H), 4.4 (m, 1H), 3.9 (m, 1H), 3.8 (m, 1H), 3.1 (m, 3H), 2.8 (br m, 1H), 2.6 (m, 1H), 2.5 (m, 3H), 2.25 (m, 1H), 1.9 (m, 3H), 1.6 (m, 1H), 1.42 (s, 9H), 1.24 (d, 3H).

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EXAMPLE 18

1,3-Dihydro-1-{1'-[1''-(3'''-pyridinecarbonyl)-2''-methyl-piperidin-4''-yl]-piperidin-4'-yl}-2H-benzimidazol-2-one

Step 1: A stirred solution of 0.13 g of 1,3-dihydro-1-{1'-[1''-(2'''-dimethylethoxycarbonyl)-2''-methylpiperidin-4''-yl]piperidin-4'-yl}-2H-benzimidazol-2-one in 15 mL of 1N HCl was heated to reflux for 3 h, cooled and concentrated to dryness. The residue was partitioned between 3 mL of 2 N NaOH and 5 x 50 mL of methylene chloride. The combined organic extracts were dried over MgSO₄ and concentrated under reduced pressure. After drying under vacuum, there was obtained 0.1 g of 1,3-dihydro-1-{1'-[2''-methylpiperidin-4''-yl]piperidin-4'-yl}-2H-benzimidazol-2-one as a gum (racemic mixture of diastereomers)..

Step 2: To a stirred solution of 0.1 g of 1,3-dihydro-1-{1'-[2''-methylpiperidin-4''-yl]piperidin-4'-yl}-2H-benzimidazol-2-one and 0.2 mL of triethylamine in 250 mL of dichloromethane was added 0.50 g of nicotinoyl chloride hydrochloride. After 2 h, 50 mL of dilute aqueous ammonia was added and the mixture stirred for an additional 30 min. The organic layer was separated, the aqueous layer saturated with sodium chloride and extracted with two additional 20 mL portions of chloroform and the combined organic extracts dried over MgSO₄ and concentrated under reduced pressure. Purification of the residue by preparative thin chromatography on silica gel eluting with 160:50:10 CHCl₃:MeOH:NH₄OH followed by trituration with ether gave 90 mg of 1,3-dihydro-1-{1'-[1''-(3'''-pyridinecarbonyl)-2''-methyl-piperidin-4''-yl]-piperidin-4'-yl}-2H-benzimidazol-2-one as a white solid: ¹H NMR (400 MHz, CDCl₃) 10.4 (s, 1H), 8.66 (d, 2H), 7.77 (dt, 1H), 7.38 (dd, 1H), 7.25 (m, 1H), 7.1 (m, 1H), 7.05 (m, 2H), 4.4 (br m, 2H), 3.7 (br m, 1H), 3.4 (br m, 1H), 3.2 (br d, 1H), 3.1 (m, 2H), 2.8 (br m, 1H), 2.65 (br m, 1H), 2.5 (m, 2H), 2.22 (q, 2H), 2.0 (br d, 1H), 1.9 (br d, 2H), 1.75 (br m, 1H), 1.4 (2 x d, 3H). The citrate salt precipitated from ethyl acetate/methanol: Analysis calculated for C₂₆H₃₄N₆O₃•1.0 C₆H₈O₇•0.8 CHCl₃•1.0 H₂O: C: 51.01, H:5.53, N: 9.66; found C:

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51.12, H: 5.76, N: 9.28. HPLC analysis on C-18 with a gradient of 60:40 to 0:100, aqueous 0.12% ammonium formate:methanol indicated a ratio of 83:17 *cis* : *trans* isomers, consistent with the known preference for equatorial attack of hydride during reductive amination of cyclic ketones.

EXAMPLE 19

1,3-Dihydro-1-(8'-azabicyclo[3.2.1]octan-3' α -yl) -2H-benzimidazol-2-one

Step 1: A mixture of 8 g of 1-carbethoxy-4-tropinone, 8 g of 1,2-phenylenediamine, 250 mL of absolute methanol, 2.5 mL of acetic acid and 2.5 g of sodium cyanoborohydride was stirred at room temperature overnight. The resulting mixture was concentrated to dryness under reduced pressure and partitioned between 3 x 200 mL of chloroform and 50 mL of sat'd. sodium carbonate in an ice bath while a solution of 50 mL of 1.93 M phosgene in toluene was added. The resulting mixture was allowed to warm to room temperature and stirred for 24 h, diluted with 250 mL of ethyl acetate. The layers were separated and the organic layer dried over MgSO₄ and concentrated under reduced pressure.

Low pressure chromatography over silica gel, eluting with ethyl acetate gave, after trituration with hexane-ethyl acetate, 7.33 g of 1,3-dihydro-1-(1'-ethoxycarbonyl-8'-azabicyclo[3.2.1]octan-3' α -yl) -2H-benzimidazol-2-one as a white solid: ¹H NMR (400 MHz, CDCl₃) 9.84 (s, 1H), 7.05 (m, 4H), 4.5 (br m, 2H), 4.4 (m, 1H), 4.2 (br m, 2H), 2.5 (br s, 2H), 2.1 (br m, 4H), 1.9 (br d, 2H), 1.5 (t, 3H).

Step 2: A stirred mixture of 7.33 g of 1,3-dihydro-1-(1'-ethoxycarbonyl-8'-azabicyclo[3.2.1]octan-3' α -yl) -2H-benzimidazol-2-one, 50 mL of dioxane and 40 mL of 2N NaOH was heated to reflux for 12 h. After cooling in an ice bath, 5 g of ammonium chloride was added and the mixture was extracted with 3 x 200 mL of 10% THF in ethyl acetate. The combined extracts were dried over MgSO₄ and concentrated under reduced pressure. Trituration with hexane-ether-ethyl acetate gave, in two crops, 5.53 g of 1,3-dihydro-1-(8'-azabicyclo[3.2.1]octan-3' α -yl)

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-2H-benzimidazol-2-one as a white solid: ^1H NMR (400 MHz, CDCl_3) 7.05 (m, 4H), 4.6 (m, 1H), 3.75 (d, 2H), 2.95 (br s, 2H), 2.35 (dt, 2H), 2.05 (t, 2H), 1.9 (m, 4H).

EXAMPLE 20

(1'''R) 1,3-Dihydro-1-{1'-[8''-(1'''-(5'''-pyrimidinyl)-1'''-ethyl)piperidin-4''-yl]-8'-azabicyclo[3.2.1]octan-3'α-yl} -2H-benzimidazol-2-one

A mixture of 0.125 g of 1-(1R-(5-pyrimidinyl)-ethyl)-2R-methyl-4-oxopiperidine, 0.240 g of 1,3-dihydro-1-(8'-azabicyclo[3.2.1]octan-3'α-yl) -2H-benzimidazol-2-one, 5 mL of 1,2-dichloroethane, 5 mL of tetrahydrofuran, 0.1 mL of glacial acetic acid and 0.4 g of sodium triacetoxyborohydride was stirred at room temperature for 24 h. The reaction mixture was partitioned between 100 mL chloroform and 5 mL 20% NaOH and the organic layer dried over MgSO_4 and concentrated under reduced pressure. Trituration with ether-ethyl acetate-hexane gave 0.275 g of (1'''R) 1,3-dihydro-1-{1'-[8''-(1'''-(5'''-pyrimidinyl)-1'''-ethyl)piperidin-4''-yl]-8'-azabicyclo[3.2.1]octan-3'α-yl} -2H-benzimidazol-2-one as a buff solid: ^1H NMR (400 MHz, CDCl_3) 9.15 (s, 1H), 8.7 (s, 2H), 7.05 (m, 3H), 4.75 (m, 1H), 3.6 (br m, 2H), 3.55 (m, 1H), 2.95 (br d, 1H), 2.8 (br d, 1H), 2.35 (dd, 2H), 2.0 (complex m, 6H), 1.8 (m, 2H), 1.7 (dd, 2H), 1.63 (br m, 1H), 1.5 (m, 2H), 1.4 (d, 3H); The citrate salt precipitated from ethyl acetate/methanol: Analysis calculated for $\text{C}_{25}\text{H}_{32}\text{N}_6\text{O} \cdot 1.05 \text{ C}_6\text{H}_8\text{O}_7 \cdot 1.0 \text{ H}_2\text{O}$: C: 57.62, H: 6.89, N: 12.88; found C: 58.01, H: 6.89, N: 12.56.

EXAMPLE 21

1,3-Dihydro-1-(8'-azabicyclo[3.2.1]octan-3'β-yl) -2H-benzimidazol-2-one

Step 1: To a stirred solution of 10 g of 1-carbethoxy-4-tropinone in 50 mL of anhydrous tetrahydrofuran cooled to -78°C under nitrogen

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atmosphere was added 75 mL of 1M lithium tri-*sec* -butylborohydride in tetrahydrofuran, keeping the internal temperature below -68°C. When the addition was complete, the mixture was stirred for 15 min at -78°C, quenched with 10 mL of water and warmed to 0°C in an ice bath. The borane complex was decomposed by dropwise addition of 30 mL of 2N NaOH and 40 mL of 15% hydrogen peroxide. After 15 min, the mixture was extracted with 6 x 200 mL of ethyl acetate and the combined organic extracts dried over MgSO₄ and concentrated under reduced pressure. Repeated azeotropic distillations with methanol to remove borate esters and trituration with hexane-ether gave 9.25 g of 1-carbethoxy-4 α -tropinol as crystalline solid: ¹H NMR (400 MHz, CDCl₃) 4.25 (br m, 2H), 4.15 (q and overlapping br m, 3H), 2.18 (q, 2H), 2.1 (br m, 2H), 1.94 (m, 2H), 1.73 (d, 2H), 1.6 (br s, 1H), 1.25 (t, 3H).

Step 2: To an ice cold, stirred solution of 4.0 g of 1-carbethoxy-4 α -tropinol and 10 mL of triethylamine in 280 mL of methylene chloride was added 2.0 mL of methanesulfonyl chloride dropwise, keeping the internal temperature below 5°C. After stirring for 30 min with the ice bath removed, the mixture was partitioned between 6 x 100 mL of chloroform and 100 mL of water. The combined extracts were washed with 100 mL of 1N HCl, 100 mL of sat'd. sodium carbonate, dried over MgSO₄ and concentrated under reduced pressure. Drying under vacuum gave 5.5 g of 1-carbethoxy-4 α -methylsulfonyloxytropane as an amber oil: ¹H NMR (400 MHz, CDCl₃) 5.0 (t, 1H), 4.3 (br m, 2H), 4.15 (q, 2H), 3.0 (s, 3H), 2.18 (br m, 2H), 2.05 (m, 6H), 1.3 (t, 3H).

Step 3: A stirred mixture of 5.5 g of 1-carbethoxy-4 α -methylsulfonyloxy-tropane, 6.0 g of sodium azide and 50 mL of anhydrous DMF was heated to 60°C for 2 days. The mixture was cooled and partitioned between 750 mL of water on 3 x 150 mL of diethyl ether. The combined ether extracts were washed 3 x 50 mL of water, dried over MgSO₄ and concentrated under reduced pressure. Drying under vacuum gave 5.5 g of 1-carbethoxy-4 β -azidotropane as a white solid: ¹H NMR (400 MHz, CDCl₃) 4.4 (br m, 2H), 4.18 (q, 2H), 3.74 (m, 1H), 2.05 (m, 2H), 1.95 (dd, 2H), 1.65 (m, 6H), 1.25 (t, 3H).

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Step 4: A mixture of 4.5 g of the 1-carbethoxy-4 β -azidotropane, 300 mL of ethanol and 1 g of 10% palladium on carbon was shaken under 1 atm of hydrogen (balloon) for 18 h. The mixture was filtered and concentrated under reduced pressure. Drying under vacuum gave 4.5 g of 1-carbethoxy-4 β -aminotropane as a gum: ¹H NMR (400 MHz, CDCl₃) 4.3 (br m, 2H), 4.18 (q, 2H), 3.15 (m, 1H), 1.95 (m, 2H), 1.82 (m, 2H), 1.7 (m, 2H), 1.4 (m, 2H), 1.30 (t, 3H).

Step 5: A mixture of 3.6 g of 2-fluoronitrobenzene, 4.5 g of 1-carbethoxy-4 β -aminotropane, 3 g of sodium carbonate, 15 mL of cyclohexanol was heated to 160°C for 2 h. After cooling, the mixture was partitioned between 4 x 100 mL of ethyl acetate and 250 mL of water. The organic extract was dried over MgSO₄ and concentrated under reduced pressure. Trituration with hexane gave, in two crops, 5.7 g of 1-carbethoxy-4 β -(2-nitrophenylamino)tropane as an orange crystalline solid: ¹H NMR (400 MHz, CDCl₃) 8.2 (d, 1H), 7.9 (d, 1H), 7.42 (m, 1H), 6.88 (d, 1H), 6.63 (m, 1H), 4.4 (br m, 2H), 4.2 (q, 2H), 4.02 (m, 1H), 2.1 (m, 4H), 1.8 (dd, 2H), 1.7 (br m, 2H), 1.3 (t, 3H).

Step 6: A mixture of 5.7 g of 1-carbethoxy-4 β -(2-nitrophenylamino)tropane, 200 mL of tetrahydrofuran, 150 mL of ethanol and 1.2 g of 5% platinum on carbon was stirred under an atmosphere of hydrogen for 7 h. The catalyst was filtered off and the filtrate concentrated to a pink solid. To an ice cold, stirred solution of the resulting crude 1-carbethoxy-4 β -(2-aminophenylamino)tropane in 500 mL of ethyl acetate was added 200 mL of saturated sodium bicarbonate followed by 20 mL of 1.9 M phosgene in toluene dropwise over 30 min. After stirring overnight at room temperature, the layers were separated, the aqueous layer extracted with 3 x 100 mL of ethyl acetate and the combined organic layers dried over MgSO₄ and concentrated to dryness. Trituration of the residue with ether gave 5.0 g of 1,3-dihydro-1-(1'-ethoxycarbonyl-8'-azabicyclo[3.2.1]octan-3' β -yl)-2H-benzimidazol-2-one as a buff solid: ¹H NMR (400 MHz, CDCl₃) 9.85 (br m, 1H), 7.15 (m, 1H), 7.05 (m, 3H), 4.95 (m, 1H), 4.5 (br d, 2H), 4.25 (br d, 2H), 2.5 (br m, 2H), 2.1 (m, 2H), 1.93 (dd, 2H), 1.77 (dd, 2H), 1.35 (t, 3H).

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Step 7: A stirred mixture of 4.74 g of 1,3-dihydro-1-(1'-ethoxycarbonyl-8'-azabicyclo[3.2.1]octan-3'β-yl) -2H-benzimidazol-2-one, 100 mL of dioxane and 40 mL of 2N NaOH was heated to reflux for 48 h. After distilling off 60 mL of dioxane over 2 h, the mixture was cooled in an ice bath and 5 g of ammonium chloride was added. The mixture extracted with 3 x 100 mL of chloroform. The combined chloroform extracts were extracted into 3 x 50 mL portions of 1N HCl. Drying the chloroform layer over MgSO₄ and concentration under reduced pressure gave back 2.3 g of 1,3-dihydro-1-(1'-ethoxycarbonyl-8'-azabicyclo[3.2.1]octan-3'β-yl) -2H-benzimidazol-2-one (starting material). The combined acidic aqueous extracts were basified with 20% NaOH to pH 10 and extracted with 3 x 100 mL of chloroform. The combined chloroform extracts were dried over MgSO₄ and concentrated under reduced pressure. Trituration with ethyl ether gave, in two crops, 1.90 g of 1,3-dihydro-1-(8'-azabicyclo[3.2.1]octan-3'β-yl) -2H-benzimidazol-2-one as a white solid: ¹H NMR (400 MHz, CDCl₃) 9.4 (br s, 1H), 7.3 (m, 1H), 7.1 (m, 1H), 7.05 (m, 2H), 4.75 (m, 1H), 3.74 (br m, 2H), 2.4 (m, 2H), 1.9 (m, 4H), 1.7 (m, 2H).

EXAMPLE 22

(1'''R) 1,3-Dihydro-1-{1'-[8''-(1'''-(5'''-pyrimidinyl)-1'''-ethyl)piperidin-4''-yl]-8'-azabicyclo[3.2.1]octan-3'β-yl} -2H-benzimidazol-2-one

A mixture of 0.25 g of 1-(1R-(5-pyrimidinyl)-ethyl)-2R-methyl-4-oxopiperidine, 0.25 g of 1,3-dihydro-1-(8'-azabicyclo[3.2.1]octan-3'β-yl) -2H-benzimidazol-2-one, 10 mL of 1,2-dichloroethane, 10 mL of tetrahydrofuran, 0.2 mL of glacial acetic acid and 0.4 g of sodium triacetoxymethylborohydride was stirred at room temperature for 48 h. The reaction mixture was partitioned between 3 x 100 mL chloroform and 50 mL sat'd. sodium carbonate and the organic layer dried over MgSO₄ and concentrated under reduced pressure. Trituration with ether-ethyl gave 0.325 g of (1'''R) 1,3-dihydro-1-{1'-[8''-(1'''-(5'''-pyrimidinyl)-1'''-ethyl)piperidin-4''-yl]-8'-azabicyclo[3.2.1]octan-3'β-yl} -2H-

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benzimidazol-2-one as a white solid: ^1H NMR (400 MHz, CDCl_3) 9.14 (br s, 1H), 9.12 (s, 1H), 8.37 (s, 2H), 7.77 (m, 1H), 7.05 (m, 3H), 4.7 (m, 1H), 3.62 (br m, 2H), 3.6 (dd, 1H), 3.0 (br d, 1H), 2.84 (br d, 1H), 2.7 (m, 1H), 2.5 (t, 2H), 2.16 (dd, 2H), 2.0 (m, 4H), 1.8 (dd, 2H), 1.75 (br m, 1H), 1.55 (m, 3H), 1.42 (d, 3H); The citrate salt precipitated from ethyl acetate/methanol: Analysis calculated for $\text{C}_{25}\text{H}_{32}\text{N}_6\text{O} \cdot 1.0 \text{ C}_6\text{H}_8\text{O}_7 \cdot 1.0 \text{ H}_2\text{O}$: C: 57.93, H: 6.59, N: 13.08; found C: 58.23, H: 6.45, N: 13.45.

EXAMPLE 23

1,3-Dihydro-1-{1'-[1''-(1'''-methylimidazol-5'''-carbonyl)-piperidin-4''-yl]-piperidin-4'-yl}-2H-benzimidazol-2-one

Step 1: A mixture of 1.5 g of ethyl 1-methyl-5-imidazolecarboxylate, 5 mL of ethanol and 5 mL of 10% NaOH was stirred at room temperature for 12 h. The mixture was acidified to pH 2 with conc. HCl, diluted with 10 mL of ice water. The precipitated product was collected by filtration in 2 crops. Drying under vacuum gave 843 mg of 1-methyl-5-imidazolecarboxylic acid as a white solid.

Step 2: To a stirred solution of 0.20 g of 1-methyl-5-imidazolecarboxylic acid in 4 mL of DMF, 4 mL of tetrahydrofuran and 0.5 mL of triethylamine cooled to 0°C was added 0.30 mL of diphenylphosphoryl chloride. A white precipitate formed immediately. After stirring in the cold for 30 min, 0.20 g of 1,3-dihydro-1-{1'-[1''-(1'''-methylimidazol-5'''-carbonyl)-piperidin-4''-yl]-piperidin-4'-yl}-2H-benzimidazol-2-one was added and the mixture allowed to warm and stir for 24 h. The mixture was partitioned between 5 mL of sat'd. sodium carbonate and 2 x 125 mL of chloroform. The combined organic extracts were dried over MgSO_4 and concentrated under reduced pressure. Purification of the residue by preparative thin chromatography on silica gel eluting with 200:50:10 CHCl_3 :MeOH: NH_4OH followed by trituration with ether gave 0.20 g of 1,3-dihydro-1-{1'-[1''-(1'''-methylimidazol-5'''-carbonyl)-piperidin-4''-yl]-piperidin-4'-yl}-2H-benzimidazol-2-one as a white solid: ^1H NMR (400 MHz, CDCl_3) 10.85 (s, 1H), 7.52 (s, 1H), 7.25 (m, 1H), 7.22

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(s, 1H), 7.15 (m, 1H), 7.05 (m, 2H), 4.55 (br m, 2H), 4.35 (m, 1H), 3.8 (s, 3H), 3.1 (d, 2H), 3.0 (br m, 2H), 2.65 (br m, 1H), 2.40 (br m, 4H), 1.95 (br d, 2H), 1.85 (br d, 2H), 1.55 (m, 2H). The citrate salt precipitated from ethyl acetate/methanol: Analysis calculated for $C_{22}H_{28}N_6O_2 \cdot 1.3 C_6H_8O_7 \cdot 1.0 CH_3CO_2CH_2CH_3$: C: 54.39, H: 6.27, N: 11.26; found C: 54.03, H: 6.48, N: 11.40.

EXAMPLE 24

(3'S,4'R,1'''S and 3'R,4'S,1'''S) 1,3-Dihydro-1-{1'-[1''-(1'''-phenylethyl)-piperidin-4''-yl]-3'-ethoxycarbonylpiperidin-4'-yl} -2H-benzimidazol-2-one

Step 1: To a stirred mixture of 1.2 g of (S)-(-)-1-phenethylamine, 17 mL of ethanol and 1.36 g of K_2CO_3 heated to reflux was added dropwise over 30 min, a solution of 4.0 g of 1,1-dimethyl-4-oxopiperidinium iodide in 20 mL of water. When the addition was complete, the mixture was heated under reflux for an additional 1.5 h, cooled, basified to pH 9 with K_2CO_3 and extracted with 3 times with 100 mL portions of methylene chloride. The combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. Chromatography over silica gel, eluting with 1:1 chloroform:ethyl acetate gave 1.2 g of 1-(1S-(phenethyl)-4-oxopiperidine) as an oil.

Step 2: A mixture of 0.350 g of 1-(1S-(phenethyl)-4-oxopiperidine), 0.170 g of ($\pm$)-*trans*-1-(3-ethoxycarbonyl-4-piperidinyl)benzimidazol-2H-one, 8 mL of 1,2-dichloroethane, 0.2 mL of glacial acetic acid and 0.8 g of sodium triacetoxyborohydride was stirred at room temperature for 48 h. The reaction mixture was partitioned between 3 x 50 mL chloroform and 20 mL saturated aqueous Na_2CO_3 . The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. Purification of the residue by preparative thin chromatography on silica gel eluting with 90:10 methylene chloride:methanol followed by trituration with ether gave 0.20 g of (3'S,4'R,1'''S and 3'R,4'S,1'''S) 1,3-dihydro-1-{1'-[1''-(1'''-phenylethyl)-piperidin-4''-yl]-3'-ethoxycarbonylpiperidin-4'-yl} -2H-

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benzimidazol-2-one as a mixture of diastereomers: ^1H NMR (400 MHz, CDCl_3) 10.45 (s, 1H), 7.3 (m, 4H), 7.25 (m, 1H), 7.2 (m, 1H), 7.1 (m, 1H), 7.05 (m, 2H), 4.4 (br m, 1H), 3.9 (q, 2H), 3.6 (br m, 1H), 3.42 (m, 1H), 3.25 (d, 1H), 3.15 (d, 1H), 3.05 (d, 1H), 2.9 (d, 1H), 2.45 (complex m, 4H), 2.0 (t, 1H), 1.85 (m, 2H), 1.7 (m, 2H), 1.6 (m, 1H), 1.38 (d, 3H), 0.95 (t, 3H); The citrate salt precipitated from ethyl acetate/methanol: Analysis calculated for $\text{C}_{24}\text{H}_{32}\text{N}_6\text{O} \cdot 1.35 \text{C}_6\text{H}_8\text{O}_7 \cdot 1.0 \text{CH}_3\text{CO}_2\text{CH}_2\text{CH}_3$: C: 58.44, H: 6.70, N: 6.80; found C: 58.37, H: 6.84, N: 6.92.

Example 25

Radioligand Binding Studies

The affinity of muscarinic antagonists for m1-m5 receptors expressed in chinese hamster ovary cells (CHO) were determined using the technique described by Dorje et al., J. Pharmacol. Exp. Ther. 256: 727-733 (1991).

When 80-100% confluent, CHO cells were harvested, and transferred to centrifuge tubes containing CHO buffer (20 mM HEPES at pH 7.4 containing 5mM MgCl_2). The cells were homogenized, using a Brinkman Polytron homogenizer for 30 seconds at a setting of 5, on ice. The homogenate was centrifuged at 40,000 x g for 15 minutes at 4°C in a Beckman J2-21M centrifuge. The supernatant was discarded and the homogenization/centrifugation step repeated once. Pelleted membranes were resuspended in CHO buffer to a concentration of one flask harvested (75 cm^2) per mL of buffer, mixed well and aliquoted in cryovials (1mL/vial). The vials were stored at -70°C until used in the assay. The binding incubation was done in polypropylene macrowell tube strips in a final volume of 0.5 mL of HEPES buffer (20 mM; pH 7.4 containing 5 mM MgCl_2) containing 0.1 mL of cell membrane suspension, ^3H -N-methylscopolamine (NEN Corporation, NET-636, 70-87 Ci/mmole) at a final concentration of approximately 0.2 nM and the competing drug in a varying range of concentrations or vehicle. After the addition of the cell homogenate the tubes were agitated on a vortex mixer and then

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placed in a water bath at 32°C. After 90 minutes of incubation, the membranes were harvested on a Skatron filtermat (#11734) or a Wallac filtermat (#205-404) using three washes of HEPES buffer (4°C). The radioactivity on the filters was counted in a Packard 2200CA scintillation counter or in a Wallac 1205 Betaplate scintillation counter. Specific binding was defined as the difference in binding observed in the presence and absence of 10 micromolar atropine and accounted for at least 80% of total binding. K_i values were calculated using the program LIGAND. Compounds displayed K_i values at m1, m2 and m4 in the range of 1 nM to 5,000 nM. All compounds described herein displayed typically greater than 300-fold less potency at the m3 receptor subtype, in the range of 300 nM to 114,000 nM.

EXAMPLE 26

m1 receptor antagonist activity on the rabbit vas deferens

The technique described by Feifel et al., Brit. J. Pharmacol. 99: 455-460 (1990) was used as follows: Male Hazelton New Zealand White rabbits weighing 1.5-3 kg, are euthanized (phenobarbital sodium, 85 mg/kg. i. v.). An abdominal incision is made and the vas deferens are removed. The tissues are placed in a Petri dish containing oxygenated Krebs solution [NaCl, 118 mM; KCl, 4.7 mM; CaCl₂, 2.5 mM; KH₂PO₄, 1.2 mM; MgSO₄, 1.2 mM; NaHCO₃, 25 mM; dextrose, 11 mM] warmed to 30°C. Each tissue is cut into three 2-cm segments: proximal to the prostate, a middle section, and distal to the prostate. Only the first two segments are used. Tissue segments are attached to platinum electrodes with 4-0 surgical silk and placed in a 10 mL jacketed tissue bath containing Krebs buffer at 30°C, bubbled with 5% CO₂ / 95% O₂. The tissues are connected to a Satham-Gould force transducer; 0.75 gram of tension is applied and the tissues are electrically stimulated. [EFS parameters are 0.05 Hz; 0.5 ms duration; voltage is set to 30% of 50 V at 25 ohms and increased until a supramaximal voltage is achieved.] The contractions are recorded on a Gould strip chart recorder. The tissues are washed every 20 minutes and allowed to

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equilibrate. A concentration response curve to the selective m1 receptor agonist McN-A-343 is determined. Tissues are washed every 20 minutes for 60 minutes. The vehicle or compound is added to the bath and the tissues are incubated for 30 minutes, then the McN-A-343 concentration response is repeated. EC₅₀ values are determined for both vehicle and tissues treated with the compound before and after treatment. Antagonist dissociation constants (K_b) are calculated by the dose-ratio method. Compounds displayed K_b values at m1 in the range of 5 to 100 nM.

EXAMPLE 27

m2 receptor antagonist activity on the guinea pig left atria

The technique described by Feifel et al., Brit. J. Pharmacol. 99: 455-460 (1990) was used as follows: Duncan-Hartley guinea pigs (Hazelton) weighing 300-600 g, are asphyxiated with CO₂. The abdomen is opened and the left atria is rapidly removed. The tissues are placed in a Petri dish containing oxygenated Krebs solution [NaCl, 118 mM; KCl, 4.7 mM; CaCl₂, 2.5 mM; KH₂PO₄, 1.2 mM; MgSO₄, 1.2 mM; NaHCO₃, 25 mM; dextrose, 11 mM] warmed to 37°C. Each atria is attached to platinum electrodes with 4-0 surgical silk and placed in a 10 mL jacketed tissue bath containing Krebs buffer at 37°C, bubbled with 5% CO₂ / 95% O₂. The tissues are connected to a Satham-Gould force transducer; 0.75 gram of tension is applied and the tissues are electrically stimulated. [EFS parameters are 3 Hz; 4 ms duration; voltage is set to 5 V.] The contractions are recorded on a Gould strip chart recorder. The tissues are washed every 20 minutes and allowed to equilibrate. A concentration response curve to the agonist carbachol is determined. Tissues are washed every 20 minutes for 60 minutes. The vehicle or compound is added to the bath and the tissues are incubated for 30 minutes, then the carbachol concentration response is repeated. EC₅₀ values are determined for both vehicle and compound treated tissues before and after treatment. Antagonist dissociation constants (K_b) are calculated by

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the dose-ratio method. Compounds displayed K_b values at M2 in the range of 5 to 100 nM.

EXAMPLE 28

M3 receptor antagonist activity on the guinea pig ileum longitudinal muscle

The technique described by Feifel et al., Brit. J. Pharmacol. 99: 455-460 (1990) was used as follows: Duncan-Hartley guinea pigs (Hazelton) weighing 300-600 g, are asphyxiated with CO₂. The abdomen is opened and the caecum and the distal end of the ileum are identified. The ileum is removed and 5 cm of the terminal end (proximal to the caecum) is discarded. The lumen of the remainder is flushed with oxygenated Krebs solution [NaCl, 118 mM; KCl, 4.7 mM; CaCl₂, 2.5 mM; KH₂PO₄, 1.2 mM; MgSO₄, 1.2 mM; NaHCO₃, 25 mM; dextrose, 11 mM] warmed to 30°C. The ileum is cut into 2.5 cm segments and each segment is mounted on a glass pipette. A scalpel is used to lightly cut the surface of the tissue and a cotton swab used to tease the longitudinal muscle free from the underlying circular muscle. Longitudinal muscle segments are attached to glass tissue holders with 4-0 surgical silk and placed in a 10 mL jacketed tissue bath containing Krebs buffer at 30°C, bubbled with 5% CO₂ / 95% O₂. The tissues are connected to a Statham-Gould force transducer. One gram of tension is applied and the contractions are recorded on a Gould strip chart recorder. The tissues are washed every 20 minutes and allowed to equilibrate. A concentration response curve to the agonist carbachol is determined. Tissues are washed every 20 minutes for 60 minutes. The vehicle or compound is added to the bath and the tissues are incubated for 30 minutes, then the carbachol concentration response is repeated. EC₅₀ values are determined for both vehicle and tissues treated with the compound before and after treatment. Antagonist dissociation constants (K_b) are calculated by the dose-ratio method. Compounds displayed K_b values at M3 in the range of 3900 to 24000 nM.

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EXAMPLE 29**m1 and m3 receptor antagonist activity on the human muscarinic receptors expressed in CHO cells**

Preconfluent CHO cells were labeled for 24 hours with 4 $\mu\text{Ci/mL}$ of [^3H] myo-inositol (specific activity 15-20 Ci /mmole). The cells were detached from flasks using 1 mM EDTA in phosphate buffer saline, centrifuged for 5 minutes at 200x g, and resuspended in assay buffer (116 mM NaCl; 10 mM LiCl; 4.7 mM KCl; 1.2 mM MgSO_4 ; 2.5 mM CaCl_2 ; 1.2 mM KH_2PO_4 ; 5 mM NaHCO_3 ; 11 mM dextrose, 20 mM HEPES; pH 7.4 at 37°C) to the desired volume. Four hundred microliters of the cell suspension (approximately 2×10^6 cells) was added to tubes containing buffer or compound and left at room temperature for 30 minutes. Muscarinic agonist (carbachol) was then added and the cells incubated for 30 minutes at 37°C. The reaction was stopped using an acid solution (12% perchloric acid / 3 mM EDTA / 1 mM diethylenetriamine pentaacetic acid) and the tubes placed on ice for 15 minutes. The samples were then neutralized with 3M KOH / 0.25 M 2-(N-morpholino)ethane sulfonic acid / 0.25 M 3-(N-morpholino) propane sulfonic acid and centrifuged at 3000x g for 15 minutes. Five hundred microliters of each supernatant was diluted to 5.5 mL with water and the entire tube contents applied to anion exchange columns. The columns are sequentially washed with 5 mL of H_2O , 15 mL of 60 mM ammonium formate / 5 mM borax and 8 mL of 200 mM ammonium formate / 5 mM borax. The radioactivity in the last eluate was determined by liquid scintillation counting and taken as the amount of [^3H]-inositol monophosphate formed during the incubation. Two different types of experiments were performed: IC_{50} values for compounds were calculated using a fixed concentration of carbachol, or K_b values were generated by performing carbachol concentration-response curves in the absence and presence of a fixed concentration of compound. Compounds displayed K_b values at m1 and m3 in the range of 1 to 100 nM at m1 and 4,000 to 20,000 at m3.

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EXAMPLE 30

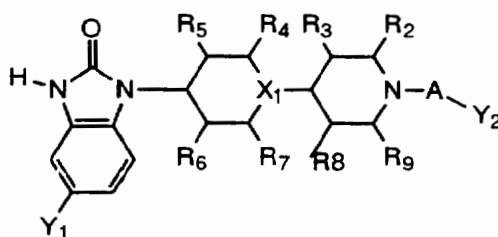
m₂ receptor antagonist activity on the human muscarinic receptors expressed in CHO cells

Preconfluent CHO cells were harvested using 1 mM EDTA in phosphate buffer saline and washed one time by centrifugation in a HEPES buffered physiological salt solution. The cell concentration was adjusted to 3.3×10^6 cells / mL in the HEPES buffer containing 1.3 micromolar isobutylmethylxanthine. Three hundred microliters of the cell suspension was added to tubes containing compound and incubated for 15 minutes at room temperature. Muscarinic agonist (50 microliters of carbachol; 1 micromolar final concentration) was then added followed by 20 microliters of 200 μ M forskolin and the tubes were incubated at 30°C for an additional 15 minutes. The reaction was stopped by placing the tubes in boiling water for 5 minutes. The tubes were cooled on ice and then centrifuged at 12,000 xg for 10 minutes. Fifty microliters of each supernatant was then analyzed for cAMP using a commercially available radioimmunoassay kit following the manufacturer's instructions. Two different types of experiments were performed: IC₅₀ values for compounds were calculated using a fixed concentration of carbachol, or K_b values were generated by performing carbachol concentration-response curves in the absence and presence of a fixed concentration of compound. Compounds displayed K_b values at m₂ in the range of 1 to 100 nM.

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WHAT IS CLAIMED IS:

1. A compound of structural formula I



I

or pharmaceutically acceptable salts thereof, or diastereomers, enantiomers or mixtures thereof;
wherein:

R₂ - R₉ are independently H, alkyl, halo, alkoxy, OH, HOCH₂-, aryl, 3-pyridyl, 5-pyrimidinyl, alkoxy carbonyl, amino, dialkylamino, alkene, thioalkyl, or alkylamino; alternatively, R₄ and R₇ or R₂ and R₉ may be connected as an ethylene bridge to form a bicyclic heterocycle;

Y₁ is H, alkyl, halo, alkylamino, dialkylamino, alkoxy, alkoxyamino, or amino;

Y₂ is heterocycle, or heterocyclyl;

A is H, CHR₁, C(R₁)₂ or carbonyl;

R₁ is alkyl, alkoxy, aryl, heteroaryl, heterocyclyl or heterocycle;
and

X₁ is N or C.

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2. The compound of Claim 1 wherein:

R₂ - R₉ are independently H, alkyl, or halo;

Y₁ is H, alkyl, or halo;

Y₂ is 5-pyrimidinyl, 3-pyridyl, or 1-methyl-5-imidazolyl;

R₁ is alkyl, alkoxy, phenyl, 5-pyrimidinyl, 3-pyridyl, or 1-methyl-5-imidazolyl; and

X₁ is N.

3. The compound:

(3''S,4''R,1'''R and 3''R,4''S,1'''R) 1,3-Dihydro-1-{1'-[1''-(1'''-(5''''-pyrimidinyl)-1'''-ethyl)-3''-methylpiperidin-4''-yl]-piperidin-4'-yl} -2H-benzimidazol-2-one;

(3'R,4'R,1'''R and 3'S,4'S,1'''R) 1,3-Dihydro-1-{1'-[1''-(1'''-(5''''-pyrimidinyl)-1'''-ethyl)-piperidin-4''-yl]-3'-methylpiperidin-4'-yl} -2H-benzimidazol-2-one;

(3'S,4'R,1'''R and 3'R,4'S,1'''R) 1,3-Dihydro-1-{1'-[1''-(1'''-(5''''-pyrimidinyl)-1'''-ethyl)-piperidin-4''-yl]-3'-methylpiperidin-4'-yl} -2H-benzimidazol-2-one;

(3'R,4'R,1'''R and 3'S,4'S,1'''R) 1,3-Dihydro-1-{1'-[1''-(1'''-(5''''-pyrimidinyl)-1'''-ethyl)-piperidin-4''-yl]-3'-ethoxycarbonylpiperidin-4'-yl} -2H-benzimidazol-2-one;

(3'S,4'R,1'''R and 3'R,4'S,1'''R) 1,3-Dihydro-1-{1'-[1''-(1'''-(5''''-pyrimidinyl)-1'''-ethyl)-piperidin-4''-yl]-3'-ethoxycarbonylpiperidin-4'-yl} -2H-benzimidazol-2-one;

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(2''R,4''S,1'''R and 2''R,4''R,1'''R) 1,3-Dihydro-1-{1'-[1''-(1'''-(5'''-pyrimidinyl)-1'''-ethyl)-2''-methyl-piperidin-4''-yl]-piperidin-4'-yl} -2H-benzimidazol-2-one;

(2''S,4''S,1'''R) 1,3-Dihydro-1-{1'-[1''-(1'''-(5'''-pyrimidinyl)-1'''-ethyl)-2''-methyl-piperidin-4''-yl]-piperidin-4'-yl} -2H-benzimidazol-2-one;

(2''S,4''R,1'''R) 1,3-Dihydro-1-{1'-[1''-(1'''-(5'''-pyrimidinyl)-1'''-ethyl)-2''-methyl-piperidin-4''-yl]-piperidin-4'-yl} -2H-benzimidazol-2-one;

(3'S,4'R,1'''S and 3'R,4'S,1'''S) 1,3-Dihydro-1-{1'-[1''-(1'''-phenylethyl)-piperidin-4''-yl]-3'-ethoxycarbonylpiperidin-4'-yl} -2H-benzimidazol-2-one;

(3''S,4''R,1'''R) 1,3-Dihydro-1-{1'-[1''-(1'''-(5'''-pyrimidinyl)-1'''-ethyl)-3''-methylpiperidin-4''-yl]-piperidin-4'-yl} -2H-benzimidazol-2-one;

(3''R,4''S,1'''R) 1,3-Dihydro-1-{1'-[1''-(1'''-(5'''-pyrimidinyl)-1'''-ethyl)-3''-methylpiperidin-4''-yl]-piperidin-4'-yl} -2H-benzimidazol-2-one;

1,3-Dihydro-1-{1'-[1''-(3'''-pyridinecarbonyl)-2''-methyl-piperidin-4''-yl]-piperidin-4'-yl} -2H-benzimidazol-2-one;

(1'''R) 1,3-dihydro-1-{1'-[8''-(1'''-(5'''-pyrimidinyl)-1'''-ethyl)piperidin-4''-yl]-8-azabicyclo[3.2.1]octan-3 α -yl} -2H-benzimidazol-2-one;

(1'''R) 1,3-dihydro-1-{1'-[8''-(1'''-(5'''-pyrimidinyl)-1'''-ethyl)piperidin-4''-yl]-8-azabicyclo[3.2.1]octan-3 β -yl} -2H-benzimidazol-2-one;

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1,3-Dihydro-1-{1'-[1''-(1'''-methylimidazol-5'''-carbonyl)-piperidin-4''-yl]-piperidin-4'-yl}-2H-benzimidazol-2-one; and

1,3-Dihydro-1-{1'-[1''-(1'''-methylimidazol-5'''-ylcarbonyl)-2''-methyl-piperidin-4''-yl]-piperidin-4'-yl}-2H-benzimidazol-2-one.

4. A method for the treatment of abnormal increase in eye axial length in an animal in need thereof, which comprises the step of ocularly administering to said animal a pharmacologically effective amount of a muscarinic pharmacological agent known to be selective for m1, m2 and m4 receptors, but less active at m3 receptors.

5. A method for the treatment of abnormal increase in eye axial length in an animal in need thereof, which comprises the step of ocularly administering to said animal a pharmacologically effective amount of a muscarinic pharmacological agent of Claim 1.

6. A method for the treatment of abnormal increase in eye axial length in an animal in need thereof, which comprises the step of ocularly administering to said animal a pharmacologically effective amount of a muscarinic pharmacological agent according to Claim 1 known to be selective for m1, m2 and m4 receptors, but less active at m3 receptors.

7. A method for the prevention of abnormal increase in eye axial length in an animal in need thereof, which comprises the step of ocularly administering to said animal a pharmacologically effective amount of a muscarinic pharmacological agent known to be selective for m1, m2 and m4 receptors, but less active at m3 receptors.

8. A method for the prevention of abnormal increase in eye axial length in an animal in need thereof, which comprises the

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step of ocularly administering to said animal a pharmacologically effective amount of a muscarinic pharmacological agent according to Claim 1.

9. A method for the prevention of abnormal increase in eye axial length in an animal in need thereof, which comprises the step of ocularly administering to said animal a pharmacologically effective amount of a muscarinic pharmacological agent according to Claim 1 known to be selective for m1, m2 and m4 receptors, but less active at m3 receptors.

10. A method of alleviating the development of amblyopia in the eye of an animal in need thereof, which comprises administering to such an animal a pharmacologically effective amount of a compound of Claim 1.

11. A method of controlling the development of amblyopia in the eye of an animal in need thereof, which comprises administering to such an animal a pharmacologically effective amount of a compound of Claim 1.

12. A composition useful for the prevention of abnormal increase in eye axial length in an animal in need thereof, which comprises a pharmacologically effective amount of a muscarinic pharmacological agent, known to be selective for m1, m2 and m4 receptors, but less active at m3 receptors, in a carrier or diluent buffered to a pH suitable for ocular administration.

13. A composition useful for the treatment of abnormal increase in eye axial length in an animal in need thereof, which comprises a pharmacologically effective amount of a muscarinic pharmacological agent, known to be selective for m1, m2 and m4 receptors, but less active at m3 receptors, in a carrier or diluent buffered to a pH suitable for ocular administration.

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14. A composition useful for the treatment of abnormal increase in eye axial length in an animal in need thereof, which comprises a pharmacologically effective amount of a muscarinic pharmacological agent of Claim 1 in a carrier or diluent buffered to a pH suitable for ocular administration.

15. A composition useful for the prevention of abnormal increase in eye axial length in an animal in need thereof, which comprises a pharmacologically effective amount of a muscarinic pharmacological agent of Claim 1, in a carrier or diluent buffered to a pH suitable for ocular administration.

16. A composition useful for the treatment of abnormal increase in eye axial length in an animal in need thereof, which comprises a pharmacologically effective amount of a muscarinic pharmacological agent of Claim 1, known to be selective for m1, m2 and m4 receptors, but less active at m3 receptors, in a carrier or diluent buffered to a pH suitable for ocular administration.

17. A composition useful for the prevention of abnormal increase in eye axial length in an animal in need thereof, which comprises a pharmacologically effective amount of a muscarinic pharmacological agent of Claim 1, known to be selective for m1, m2 and m4 receptors, but less active at m3 receptors, in a carrier or diluent buffered to a pH suitable for ocular administration.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/17446

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 31/505, 31/445; C07D 401/02, 401/14

US CL : 514/269, 316, 322; 544/333; 546/187, 199

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/269, 316, 322; 544/333; 546/187, 199

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4,329,353 (STOKBROEKX ET AL.) 11 May 1982, page 1, abstract.	1-17
A	US 5,403,845 (DUNBAR ET AL.) 04 April 1995, column 1, lines 10-23.	1-17
E, A	US 5,574,044 (THOMPSON ET AL.) 12 November 1996, page 1, abstract.	1-17

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

24 DECEMBER 1996

Date of mailing of the international search report

19 FEB 1997

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

EBENEZER SACKNEY

Telephone No. (703) 308-1235

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To:

HOSTETLER, MICHAEL J.

WILSON SONSINI GOODRICH & ROSATI 650 PAGE MILL
ROAD PALO ALTO CA 94304 USA**PCT****NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL SEARCH REPORT AND
THE WRITTEN OPINION OF THE INTERNATIONAL
SEARCHING AUTHORITY, OR THE DECLARATION**

(PCT Rule 44.1)

Date of mailing
(day/month/year) 30 September 2015 (30.09.2015)Applicant's or agent's file reference
46682-701601**FOR FURTHER ACTION** See paragraphs 1 and 4 belowInternational application No.
PCT/US2015/037249International filing date
(day/month/year) **23 June 2015 (23.06.2015)**

Applicant

SYDNEXIS, INC.

1. ☒ The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

Filing of amendments and statement under Article 19:

The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

When? The time limit for filing such amendments is normally two months from the date of transmittal of the international search report.**How?** Directly to the International Bureau of WIPO preferably through ePCT or on paper to, 34 chemin des Colombettes 1211 Geneva 20, Switzerland, Facsimile No.: +41 22 338 82 70**For more detailed instructions,** see *PCT Applicant's Guide*, International Phase, paragraphs 9.004 - 9-011.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. ☐ **With regard to any protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:
☐ the protest together with the decision thereon has been transmitted to the International Bureau together with any request to forward the texts of both the protest and the decision thereon to the designated Offices.

☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. Reminders

The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established. Following the expiration of 30 months from the priority date, these comments will also be made available to the public.

Shortly after the expiration of **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau before the completion of the technical preparations for international publication (Rules 90bis.1 and 90bis.3).

Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase **until 30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices. In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months. For details about the applicable time limits, Office by Office, see www.wipo.int/pct/en/texts/time_limits.html and the *PCT Applicant's Guide*, National Chapters.

Within **19 months** from the priority date, the applicant may request that a supplementary international search be carried out by an International Searching Authority, that offers this service (Rule 45bis.1). The procedure for requesting supplementary international search is described in the *PCT Applicant's Guide*, International Phase, paragraphs 8.006-8.032.

Name and mailing address of the ISA/KR

International Application Division
Korean Intellectual Property Office
189 Cheongsa-ro, Seo-gu, Daejeon Metropolitan City, 35208,
Republic of Korea

Facsimile No. 82-42-472-7140

Authorized officer

COMMISSIONER

Telephone No. 82-42-481-5875



* Attention

Copies of the documents cited in the international search report can be searched in the following Korean Intellectual Property Office English website for six months(expire date : **2016.03.30**) from the date of mailing of the international search report.

<http://www.kipo.go.kr/en/> => PCT Services => PCT Services

ID : PCT international application number

PW : **P324W86Q**

Inquiries related to PCT International Search Report or Written Opinion prepared by KIPO as an International Searching Authority can be answered not only by KIPO but also through IPKC (Intellectual Property Korea Center), located in Vienna, VA, which functions as a PCT Help Desk for PCT applicants.

Homepage: <http://www.ipkcenter.com>

Email: ipkc@ipkcenter.com

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 46682-701601	FOR FURTHER ACTION see Form PCT/ISA/220 as well as, where applicable, item 5 below.	
International application No. PCT/US2015/037249	International filing date (<i>day/month/year</i>) 23 June 2015 (23.06.2015)	(Earliest) Priority Date (<i>day/month/year</i>) 24 June 2014 (24.06.2014)
Applicant SYDNEXIS, INC.		

This International search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 4 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of :

☒ the international application in the language in which it was filed

☐ a translation of the international application into _____, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b))

b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6bis(a)).

c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☒ **Certain claims were found unsearchable** (See Box No. II)

3. ☐ **Unity of invention is lacking** (See Box No. III)

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the drawings,

a. the figure of the **drawings** to be published with the abstract is Figure No. 10

☒ as suggested by the applicant.

☐ as selected by this Authority, because the applicant failed to suggest a figure.

☐ as selected by this Authority, because this figure better characterizes the invention.

b. ☐ none of the figures is to be published with the abstract.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2015/037249

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 19-21
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 19-21 pertain to methods for treatment of the human body by therapy, and thus relate to a subject matter which this International Searching Authority is not required, under PCT Article 17(2)(a)(i) and PCT Rule 39.1(iv), to search.
2. ☒ Claims Nos.: 13, 20
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
Claims 13 and 20 are unclear, since they refer to one of claims which are not drafted in accordance with PCT Rule 6.4(a) (PCT Article 6).
3. ☒ Claims Nos.: 5-12, 14-19, 21, 25-30
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of any additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2015/037249**A. CLASSIFICATION OF SUBJECT MATTER****A61K 31/46(2006.01)i, A61K 31/245(2006.01)i, A61K 9/08(2006.01)i, A61P 27/02(2006.01)i**

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K 31/46; A61K 31/135; A61P 29/00; A61K 31/55; A61K 31/505; A61K 9/50; A61K 47/32; A61K 47/38; A61K 47/42; A61K 38/13; C07D 401/14; A61K 31/245; A61K 9/08; A61P 27/02

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

eKOMPASS(KIPO internal) & Keywords: ophthalmic composition, muscarinic antagonist, deuterated water, shelf life, stability

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5716952 A (WOLDEMUSSE, E. et al.) 10 February 1998 See columns 2, 4; and table 1.	22-24
A		1-4
X	WO 97-16192 A1 (MERCK & CO., INC.) 09 May 1997 See pages 2-4, 12.	22-24
X	US 2012-0015035 A1 (WILDSOET, C. F. et al.) 19 January 2012 See paragraphs [0030], [0031], [0075]; and claims 1, 13, 14.	22-24
A	WO 2011-019940 A2 (SEROS MEDICAL, LLC) 17 February 2011 See the whole document.	1-4, 22-24
A	US 2012-0028910 A1 (COMBAL, J.-P. et al.) 02 February 2012 See the whole document.	1-4, 22-24

☐ Further documents are listed in the continuation of Box C.☒ See patent family amex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

30 September 2015 (30.09.2015)

Date of mailing of the international search report

30 September 2015 (30.09.2015)

Name and mailing address of the ISA/KR

International Application Division
Korean Intellectual Property Office
189 Cheongsa-ro, Seo-gu, Daejeon Metropolitan City, 35208,
Republic of Korea

Facsimile No. +82-42-472-7140

Authorized officer

LEE, Jeong A

Telephone No. +82-42-481-8740



INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2015/037249

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5716952 A	10/02/1998	CA 2132007 C EP 0634932 A1 EP 0634932 B1 WO 93-18772 A1	18/07/2006 04/10/2001 19/06/2002 30/09/1993
WO 97-16192 A1	09/05/1997	AU 7528696 A	22/05/1997
US 2012-0015035 A1	19/01/2012	US 8883214 B2 WO 2010-083129 A2 WO 2010-083129 A9	11/11/2014 22/07/2010 09/12/2010
WO 2011-019940 A2	17/02/2011	EP 2464387 A2 EP 2464387 A4 US 2012-0203161 A1 WO 2011-019940 A3	20/06/2012 15/05/2013 09/08/2012 16/06/2011
US 2012-0028910 A1	02/02/2012	AU 2008-309923 A1 AU 2008-309923 B2 BR PI0819081 A2 CA 2702082 A1 CN 101820917 A CN 101820917 B EA 019867 B1 EA 201000441 A1 EP 2195033 A1 HK 1147937 A1 JP 2010-540671 A JP 5640207 B2 MX 2010003774 A NZ 584275 A WO 2009-046967 A1	16/04/2009 03/04/2014 22/04/2015 16/04/2009 01/09/2010 02/01/2013 30/06/2014 29/10/2010 16/06/2010 13/09/2013 24/12/2010 17/12/2014 27/04/2010 29/06/2012 16/04/2009

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43bis.1)

To: HOSTETLER, MICHAEL J. WILSON SONSINI GOODRICH & ROSATI 650 PAGE MILL ROAD PALO ALTO CA 94304 USA

Date of mailing (day/month/year)	30 September 2015 (30.09.2015)

Applicant's or agent's file reference 46682-701601	FOR FURTHER ACTION See paragraph 2 below

International application No. PCT/US2015/037249	International filing date (day/month/year) 23 June 2015 (23.06.2015)	Priority date(day/month/year) 24 June 2014 (24.06.2014)

International Patent Classification (IPC) or both national classification and IPC A61K 31/46(2006.01)i, A61K 31/245(2006.01)i, A61K 9/08(2006.01)i, A61P 27/02(2006.01)i

Applicant SYDNEXIS, INC.

1. This opinion contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☒	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/KR International Application Division Korean Intellectual Property Office 189 Cheongsu-ro, Seo-gu, Daejeon Metropolitan City, 35208, Republic of Korea Facsimile No. +82-42-472-7140	Date of completion of this opinion 30 September 2015 (30.09.2015)	Authorized officer LEE, Jeong A Telephone No. +82-42-481-8740



**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US2015/037249

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of :
- ☒ the international application in the language in which it was filed
 - ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b))
2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))
3. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing:
- a. ☐ forming part of the international application as filed:
 - ☐ in the form of an Annex C/ST.25 text file.
 - ☐ on paper or in the form of an image file.
 - b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
 - ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).
4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
5. Additional comments:

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US2015/037249

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application

☒ claims Nos. 5-21, 25-30

because:

☒ the said international application, or the said claims Nos. 19-21

relate to the following subject matter which does not require an international search (*specify*):

The subject matter of claims 19-21 relates to a method for treatment of the human body by therapy (PCT Rule 43bis.1(b), Rule 67.1(iv)).

☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 13, 20
are so unclear that no meaningful opinion could be formed (*specify*):

Claims 13 and 20 are unclear since they refer to one of the claims which are not drafted in accordance with PCT Rule 6.4(a) (PCT Article 6).

☐ the claims, or said claims Nos. _____ are so inadequately supported
by the description that no meaningful opinion could be formed (*specify*):

☒ no international search report has been established for said claims Nos. 5-21, 25-30

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

☐ furnish a sequence listing in the form of an Annex C/ST.25 text file, and such listing was not available to the International Searching Authority in the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.

☐ furnish a sequence listing on paper or in the form of an image file complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.

☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13ter.1(a) or (b).

☐ See Supplemental Box for further details.

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.

PCT/US2015/037249

Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-4,22-24	YES
	Claims	NONE	NO
Inventive step (IS)	Claims	1-4	YES
	Claims	22-24	NO
Industrial applicability (IA)	Claims	1-4,22-24	YES
	Claims	NONE	NO

2. Citations and explanations :

※ The word “pirenzapine” of claims 2 and 23 is considered to be a typo for “pirenzepine”.

Reference is made to the following documents:

D1: US 5716952 A (WOLDEMUSSIE, E. et al.) 10 February 1998

D2: WO 97-16192 A1 (MERCK & CO., INC.) 09 May 1997

D3: US 2012-0015035 A1 (WILDSOET, C. F. et al.) 19 January 2012

D4: WO 2011-019940 A2 (SEROS MEDICAL, LLC) 17 February 2011

D5: US 2012-0028910 A1 (COMBAL, J.-P. et al.) 02 February 2012

2.1. Novelty and Inventive Step

2.1.1. Claim 1

Claim 1 relates to an ophthalmic composition comprising from about 0.001 wt% to about 0.05 wt% of a muscarinic antagonist and deuterated water, at a pD of from about 4.2 to about 7.9.

D1, which is considered to be the closest prior art to the subject matter of claim 1, discloses an ophthalmic formulation comprising about 0.0001 to about 0.1 %W/V of a muscarinic antagonist compound, pH adjustor, and purified water (see column 2, lines 50, 51; column 4, lines 50-54; and table 1 in D1).

Claim 1 differs from D1 in that the ophthalmic composition comprises deuterated water, and said difference is not disclosed in any other available prior art documents D2-D5. Furthermore, the applicant has shown that the ophthalmic composition comprising deuterated water in the present claim provides a more stabilized ophthalmic composition with extended shelf life upon

Continued on Supplemental Box

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US2015/037249

Box No. VII Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

Claims 5-12, 14-19, 21 and 25-30 do not comply with PCT Rule 6.4(a) because multiple dependent claims shall not serve as a basis for any other multiple dependent claim.

Claim 19 does not comply with PCT Rule 6.4(a) because the multiple dependent claim does not refer to other claims in the alternative.

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of : Box No. V

storage, compared to an ophthalmic composition comprising regular water. Accordingly, claim 1 is not anticipated by any of the documents D1-D5, nor is it obvious from the documents, taken individually or in any combination. Therefore, claim 1 is novel and involves an inventive step under PCT Article 33(2) and (3).

2.1.2. Claims 2-4

Claims 2-4 are essentially dependent on claim 1 and therefore meet the requirements of PCT Article 33(2) and (3).

2.1.3. Claim 22

Claim 22 relates to an ophthalmic composition comprising from about 0.001 wt% to about 0.05 wt% of a muscarinic antagonist and water, at a pH of from about 3.8 to about 7.5.

D1, which is considered to be the closest prior art to the subject matter of claim 22, discloses an ophthalmic formulation comprising about 0.0001 to about 0.1 %W/V of a muscarinic antagonist compound, a pH adjustor, and purified water (see column 2, lines 50, 51; column 4, lines 50-54; and table 1 in D1).

Claim 22 differs from D1 in the ranges of the amount of the muscarinic antagonist and pH. However, said differences appear to come within the scope of the customary practice followed by a person skilled in the art. Moreover, the applicant has not shown that the ranges of amount and pH defined in the present claim produce an unexpected beneficial effect compared to those in D1. Therefore, claim 22 lacks an inventive step under PCT Article 33(3) as being obvious over D1.

2.1.4. Claim 23

Claim 23, dependent on claim 22, defines the sort of muscarinic antagonist. As D1 discloses pirenzepine as the muscarinic antagonist compound (see column 2, lines 50, 51 in D1), it would have been obvious for a person skilled in the art to arrive at the invention of claim 23. Therefore, claim 23 lacks an inventive step under PCT Article 33(3) as being obvious over D1.

Continued on The Next Page

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of : Previous Page

2.1.5. Claim 24

Claim 24, dependent on claim 22 or 23, defines the ophthalmic composition as comprising at least about 80% of the muscarinic antagonist based on initial concentration after extended period of time under storage condition. Although D1 does not disclose the additional feature of claim 24, said feature is merely a property of the composition, and a person skilled in the art would easily arrive at the claimed invention by general experimentation alone without exercising any ingenuity. Therefore, claim 24 lacks an inventive step under PCT Article 33(3) as being obvious over D1.

2.2. Industrial Applicability

Claims 1-4 and 22-24 are industrially applicable under PCT Article 33(4).

Electronic Acknowledgement Receipt

EFS ID:	24563015
Application Number:	14726139
International Application Number:	
Confirmation Number:	7389
Title of Invention:	OPHTHALMIC COMPOSITION
First Named Inventor/Applicant Name:	Gregory I. Ostrow
Customer Number:	21971
Filer:	Michael J. Hostetler/Jennifer Vail
Filer Authorized By:	Michael J. Hostetler
Attorney Docket Number:	46682-701.201
Receipt Date:	07-JAN-2016
Filing Date:	29-MAY-2015
Time Stamp:	18:15:35
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		46682-701-201_IDS.pdf	238055 4eb9e4a21d08bf762c2f9478975eed6564eafec9	yes	11

	Multipart Description/PDF files in .zip description				
	Document Description		Start	End	
	Transmittal Letter		1	4	
	Information Disclosure Statement (IDS) Form (SB08)		5	11	
Warnings:					
Information:					
2	Foreign Reference	CN101327216A_EFS.PDF	816530	no	17
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Warnings:					
Information:					
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Warnings:					
Information:					
4	Foreign Reference	EP0332826A1_EFS.PDF	557372	no	9
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Information:					
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27	Non Patent Literature	KREZEL.PDF	297359	no	6
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28	Non Patent Literature	LAI_RAPIDTRANSPORT_1482-1487.PDF	228557	no	6
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29	Non Patent Literature	LUND.PDF	1200284	no	13
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31	Non Patent Literature	OBATA_DEUTERIUM.PDF	355853	no	3
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32	Non Patent Literature	OBATA_DEUTERIUM_MR_IN_VO.PDF	3317251	no	4
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Information:					
33	Non Patent Literature	PCTUS2015037249_ISRWO_30 SEPT2015.PDF	1463878	no	13
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34	Non Patent Literature	RICHARD_INTLJOURNALPHARMA_144.PDF	1744340	no	7
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Information:					

36	Non Patent Literature	TAKTAK_JPHARMPHARMACAL_578.PDF	884753 0a9a8e7576e997508c05ca9bfe0896976f3180af	no	5
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37	Non Patent Literature	THE_FDA_GUIDANCE.PDF	8549896 7244c38b4e7538c9eb449f54705a231429fd48cb	no	63
Warnings:					
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38	Non Patent Literature	VIEGAS_OSMOTICBEHAVIOROF_POLOXAMER_157-162.PDF	230179 d4fe624a3ef2e24e0bc2a3d54f687698782e5711	no	7
Warnings:					
Information:					
Total Files Size (in bytes):			44271842		
This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503. <u>New Applications Under 35 U.S.C. 111</u> If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application. <u>National Stage of an International Application under 35 U.S.C. 371</u> If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course. <u>New International Application Filed with the USPTO as a Receiving Office</u> If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.					

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor:	OSTROW; Gregory I. et al.	Group Art Unit:	1621
Serial Number:	14/726,139	Examiner:	Fay, Zohreh A
Filing or 371 (c) Date:	2015-05-29	CONFIRMATION NO:	7389
Title:	OPHTHALMIC COMPOSITION		

FILED ELECTRONICALLY ON: January 7, 2016

Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

INFORMATION DISCLOSURE STATEMENT
UNDER 37 CFR § 1.97

Commissioner for Patents:

An Information Disclosure Statement along with attached PTO/SB/08 is hereby submitted. A copy of each listed publication is submitted, if required, pursuant to 37 CFR §§1.97-1.98, as indicated below.

The Examiner is requested to review the information provided and to make the information of record in the above-identified application. The Examiner is further requested to initial and return the attached PTO/SB/08 in accordance with MPEP § 609.

The right to establish the patentability of the claimed invention over any of the information provided herewith, and/or to prove that this information may not be prior art, and/or to prove that this information may not be enabling for the teachings purportedly offered, is hereby reserved.

This statement is not intended to represent that a search has been made or that the information cited in the statement is, or is considered to be, prior art or material to patentability as defined in § 1.56.

- A. ☒ 37 CFR § 1.97 (b). This Information Disclosure Statement should be considered by the Office because:
- ☐ (1) It is being filed within 3 months of the filing date of a national application and is other than a continued prosecution application under § 1.53 (d);
-- OR --
 - ☐ (2) It is being filed within 3 months of entry of the national stage as set forth in § 1.491 in an international application;
-- OR --
 - ☒ (3) It is being filed before the mailing of a first Office action on the merits;
-- OR --
 - ☐ (4) It is being filed before the mailing of a first Office action after the filing of a request for continued examination under § 1.114.
- B. ☐ 37 CFR § 1.97(c). Although this Information Disclosure Statement is being filed after the period specified in 37 CFR § 1.97(b), above, it is filed before the mailing date of the earlier of (1) a final office action under § 1.113, (2) a notice of allowance under § 1.311, or (3) an action that otherwise closes prosecution on the merits, this Information Disclosure Statement should be considered because it is accompanied by one of:
- ☐ a statement as specified in §1.97 (e) provided concurrently herewith;
-- OR --
 - ☐ a fee of \$180.00 as set forth in § 1.17 (p) authorized below, enclosed, or included with the payment of other papers filed together with this statement.
- C. ☐ 37 CFR § 1.97 (d). Although this Information Disclosure Statement is being filed after the mailing date of the earlier of (1) a final office action under § 1.113 or (2) a notice of allowance under § 1.311, it is being filed before payment of the issue fee and should be considered because it is accompanied by:
- i. a statement as specified in § 1.97 (e);
-- AND --
 - ii. a fee of \$180.00 as set forth in §1.17(p) is authorized below, enclosed, or included with the payment of other papers filed together with this Statement.
- D. ☐ 37 CFR §1.97 (e). Statement.
- ☐ A statement is provided herewith to satisfy the requirement under 37Â CFR §§ 1.97 (c);
-- AND/OR --
 - ☐ A statement is provided herewith to satisfy the requirement under 37Â CFR §§ 1.97 (d);
-- AND/OR --
 - ☐ A copy of a dated communication from a foreign patent office clearly showing that the information disclosure statement is being submitted within 3 months of the filing date on the communication is provided in lieu of a statement under 37 C.F.R. § 1.97(e) (1) as provided for under MPEP 609.04(b) V.

- E. ☐ *Statement Under 37 C.F.R. §1.704(d)*. Each item of information contained in the information disclosure statement was first cited in any communication from a patent office in a counterpart foreign or international application or from the Office or is a communication that was issued by a patent office in a counterpart foreign or international application or by the Office that was received by an individual designated in § 1.56(c) not more than thirty (30) days prior to the filing of this information disclosure statement. This statement is made pursuant to the requirements of 37 C.F.R. §1.704(d) to avoid reduction of the period of adjustment of the patent term for Applicant(s) delay.
- F. ☒ *37 CFR §1.98 (a) (2)*. The content of the Information Disclosure Statement is as follows:
- ☐ Copies of each of the references listed on the attached Form PTO/SB/08 are enclosed herewith.
- OR --
- ☒ Copies of U.S. Patent Documents (issued patents and patent publications) listed on the attached Form PTO/SB/08 is NOT enclosed.
- AND/OR --
- ☒ Copies of Foreign Patent Documents and/or Non Patent Literature Documents listed on the attached Form PTO/SB/08 are enclosed in accordance with 37 CFR §1.98 (a)(2).
- AND/OR --
- ☒ Copies of pending unpublished U.S. patent applications are enclosed in accordance with 37 CFR §1.98 (a) (2) (iii).
- G. ☒ *37 CFR §1.98(a)(3)*. The Information Disclosure Statement includes non-English patents and/or references.
- ☐ Pursuant to 37 CFR §1.98(a)(3)(i), a concise explanation of the relevance of each patent, publication or other information provided that is not in English is provided herewith.
- ☐ Pursuant to MPEP 609(B), an English language copy of a foreign search report is submitted herewith to satisfy the requirement for a concise explanation where non-English language information is cited in the search report.
- OR --
- ☐ A concise explanation of the relevance of each patent, publication or other information provided that is not in English is as follows: _____
- ☒ Pursuant to 37 CFR §1.98(a) (3) (ii), a copy of a translation, or a portion thereof, of the non-English language reference(s) is provided herewith.
- H. ☐ *37 CFR §1.98(d)*. Copies of patents, publications and pending U.S. patent applications, or other information specified in 37 C.F.R. § 1.98(a) are not provided herewith because:
- ☐ Pursuant to 37 CFR §1.98(d)(1) the information was previously submitted in an Information Disclosure Statement, or cited by examiner for another application under which this application claims priority for an earlier effective filing date under 35 U.S.C. 120.
- Application in which the information was submitted: _____
- Information Disclosure Statement(s) filed on: _____
- AND
- ☐ The information disclosure statement submitted in the earlier application complied with paragraphs (a) through (c) of 37 CFR §1.98.

- I. ☒ *Fee Authorization.* The Commissioner is hereby authorized to charge the above-referenced fees of \$0.00 and charge any additional fees or credit any overpayment associated with this communication to Deposit Account No. 23-2415 (Docket No.46682-701.201).

Respectfully submitted,

WILSON SONSINI GOODRICH & ROSATI

Dated: January 7, 2016

By: /Michael Hostetler/
Michael Hostetler, Reg. No. 47664

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Customer No. 21971



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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
14/726,139	05/29/2015	Gregory I. Ostrow	46682-701.201

CONFIRMATION NO. 7389

21971
WILSON, SONSINI, GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304-1050

PUBLICATION NOTICE



Title:OPHTHALMIC COMPOSITION

Publication No.US-2015-0366854-A1

Publication Date:12/24/2015

NOTICE OF PUBLICATION OF APPLICATION

The above-identified application will be electronically published as a patent application publication pursuant to 37 CFR 1.211, et seq. The patent application publication number and publication date are set forth above.

The publication may be accessed through the USPTO's publically available Searchable Databases via the Internet at www.uspto.gov. The direct link to access the publication is currently <http://www.uspto.gov/patft/>.

The publication process established by the Office does not provide for mailing a copy of the publication to applicant. A copy of the publication may be obtained from the Office upon payment of the appropriate fee set forth in 37 CFR 1.19(a)(1). Orders for copies of patent application publications are handled by the USPTO's Office of Public Records. The Office of Public Records can be reached by telephone at (703) 308-9726 or (800) 972-6382, by facsimile at (703) 305-8759, by mail addressed to the United States Patent and Trademark Office, Office of Public Records, Alexandria, VA 22313-1450 or via the Internet.

In addition, information on the status of the application, including the mailing date of Office actions and the dates of receipt of correspondence filed in the Office, may also be accessed via the Internet through the Patent Electronic Business Center at www.uspto.gov using the public side of the Patent Application Information and Retrieval (PAIR) system. The direct link to access this status information is currently <http://pair.uspto.gov/>. Prior to publication, such status information is confidential and may only be obtained by applicant using the private side of PAIR.

Further assistance in electronically accessing the publication, or about PAIR, is available by calling the Patent Electronic Business Center at 1-866-217-9197.

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of: Inventors: Gregory I. Ostrow, <i>et al.</i> Serial No.: 14/726,139 Filing Date: May 29, 2015 Title: OHTHALMIC COMPOSITION	Art Unit: 1621 Examiner: Fay, Zohreh A. Confirmation No.: 7389 Customer No.: 021971 <u>Certificate of Electronic Filing</u> I hereby certify that this Response to Restriction Requirement and all marked attachments are being deposited by Electronic Filing on December 8, 2015 by using the EFS-Web patent filing system and addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450. By: <u>/Linda Anders/</u>

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

RESPONSE TO RESTRICTION REQUIREMENT

Dear Madam:

This paper is being submitted in response to the Restriction Requirement dated December 2, 2015. This Response is timely filed; therefore, no fees are believed to be due. However, the Commissioner is hereby authorized to charge any fees that may be required, or credit any overpayment to Deposit Account No. 23-2415, referencing Attorney Docket No. 46682-701.201. Applicants respectfully request entry of the proposed amendments prior to examination and allowance of the pending claims.

Amendments to the Claims begin on page 2.

Remarks begin on page 6.

The Conclusion is on page 7.

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings in the above-referenced patent application. The foregoing amendments are without prejudice and do not constitute an admission regarding the patentability of the amended subject matter and should not so be construed.

Listing of Claims

1. (Original) An ophthalmic composition, comprising from about 0.001 wt% to about 0.05 wt% of a muscarinic antagonist and deuterated water, at a pD of from about 4.2 to about 7.9.
2. (Original) The ophthalmic composition of claim 1, wherein the muscarinic antagonist comprises atropine, atropine sulfate, noratropine, atropine-N-oxide, tropine, tropic acid, hyoscine, scopolomine, tropicamide, cyclopentolate, pirenzapine, homatropine, or a combination thereof.
3. (Original) The ophthalmic composition of claim 2, wherein the muscarinic antagonist is atropine, or atropine sulfate.
4. (Original) The ophthalmic composition of claim 1, wherein the ophthalmic composition has a pD of one of: less than about 7.3, less than about 7.2, less than about 7.1, less than about 7, less than about 6.8, less than about 6.5, less than about 6.4, less than about 6.3, less than about 6.2, less than about 6.1, less than about 6, less than about 5.9, less than about 5.8, less than about 5.2, or less than about 4.8 after extended period of time under storage condition.
5. (Original) The ophthalmic composition of claim 1, wherein the ophthalmic composition comprises one of: at least about 80%, at least about 85%, at least about 90%, at least about 93%, at least about 95%, at least about 97%, at least about 98%, or at least about 99% of the muscarinic antagonist based on initial concentration after extended period of time under storage condition.
6. (Original) The ophthalmic composition of claim 1, wherein the ophthalmic composition further has a potency of one of: at least 80%, at least 85%, at least 90%, at least 93%, at least 95%, at least 97%, at least 98%, or at least 99% after extended period of time under storage condition.
7. (Original) The ophthalmic composition of claim 1, wherein the extended period of time is one of: about 1 week, about 2 weeks, about 3 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 8 months, about 10 months, about 12 months, about 18 months, about 24 months, about 36 months, about 4 years, or about 5 years.

8. (Original) The ophthalmic composition of claim 1, wherein the storage condition has a storage temperature of from about 2°C to about 10°C or from about 16°C to about 26°C.
9. (Original) The ophthalmic composition of claim 1, wherein the muscarinic antagonist is present in the composition at a concentration of one of: from about 0.001 wt% to about 0.04 wt%, from about 0.001 wt% to about 0.03 wt%, from about 0.001 wt% to about 0.025 wt%, from about 0.001 wt% to about 0.02 wt%, from about 0.001 wt% to about 0.01 wt%, from about 0.001 wt% to about 0.008 wt%, or from about 0.001 wt% to about 0.005 wt%.
10. (Original) The ophthalmic composition of claim 1, wherein the ophthalmic composition further comprises an osmolarity adjusting agent.
11. (Original) The ophthalmic composition of claim 10, wherein the osmolarity adjusting agent is sodium chloride.
12. (Original) The ophthalmic composition of claim 1, wherein the ophthalmic composition further comprises a preservative.
13. (Original) The ophthalmic composition of claim 12, wherein the preservative is selected from benzalkonium chloride, cetrimonium, sodium perborate, stabilized oxychloro complex, SofZia, polyquaternium-1, chlorobutanol, edetate disodium, polyhexamethylene biguanide, or combinations thereof.
14. (Original) The ophthalmic composition of claim 1, wherein the ophthalmic composition further comprises a buffer agent.
15. (Original) The ophthalmic composition of claim 14, wherein the buffer agent is selected from borates, borate-polyol complexes, phosphate buffering agents, citrate buffering agents, acetate buffering agents, carbonate buffering agents, organic buffering agents, amino acid buffering agents, or combinations thereof.
16. (Original) The ophthalmic composition of claim 1, wherein the ophthalmic composition is essentially free of procaine and benactyzine, or pharmaceutically acceptable salts thereof.

17. (Original) The ophthalmic composition of claim 1, wherein the ophthalmic composition has a dose-to-dose muscarinic antagonist concentration variation of one of: less than 50%, less than 40%, less than 30%, less than 20%, less than 10%, or less than 5%.
18. (Original) The ophthalmic composition of claim 17, wherein the dose-to-dose muscarinic antagonist concentration variation is based on one of: 10 consecutive doses, 8 consecutive doses, 5 consecutive doses, 3 consecutive doses, or 2 consecutive doses.
19. (Original) The ophthalmic composition of claim 1, wherein the ophthalmic composition further comprises a pD adjusting agent.
20. (Original) The ophthalmic composition of claim 19, wherein the pD adjusting agent comprises DCl, NaOD, CD₃COOD, or C₆D₈O₇.
21. (Original) The ophthalmic composition of claim 1, wherein the ophthalmic composition comprises one of: less than 5% of H₂O, less than 4% of H₂O, less than 3% of H₂O, less than 2% of H₂O, less than 1% of H₂O, less than 0.5% of H₂O, less than 0.1% of H₂O, or 0% of H₂O.
22. (Original) The ophthalmic composition of claim 1, wherein the ophthalmic composition is not formulated as an injectable formulation.
23. (Original) The ophthalmic composition of claim 1, wherein the ophthalmic composition is formulated as an ophthalmic solution for the treatment of pre-myopia, myopia, or progression of myopia.
24. (Original) An ophthalmic solution, comprising from about 0.001 wt% to about 0.05 wt% of a muscarinic antagonist and deuterated water, at a pD of from about 4.2 to about 7.9.
25. (Original) The ophthalmic solution of claim 24, wherein the ophthalmic solution has a pD of one of: less than about 7.3, less than about 7.2, less than about 7.1, less than about 7, less than about 6.8, less than about 6.5, less than about 6.4, less than about 6.3, less than about 6.2, less than about 6.1, less than about 6, less than about 5.9, less than about 5.8, less than about 5.2, or less than about 4.8 after extended period of time under storage condition.

26. (Original) The ophthalmic solution of claim 24, wherein the muscarinic antagonist comprises atropine, atropine sulfate, noratropine, atropine-N-oxide, tropine, tropic acid, hyoscine, scopolomine, tropicamide, cyclopentolate, pirenzapine, homatropine, or a combination thereof.
27. (Original) The ophthalmic solution of claim 24, wherein the ophthalmic solution comprises one of: less than 5% of H₂O, less than 4% of H₂O, less than 3% of H₂O, less than 2% of H₂O, less than 1% of H₂O, less than 0.5% of H₂O, less than 0.1% of H₂O, or 0% of H₂O.
28. (Withdrawn) A method of arresting or preventing myopia development, comprising administering to an eye of an individual in need thereof an effective amount of an ophthalmic composition comprising from about 0.001 wt% to about 0.05 wt% of a muscarinic antagonist and deuterated water, at a pD of from about 4.2 to about 7.9.
29. (Withdrawn) The method of claim 28, wherein the ophthalmic composition is stored at between about 2 °C to about 10 °C prior to first use.
30. (Withdrawn) The method of claim 28, wherein the ophthalmic composition is stored at between about 16 °C to about 26 °C after first use.

REMARKS

Claims

Claims 1-30 are pending and subject to examination. Claims 28-30 are marked as withdrawn and are retained for possible rejoinder upon allowance of a generic claim. Applicants reserve the right to pursue any withdrawn or cancelled subject matter, or no longer claimed or as-yet unclaimed subject matter, in this or a related application.

Restriction Requirement

In the Office Action mailed December 2, 2015, the Examiner has set forth an election of species requirement as follows:

- I. Claims 1-27, drawn to an ophthalmic composition comprising muscarinic antagonist and deuterated water; and
- II. Claims 28-30, drawn to a method of arresting or preventing myopia using the composition of Group I.

Applicants hereby elect **Group I**, claims 1-27, drawn to an ophthalmic composition comprising muscarinic antagonist and deuterated water.

Species Election

The Office has additionally required the Applicants to elect a single disclosed species from within the elected group of atropine, atropine sulfate, noratropine, atropine-n-oxide, atropic acid, hyoscine, scopolamine, tropicamide, cyclopentolate, pirenzapine, and homatropine.

Applicants are required under 35 U.S.C. 121 to elect a single disclosed species or a single grouping of patentably indistinct species, for prosecution on the merits to which the claims shall be restricted if no generic claim is finally held to be allowable.

For search purposes only, Applicants elect the following species: **atropine**. This election encompasses claims 1-27.

CONCLUSION

Applicants submit that this response fully addresses the Restriction Requirement mailed December 2, 2015, and respectfully request consideration and allowance of the claims. Applicants reserve the right pursuant to 35 U.S.C. §121 to file one or more divisional applications directed to one or more of the non-elected inventions during the pendency of the present application.

Should the Examiner have any questions, the Examiner is encouraged to contact the undersigned attorney at (858) 350-2306. If additional fees are believed to be required, the Commissioner is authorized to charge any additional fees to Deposit Account No. 46682-701.201.

Respectfully submitted,

WILSON SONSINI GOODRICH & ROSATI
A Professional Corporation

Date: December 8, 2015

By: /Michael Hostetler/
Michael J. Hostetler
Registration No. 47,664

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Electronic Acknowledgement Receipt

EFS ID:	24301406
Application Number:	14726139
International Application Number:	
Confirmation Number:	7389
Title of Invention:	OPHTHALMIC COMPOSITION
First Named Inventor/Applicant Name:	Gregory I. Ostrow
Customer Number:	21971
Filer:	Michael J. Hostetler/Linda Anders
Filer Authorized By:	Michael J. Hostetler
Attorney Docket Number:	46682-701.201
Receipt Date:	08-DEC-2015
Filing Date:	29-MAY-2015
Time Stamp:	18:50:54
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		46682-701-201-responseRR.pdf	141324 44a28f5e53adb3f45d911f8e522e9280a100785e	yes	7

Multipart Description/PDF files in .zip description			
Document Description	Start	End	
Response to Election / Restriction Filed	1	1	
Claims	2	5	
Applicant Arguments/Remarks Made in an Amendment	6	7	

Warnings:

Information:

Total Files Size (in bytes):	141324

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875					Application or Docket Number 14/726,139		Filing Date 05/29/2015		☐ To be Mailed				
ENTITY: ☐ LARGE ☒ SMALL ☐ MICRO													
APPLICATION AS FILED – PART I													
(Column 1)			(Column 2)										
FOR		NUMBER FILED		NUMBER EXTRA		RATE (\$)		FEE (\$)					
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))		N/A		N/A		N/A							
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))		N/A		N/A		N/A							
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))		N/A		N/A		N/A							
TOTAL CLAIMS (37 CFR 1.16(i))		minus 20 =		*		X \$ =							
INDEPENDENT CLAIMS (37 CFR 1.16(h))		minus 3 =		*		X \$ =							
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))		If the specification and drawings exceed 100 sheets of paper, the application size fee due is \$310 (\$155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).											
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))													
* If the difference in column 1 is less than zero, enter "0" in column 2.						TOTAL							
APPLICATION AS AMENDED – PART II													
(Column 1)			(Column 2)			(Column 3)							
AMENDMENT	12/08/2015		CLAIMS REMAINING AFTER AMENDMENT			HIGHEST NUMBER PREVIOUSLY PAID FOR		PRESENT EXTRA		RATE (\$)		ADDITIONAL FEE (\$)	
	Total (37 CFR 1.16(i))		* 30		Minus	** 30		= 0		X \$40 =		0	
	Independent (37 CFR 1.16(h))		* 3		Minus	*** 3		= 0		X \$210 =		0	
	☐ Application Size Fee (37 CFR 1.16(s))												
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))												
										TOTAL ADD'L FEE		0	
(Column 1)			(Column 2)			(Column 3)							
AMENDMENT			CLAIMS REMAINING AFTER AMENDMENT			HIGHEST NUMBER PREVIOUSLY PAID FOR		PRESENT EXTRA		RATE (\$)		ADDITIONAL FEE (\$)	
	Total (37 CFR 1.16(i))		*		Minus	**		=		X \$ =			
	Independent (37 CFR 1.16(h))		*		Minus	***		=		X \$ =			
	☐ Application Size Fee (37 CFR 1.16(s))												
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))												
										TOTAL ADD'L FEE			
* If the entry in column 1 is less than the entry in column 2, write "0" in column 3. ** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20". *** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3". The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.											LIE /KIM P. DOZIER/		

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.



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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/726,139	05/29/2015	Gregory I. Ostrow	46682-701.201	7389

21971 7590 12/02/2015
WILSON, SONSINI, GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304-1050

EXAMINER

FAY, ZOHREH A

ART UNIT	PAPER NUMBER

1621

NOTIFICATION DATE	DELIVERY MODE

12/02/2015

ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

patentdocket@wsgr.com

Office Action Summary	Application No. 14/726,139	Applicant(s) OSTROW ET AL.	
	Examiner ZOHREH FAY	Art Unit 1621	AIA (First Inventor to File) Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 2 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☐ Responsive to communication(s) filed on _____.
☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.
- 2a) ☐ This action is **FINAL**. 2b) ☐ This action is non-final.
- 3) ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.
- 4) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

- 5) ☒ Claim(s) 1-30 is/are pending in the application.
5a) Of the above claim(s) _____ is/are withdrawn from consideration.
- 6) ☐ Claim(s) _____ is/are allowed.
- 7) ☐ Claim(s) _____ is/are rejected.
- 8) ☐ Claim(s) _____ is/are objected to.
- 9) ☒ Claim(s) 1-30 are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

- 10) ☐ The specification is objected to by the Examiner.
- 11) ☐ The drawing(s) filed on _____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

- a) ☐ All b) ☐ Some** c) ☐ None of the:
1. ☐ Certified copies of the priority documents have been received.
 2. ☐ Certified copies of the priority documents have been received in Application No. _____.
 3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)	3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____
2) ☐ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____	4) ☐ Other: _____

DETAILED ACTION

Election/Restrictions

Restriction to one of the following inventions is required under 35 U.S.C. 121:

I. Claims 1-27 are, drawn to an ophthalmic composition comprising muscarinic antagonist and deuterated water, classified in class A61K 31/415.

II. Claims 28-30 are, drawn to a method of arresting or preventing myopia using the composition of Group I, classified in class A61k 31/415.

The inventions are distinct, each from the other because of the following reasons:

Inventions Group I and Group II are related as product and process of use. The inventions can be shown to be distinct if either or both of the following can be shown: (1) the process for using the product as claimed can be practiced with another materially different product or (2) the product as claimed can be used in a materially different process of using that product. See MPEP § 806.05(h). In the instant case the product as claimed can be used in a materially different process of using that product.

Restriction for examination purposes as indicated is proper because all these inventions listed in this action are independent or distinct for the reasons given above and there would be a serious search and/or examination burden if restriction were not required because one or more of the following reasons apply:

(a) the inventions have acquired a separate status in the art in view of their different classification;

(b) the inventions have acquired a separate status in the art due their recognized divergent subject matter;

(c) the inventions have acquired a different field of search (for example searching different classes/subclasses or electronic resources, or employing different search queries);

(d) the prior art applicable to one invention would not likely be applicable to another invention;

(e) the inventions are likely to raise different non-prior art issues under 35 U.S.C. 101 and/or 35 U.S.C. 112 first paragraph.

Applicant is advised that the reply to this requirement to be complete must include (i) an election of an invention to be examined even though the requirement may be traversed (37 CFR 1.143) and (ii) identification of the claims encompassing the elected invention.

The election of an invention may be made with or without traverse. To reserve a right to petition, the election must be made with traverse. If the reply does not distinctly and specifically point out supposed errors in the restriction requirement, the election shall be treated as an election without traverse. Traversal must be presented at the time of election in order to be considered timely. Failure to timely traverse the requirement will result in the loss of right to petition under 37 CFR 1.144. If claims are added after the election, applicant must indicate which of these claims are readable upon the elected invention.

Should applicant traverse on the ground that the inventions are not patentably distinct, applicant should submit evidence or identify such evidence now of record showing the inventions to be obvious variants or clearly admit on the record that this is the case. In either instance, if the examiner finds one of the inventions unpatentable over the prior art, the evidence or admission may be used in a rejection under 35 U.S.C. 103 or pre-AIA 35 U.S.C. 103(a) of the other invention.

This application contains claims directed to the following patentably distinct species atropine, atropine sulfate, noratropine, atropine-n-oxide, atropic acid, hyoscine, scopolamine, tropicamide, cyclopentolate, pirenzapine and homatropine. The species are independent or distinct because they are drawn to different compounds with different chemical structure. In addition, these species are not obvious variants of each other based on the current record.

Applicant is required under 35 U.S.C. 121 to elect a single disclosed species, or a single grouping of patentably indistinct species, for prosecution on the merits to which the claims shall be restricted if no generic claim is finally held to be allowable. Currently, claims 1 and 28 are generic.

There is a search and/or examination burden for the patentably distinct species as set forth above because at least the following reason(s) apply:

(a) the inventions have acquired a separate status in the art in view of their different classification;

(b) the inventions have acquired a separate status in the art due their recognized divergent subject matter;

(c) the inventions have acquired a different field of search (for example searching different classes/subclasses or electronic resources, or employing different search queries);

(d) the prior art applicable to one invention would not likely be applicable to another invention;

(e) the inventions are likely to raise different non-prior art issues under 35 U.S.C. 101 and/or 35 U.S.C. 112 first paragraph .

Applicant is advised that the reply to this requirement to be complete must include (i) an election of a species to be examined even though the requirement may be traversed (37 CFR 1.143) **and (ii) identification of the claims encompassing the elected species or grouping of patentably indistinct species**, including any claims subsequently added. An argument that a claim is allowable or that all claims are generic is considered nonresponsive unless accompanied by an election.

The election may be made with or without traverse. To preserve a right to petition, the election must be made with traverse. If the reply does not distinctly and specifically point out supposed errors in the election of species requirement, the election shall be treated as an election without traverse. Traversal must be presented at the time of election in order to be considered timely. Failure to timely traverse the requirement will result in the loss of right to petition under 37 CFR 1.144. If claims are added after the election, applicant must indicate which of these claims are readable on the elected species or grouping of patentably indistinct species.

Should applicant traverse on the ground that the species, or groupings of patentably indistinct species from which election is required, are not patentably distinct, applicant should submit evidence or identify such evidence now of record showing them to be obvious variants or clearly admit on the record that this is the case. In either instance, if the examiner finds one of the species unpatentable over the prior art, the evidence or admission may be used in a rejection under 35 U.S.C. 103 or pre-AIA 35 U.S.C. 103(a) of the other species.

Upon the allowance of a generic claim, applicant will be entitled to consideration of claims to additional species which depend from or otherwise require all the limitations of an allowable generic claim as provided by 37 CFR 1.141.

Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be corrected in compliance with 37 CFR 1.48(a) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. A request to correct inventorship under 37 CFR 1.48(a) must be accompanied by an application data sheet in accordance with 37 CFR 1.76 that identifies each inventor by his or her legal name and by the processing fee required under 37 CFR 1.17(i).

The examiner has required restriction between product or apparatus claims and process claims. Where applicant elects claims directed to the product/apparatus, and all product/apparatus claims are subsequently found allowable, withdrawn process claims that include all the limitations of the allowable product/apparatus claims should be considered for rejoinder. All claims directed to a nonelected process invention must

include all the limitations of an allowable product/apparatus claim for that process invention to be rejoined.

In the event of rejoinder, the requirement for restriction between the product/apparatus claims and the rejoined process claims will be withdrawn, and the rejoined process claims will be fully examined for patentability in accordance with 37 CFR 1.104. Thus, to be allowable, the rejoined claims must meet all criteria for patentability including the requirements of 35 U.S.C. 101, 102, 103 and 112. Until all claims to the elected product/apparatus are found allowable, an otherwise proper restriction requirement between product/apparatus claims and process claims may be maintained. Withdrawn process claims that are not commensurate in scope with an allowable product/apparatus claim will not be rejoined. See MPEP § 821.04. Additionally, in order for rejoinder to occur, applicant is advised that the process claims should be amended during prosecution to require the limitations of the product/apparatus claims. **Failure to do so may result in no rejoinder.** Further, note that the prohibition against double patenting rejections of 35 U.S.C. 121 does not apply where the restriction requirement is withdrawn by the examiner before the patent issues. See MPEP § 804.01.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ZOHREH FAY whose telephone number is (571)272-0573. The examiner can normally be reached on Monday to Friday 9:30-6:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Melenie McCormick can be reached on (571) 272-8037. The fax phone

Art Unit: 1621

number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

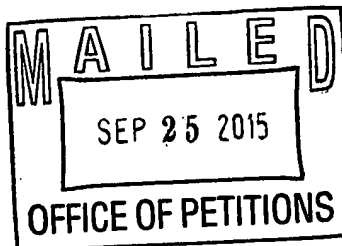
ZF
/ZOHREH FAY/
Primary Examiner, Art Unit 1621



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PALO ALTO CA 94304-1050



Doc Code: TRACK1.GRANT

**Decision Granting Request for
Prioritized Examination
(Track I or After RCE)**

Application No.: 14/726,139

1. THE REQUEST FILED May 29, 2015 IS **GRANTED**.

The above-identified application has met the requirements for prioritized examination

- A. ☒ for an original nonprovisional application (Track I).
B. ☐ for an application undergoing continued examination (RCE).

2. **The above-identified application will undergo prioritized examination.** The application will be accorded special status throughout its entire course of prosecution until one of the following occurs:

- A. filing a **petition for extension of time** to extend the time period for filing a reply;
B. filing an **amendment to amend the application to contain more than four independent claims, more than thirty total claims**, or a multiple dependent claim;
C. filing a **request for continued examination**;
D. filing a notice of appeal;
E. filing a request for suspension of action;
F. mailing of a notice of allowance;
G. mailing of a final Office action;
H. completion of examination as defined in 37 CFR 41.102; or
I. abandonment of the application.

Telephone inquiries with regard to this decision should be directed to Irvin Dingle at (571)272-3210, Office of Petitions.

Irvin Dingle
/Irvin Dingle/
[Signature]

Lead Paralegal Specialist
(Title)



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
14/726,139	05/29/2015	Gregory I. Ostrow	46682-701.201

21971
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CONFIRMATION NO. 7389
POA ACCEPTANCE LETTER



Date Mailed: 08/05/2015

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 07/07/2015.

The Power of Attorney in this application is accepted. Correspondence in this application will be mailed to the above address as provided by 37 CFR 1.33.

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at (571) 272-4000 or (571) 272-4200 or 1-888-786-0101.

/dtdinh/



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UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY. DOCKET NO	TOT CLAIMS	IND CLAIMS
14/726,139	05/29/2015	1629	1200	46682-701.201	30	3

CONFIRMATION NO. 7389

UPDATED FILING RECEIPT

21971
WILSON, SONSINI, GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304-1050



Date Mailed: 08/05/2015

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Inventor(s)

Gregory I. Ostrow, San Diego, CA;
Kenneth J. Widder, Rancho Santa Fe, CA;
David S. Baker, Carlsbad, CA;

Applicant(s)

Sydnexis, Inc., San Diego, CA;

Power of Attorney: The patent practitioners associated with Customer Number 21971

Domestic Priority data as claimed by applicant

This appln claims benefit of 62/151,926 04/23/2015
and claims benefit of 62/096,433 12/23/2014
and claims benefit of 62/016,502 06/24/2014

Foreign Applications for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see <http://www.uspto.gov> for more information.) - None.

Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

Permission to Access - A proper **Authorization to Permit Access to Application by Participating Offices** (PTO/SB/39 or its equivalent) has been received by the USPTO.

If Required, Foreign Filing License Granted: 06/08/2015

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 14/726,139**

Projected Publication Date: 12/24/2015

Non-Publication Request: No

Early Publication Request: No

**** SMALL ENTITY ****

Title

OPHTHALMIC COMPOSITION

Preliminary Class

514

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at <http://www.uspto.gov/web/offices/pac/doc/general/index.html>.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, <http://www.stopfakes.gov>. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

LICENSE FOR FOREIGN FILING UNDER
Title 35, United States Code, Section 184
Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign Assets Control, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

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The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The U.S. offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to promote and facilitate business investment. SelectUSA provides information assistance to the international investor community; serves as an ombudsman for existing and potential investors; advocates on behalf of U.S. cities, states, and regions competing for global investment; and counsels U.S. economic development organizations on investment attraction best practices. To learn more about why the United States is the best country in the world to develop technology, manufacture products, deliver services, and grow your business, visit <http://www.SelectUSA.gov> or call +1-202-482-6800.

TRANSMITTAL FOR POWER OF ATTORNEY TO ONE OR MORE REGISTERED PRACTITIONERS

NOTE: This form is to be submitted with the Power of Attorney by Applicant form (PTO/AIA/82B or equivalent) to identify the application to which the Power of Attorney is directed, in accordance with 37 CFR 1.5. If the Power of Attorney by Applicant form is not accompanied by this transmittal form or an equivalent, the Power of Attorney will not be recognized in the application.

Application Number	14/726,139		
Filing Date	May 29, 2015		
First Named Inventor	Gregory I. Ostrow		
Title	OPHTHALMIC COMPOSITION		
Art Unit	1629		
Examiner Name	Not yet assigned		
Attorney Docket Number	46682-701.201		
SIGNATURE of Applicant or Patent Practitioner			
Signature	/Michael Hostetler/	Date	
Name	Michael J. Hostetler	Telephone	858-350-2339
Registration Number	47,664		
NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications.			
☒ *Total of <u>2</u> forms are submitted.			

This collection of information is required by 37 CFR 1.31, 1.32 and 1.33. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 3 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Doc Code: PA..

Document Description: Power of Attorney

PTO/AIA/82B(07-12)

Approved for use through 11/30/2014. OMB 0651-0035

U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

POWER OF ATTORNEY BY APPLICANT

I hereby revoke all previous powers of attorney given in the application identified in the attached transmittal letter.

- ☒ I hereby appoint Practitioner(s) associated with the following Customer Number as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the application referenced in the attached transmittal letter (form PTO/AIA/82A or equivalent):

21971

OR

- ☐ I hereby appoint Practitioner(s) named below as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the application referenced in the attached transmittal letter (form PTO/AIA/82A or equivalent):

Name	Registration Number	Name	Registration Number

Please recognize or change the correspondence address for the application identified in the attached transmittal letter to:

- ☐ The address associated with the above-mentioned Customer Number.

OR

- ☐ The address associated with Customer Number:

OR

☐ Firm or
Individual Name

Address

City

State

Zip

Country

Telephone

Email

I am the Applicant:

- ☐ Inventor or Joint Inventor
- ☐ Legal Representative of a Deceased or Legally Incapacitated Inventor
- ☒ Assignee or Person to Whom the Inventor is Under an Obligation to Assign
- ☐ Person Who Otherwise Shows Sufficient Proprietary Interest (e.g., a petition under 37 CFR 1.46(b)(2) was granted in the application or is concurrently being filed with this document)

SIGNATURE of Applicant for Patent

Signature

Date

Name

Telephone

Title and Company

NOTE: Signature - This form must be signed by the applicant in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. Submit multiple forms for more than one signature, see below *.

☒ *Total of 2 forms are submitted.

This collection of information is required by 37 CFR 1.31, 1.32 and 1.33. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 3 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

STATEMENT UNDER 37 CFR 3.73(c)Applicant/Patent Owner: Sydnexis, Inc.Application No./Patent No.: 14/726,139Filed/Issue Date: May 29, 2015Titled: OPHTHALMIC COMPOSITIONSydnexis, Inc., a California corporation

(Name of Assignee)

(Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that, for the patent application/patent identified above, it is (choose one of options 1, 2, 3 or 4 below):

1. ☐ The assignee of the entire right, title, and interest.
2. ☐ An assignee of less than the entire right, title, and interest (check applicable box):
- ☐ The extent (by percentage) of its ownership interest is ____%. Additional Statement(s) by the owners holding the balance of the interest must be submitted to account for 100% of the ownership interest.
- ☐ There are unspecified percentages of ownership. The other parties, including inventors, who together own the entire right, title and interest are:

Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

3. ☐ The assignee of an undivided interest in the entirety (a complete assignment from one of the joint inventors was made). The other parties, including inventors, who together own the entire right, title, and interest are:

Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

4. ☐ The recipient, via a court proceeding or the like (e.g., bankruptcy, probate), of an undivided interest in the entirety (a complete transfer of ownership interest was made). The certified document(s) showing the transfer is attached.

The interest identified in option 1, 2 or 3 above (not option 4) is evidenced by either (choose one of options A or B below):

- A. ☒ An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel _____, Frame _____, or for which a copy thereof is attached.
- B. ☐ A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

1. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.

2. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.

[Page 1 of 2]

This collection of information is required by 37 CFR 3.73(b). The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

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STATEMENT UNDER 37 CFR 3.73(c)

3. From: _____ To: _____

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Reel _____, Frame _____, or for which a copy thereof is attached.

4. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.

5. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.

6. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.☐ Additional documents in the chain of title are listed on a supplemental sheet(s).☐ As required by 37 CFR 3.73(c)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (i.e., a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

/Michael Hostetler/

06/19/2015

Signature

Date

Michael J. Hostetler

47,664

Printed or Typed Name

Title or Registration Number

WHEREAS, the undersigned:

1. OSTROW, Gregory I. 14051 Caminito Vistana San Diego, CA 92130	2. WIDDER, Kenneth J. P.O. Box 676250 Rancho Santa Fe, CA 92067	3. BAKER, David S. 7205 Aviara Drive Carlsbad, CA 92011

(hereinafter "Inventor(s)), have invented certain new and useful improvements in

OPHTHALMIC COMPOSITION

- ☐ for which a United States patent application is executed on even date herewith;
☒ for which application serial number 14/726,139 was filed on May 29, 2015 in the United States Patent and Trademark Office;
☐ for which application serial number ____ was filed on ____ in the U.S. Receiving Office of the Patent Cooperation Treaty;
☐ for which application serial number ____ was filed on ____ in the ____ Patent Office; and/or
☐ for which an application was filed upon which a United States Patent issued on ____, as U.S. Patent No. (hereinafter, "Application(s)"). The term "Application(s)" also includes all patent applications that share or claim priority to or from the above application(s).

WHEREAS, Sydnexis, Inc., a corporation of the State OR Commonwealth of California, having a place of business at 14051 Caminito Vistana, San Diego, CA 92130, (hereinafter "Assignee"), is desirous of acquiring the entire right, title and interest in and to said Application(s), and the inventions disclosed therein, and in and to all embodiments of the inventions, heretofore conceived, made or discovered, whether jointly or severally, by said Inventor(s) (hereinafter collectively referred to as "Inventions"), and in and to any and all patents, inventor's certificates and other forms of protection thereon granted in the United States, foreign countries, or under any international convention, agreement, protocol, or treaty, including those filed under the Paris Convention for the Protection of Industrial Property, The Patent Cooperation Treaty or otherwise (hereinafter "Patent(s)").

NOW, THEREFORE, in consideration of good and valuable consideration acknowledged by said Inventor(s) to have been received in full from said Assignee:

1. Said Inventor(s) do hereby sell, assign, transfer and convey unto said Assignee the entire right, title and interest (a) in and to said Inventions; (b) in and to said Applications, including the right to claim priority to and from said Application(s); (c) in and to each and every application that is a divisional, substitution, continuation, or continuation-in-part of any of said Application(s); (d) in and to said Patent(s) and each and every patent issuing or reissuing from any of the foregoing; (e) in and to each and every reissue, reexamination, renewal or extension of any kind of any of the foregoing; (f) in and to each and every patent and application filed outside the United States and corresponding to any of the foregoing; and (g) in and to all claims for past, present and future infringement of the Patent(s), including all rights to sue for and to receive and recover for Assignee's own use all past, present, and future lost profits, royalties, and damages of whatever nature recoverable from an infringement of the Patent(s).

2. Said Inventor(s) hereby covenant and agree to cooperate with said Assignee to enable said Assignee to enjoy to the fullest extent the right, title and interest herein conveyed in the United States, foreign countries, or under any international convention, agreement, protocol, or treaty. Such cooperation by said Inventor(s) shall include prompt production of pertinent facts and documents, giving of testimony, execution of petitions, oaths, specifications, declarations or other papers, and other assistance all to the extent deemed necessary or desirable by said Assignee (a) for perfecting in said Assignee the right, title and interest herein conveyed; (b) for prosecuting any applications covering said Inventions; (c) for filing and prosecuting substitute, divisional, continuing or additional applications covering said Inventions; (d) for filing and prosecuting applications for reissuance of any said Patent(s); (e) for interference or other priority proceedings involving said Inventions; and (f) for legal proceedings involving said Inventions and any applications therefor and any Patent(s) granted thereon, including without limitation reissues and reexaminations, opposition proceedings, cancellation proceedings, priority contests, public use proceedings, infringement actions and court actions; provided, however, that reasonable expenses incurred by said Inventor(s) in providing such cooperation shall be paid for by said Assignee.

3. The terms and covenants of this assignment shall inure to the benefit of said Assignee, its successors, assigns and other legal representatives, and shall be binding upon said Inventor(s), their respective heirs, legal representatives and assigns.

PATENT ASSIGNMENT

Docket Number 46682-701.201

4. Said Inventor(s) hereby warrant, represent and covenant that said Inventor(s) have not entered and will not enter into any assignment, contract, or understanding in conflict herewith.

5. Said Inventor(s) hereby request that any Patent(s) issuing in the United States, foreign countries, or under any international convention, agreement, protocol, or treaty, be issued in the name of the Assignee, or its successors and assigns, for the sole use of said Assignee, its successors, legal representatives and assigns.

6. This instrument will be interpreted and construed in accordance with the laws of the State of California, without regard to conflict of law principles. If any provision of this instrument is found to be illegal or unenforceable, the other provisions shall remain effective and enforceable to the greatest extent permitted by law. This instrument may be executed in counterparts, each of which is deemed an original, but all of which together constitute one and the same agreement.

IN WITNESS WHEREOF, said Inventor(s) have executed and delivered this instrument to said Assignee as of the dates written below:

Date:

6/1/15

Gregory I. Ostrow

Date:

Kenneth J. Widder

Date:

David S. Baker

PATENT ASSIGNMENT

Docket Number 46682-701.201

4. Said Inventor(s) hereby warrant, represent and covenant that said Inventor(s) have not entered and will not enter into any assignment, contract, or understanding in conflict herewith.

5. Said Inventor(s) hereby request that any Patent(s) issuing in the United States, foreign countries, or under any international convention, agreement, protocol, or treaty, be issued in the name of the Assignee, or its successors and assigns, for the sole use of said Assignee, its successors, legal representatives and assigns.

6. This instrument will be interpreted and construed in accordance with the laws of the State of California, without regard to conflict of law principles. If any provision of this instrument is found to be illegal or unenforceable, the other provisions shall remain effective and enforceable to the greatest extent permitted by law. This instrument may be executed in counterparts, each of which is deemed an original, but all of which together constitute one and the same agreement.

IN WITNESS WHEREOF, said Inventor(s) have executed and delivered this instrument to said Assignee as of the dates written below:

Date: _____
Gregory I. Ostrow

Date: _____
Kenneth J. Widder

Date: 6/2/15 DSB
David S. Baker

PATENT ASSIGNMENT

Docket Number 46682-701.201

4. Said Inventor(s) hereby warrant, represent and covenant that said Inventor(s) have not entered and will not enter into any assignment, contract, or understanding in conflict herewith.

5. Said Inventor(s) hereby request that any Patent(s) issuing in the United States, foreign countries, or under any international convention, agreement, protocol, or treaty, be issued in the name of the Assignee, or its successors and assigns, for the sole use of said Assignee, its successors, legal representatives and assigns.

6. This instrument will be interpreted and construed in accordance with the laws of the State of California, without regard to conflict of law principles. If any provision of this instrument is found to be illegal or unenforceable, the other provisions shall remain effective and enforceable to the greatest extent permitted by law. This instrument may be executed in counterparts, each of which is deemed an original, but all of which together constitute one and the same agreement.

IN WITNESS WHEREOF, said Inventor(s) have executed and delivered this instrument to said Assignee as of the dates written below:

Date: _____
Gregory I. Ostrow

Date: 6/10/15 Kenneth J. Widder
Kenneth J. Widder

Date: _____
David S. Baker

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	46682-701.201
		Application Number	
Title of Invention	OPHTHALMIC COMPOSITION		
The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76. This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.			

Secrecy Order 37 CFR 5.2

☐	Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor 1					Remove	
Legal Name						
Prefix	Given Name	Middle Name	Family Name	Suffix		
	Gregory	I.	Ostrow			
Residence Information (Select One) ☒ US Residency ☐ Non US Residency ☐ Active US Military Service						
City	San Diego	State/Province	CA	Country of Residence	US	
Mailing Address of Inventor:						
Address 1		14051 Caminito Vistana				
Address 2						
City	San Diego	State/Province	CA			
Postal Code	92130	Country	US			
Inventor 2					Remove	
Legal Name						
Prefix	Given Name	Middle Name	Family Name	Suffix		
	Kenneth	J.	Widder			
Residence Information (Select One) ☒ US Residency ☐ Non US Residency ☐ Active US Military Service						
City	Rancho Santa Fe	State/Province	CA	Country of Residence	US	
Mailing Address of Inventor:						
Address 1		P.O. Box 676250				
Address 2						
City	Rancho Santa Fe	State/Province	CA			
Postal Code	92067	Country	US			
Inventor 3					Remove	
Legal Name						
Prefix	Given Name	Middle Name	Family Name	Suffix		
	David	S.	Baker			
Residence Information (Select One) ☒ US Residency ☐ Non US Residency ☐ Active US Military Service						

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number		46682-701.201	
		Application Number			
Title of Invention	OPHTHALMIC COMPOSITION				
City	Carlsbad	State/Province	CA	Country of Residence	US
Mailing Address of Inventor:					
Address 1	7205 Aviara Dr.				
Address 2					
City	Carlsbad	State/Province	CA		
Postal Code	92011	Country	US		
All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the Add button.					Add

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below. For further information see 37 CFR 1.33(a).			
☐ An Address is being provided for the correspondence Information of this application.			
Customer Number	21971		
Email Address	patentdocket@wsgr.com	Add Email	Remove Email

Application Information:

Title of the Invention	OPHTHALMIC COMPOSITION		
Attorney Docket Number	46682-701.201	Small Entity Status Claimed	☒
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	10	Suggested Figure for Publication (if any)	
Filing By Reference :			
Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").			
For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).			
Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country	

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)
☐ Request Not to Publish. I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application has not and will not be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	46682-701.201
		Application Number	
Title of Invention	OPHTHALMIC COMPOSITION		

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32). Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	☒ Customer Number	☐ US Patent Practitioner	☐ Limited Recognition (37 CFR 11.9)
Customer Number	21971		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) or indicate National Stage entry from a PCT application. Providing this information in the application data sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.

When referring to the current application, please leave the application number blank.

Prior Application Status	Pending	Remove	
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
	Claims benefit of provisional	62151926	2015-04-23
Prior Application Status	Pending	Remove	
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
	Claims benefit of provisional	62096433	2014-12-23
Prior Application Status	Pending	Remove	
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
	Claims benefit of provisional	62016502	2014-06-24
Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the Add button.			

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55(d). When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)ⁱ the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(h)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

			Remove
Application Number	Country ⁱ	Filing Date (YYYY-MM-DD)	Access Code ⁱ (if applicable)

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	46682-701.201
		Application Number	
Title of Invention	OPHTHALMIC COMPOSITION		

Additional Foreign Priority Data may be generated within this form by selecting the **Add** button.

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013. ☐ NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

Authorization to Permit Access:

☒ Authorization to Permit Access to the Instant Application by the Participating Offices
If checked, the undersigned hereby grants the USPTO authority to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the World Intellectual Property Office (WIPO), and any other intellectual property offices in which a foreign application claiming priority to the instant patent application is filed access to the instant patent application. See 37 CFR 1.14(c) and (h). This box should not be checked if the applicant does not wish the EPO, JPO, KIPO, WIPO, or other intellectual property office in which a foreign application claiming priority to the instant patent application is filed to have access to the instant patent application. In accordance with 37 CFR 1.14(h)(3), access will be provided to a copy of the instant patent application with respect to: 1) the instant patent application-as-filed; 2) any foreign application to which the instant patent application claims priority under 35 U.S.C. 119(a)-(d) if a copy of the foreign application that satisfies the certified copy requirement of 37 CFR 1.55 has been filed in the instant patent application; and 3) any U.S. application-as-filed from which benefit is sought in the instant patent application. In accordance with 37 CFR 1.14(c), access may be provided to information concerning the date of filing this Authorization.

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	46682-701.201
		Application Number	
Title of Invention	OPHTHALMIC COMPOSITION		

Applicant 1			
If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section. Clear			
☒ Assignee		☐ Legal Representative under 35 U.S.C. 117	
☐ Person to whom the inventor is obligated to assign.		☐ Joint Inventor	
☐ Person who shows sufficient proprietary interest			
If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:			
Name of the Deceased or Legally Incapacitated Inventor :			
If the Applicant is an Organization check here. ☒			
Organization Name		Sydnexis, Inc.	
Mailing Address Information For Applicant:			
Address 1		14051 Caminito Vistana	
Address 2			
City	San Diego	State/Province	CA
Country	US	Postal Code	92130
Phone Number		Fax Number	
Email Address			
Additional Applicant Data may be generated within this form by selecting the Add button.			

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Assignee 1
Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.
If the Assignee or Non-Applicant Assignee is an Organization check here. ☐

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	46682-701.201	
		Application Number		
Title of Invention	OPHTHALMIC COMPOSITION			

Prefix	Given Name	Middle Name	Family Name	Suffix

Mailing Address Information For Assignee including Non-Applicant Assignee:

Address 1				
Address 2				
City		State/Province		
Country i	Postal Code			
Phone Number		Fax Number		
Email Address				

Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button.

Signature:

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Signature	/Xiaofan Yang/		Date (YYYY-MM-DD)	2015-05-29
First Name	Xiaofan	Last Name	Yang	Registration Number 61459

Additional Signature may be generated within this form by selecting the Add button.

This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Electronic Acknowledgement Receipt

EFS ID:	22849928
Application Number:	14726139
International Application Number:	
Confirmation Number:	7389
Title of Invention:	OPHTHALMIC COMPOSITION
First Named Inventor/Applicant Name:	Gregory I. Ostrow
Customer Number:	21971
Filer:	Michael J. Hostetler/Linda Anders
Filer Authorized By:	Michael J. Hostetler
Attorney Docket Number:	46682-701.201
Receipt Date:	07-JUL-2015
Filing Date:	29-MAY-2015
Time Stamp:	18:36:21
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		46682-701-201-DecsPOA.pdf	672953 e55849e06ea144aa00442dedb215707f1b2 db3c1	yes	17

	Multipart Description/PDF files in .zip description		
	Document Description	Start	End
	Oath or Declaration filed	1	3
	Power of Attorney	4	17
Warnings:			
Information:			
Total Files Size (in bytes):		672953	
This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503. <u>New Applications Under 35 U.S.C. 111</u> If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application. <u>National Stage of an International Application under 35 U.S.C. 371</u> If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course. <u>New International Application Filed with the USPTO as a Receiving Office</u> If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.			

PTO/AIA/01 (08-12)

Approved for use through 01/31/2014, OMB 0551-0032

U.S. Patent and Trademark Office, U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	OPHTHALMIC COMPOSITION	
As the below named inventor, I hereby declare that: This declaration is directed to: ☐ The attached application, or ☒ United States application or PCT (international) application number <u>14/726,139</u> filed on <u>May 29, 2015</u> The above-identified application was made or authorized to be made by me. I believe that I am the original inventor or an original joint inventor of a claimed invention in the application. I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both. WARNING: Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.		
LEGAL NAME OF INVENTOR		
Inventor:	Gregory I. Ostrow	Date (Optional): <u>6/1/15</u>
Signature:	<i>Gregory I. Ostrow</i>	
Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.		

This collection of information is required by 35 U.S.C. 115 and 37 CFR 1.63. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 1 minute to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of
Invention

OPHTHALMIC COMPOSITION

As the below named inventor, I hereby declare that:

This declaration
is directed to:☐

The attached application, or

☒United States application or PCT International application number 14/726,139filed on May 29, 2015

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

WARNING:

Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

LEGAL NAME OF INVENTOR

Inventor: Kenneth J. Widder

Date (Optional): _____

Signature: Kenneth J. Widder

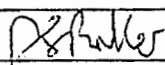
Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.

This collection of information is required by 35 U.S.C. 115 and 37 CFR 1.63. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 1 minute to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing this form, call 1-800-PTO-9199 and select option 2.

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**DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN
APPLICATION DATA SHEET (37 CFR 1.76)**

Title of Invention	OPHTHALMIC COMPOSITION
As the below named inventor, I hereby declare that: This declaration is directed to: ☐ The attached application, or ☒ United States application or PCT international application number <u>14/726,139</u> filed on <u>May 29, 2015</u> The above-identified application was made or authorized to be made by me. I believe that I am the original inventor or an original joint inventor of a claimed invention in the application. I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both. WARNING: Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.	
LEGAL NAME OF INVENTOR	
Inventor: <u>David S. Baker</u> Date (Optional): <u>6/3/15</u> Signature: <u></u>	
Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.	

This collection of information is required by 35 U.S.C. 115 and 37 CFR 1.63. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 1 minute to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Electronic Patent Application Fee Transmittal

Application Number:	14726139			
Filing Date:	29-May-2015			
Title of Invention:	OPHTHALMIC COMPOSITION			
First Named Inventor/Applicant Name:	Gregory I. Ostrow			
Filer:	Michael J. Hostetler/Linda Anders			
Attorney Docket Number:	46682-701.201			
Filed as Small Entity				
Filing Fees for Utility under 35 USC 111(a)				
Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Late Filing Fee for Oath or Declaration	2051	1	70	70
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Extension-of-Time:				
Miscellaneous:				
Total in USD (\$)				70

Electronic Acknowledgement Receipt

EFS ID:	22656350
Application Number:	14726139
International Application Number:	
Confirmation Number:	7389
Title of Invention:	OPHTHALMIC COMPOSITION
First Named Inventor/Applicant Name:	Gregory I. Ostrow
Customer Number:	21971
Filer:	Michael J. Hostetler/Linda Anders
Filer Authorized By:	Michael J. Hostetler
Attorney Docket Number:	46682-701.201
Receipt Date:	17-JUN-2015
Filing Date:	29-MAY-2015
Time Stamp:	12:52:05
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	\$ 70
RAM confirmation Number	852
Deposit Account	232415
Authorized User	ADVANI, RAJ J.

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Fee Worksheet (SB06)	fee-info.pdf	30225	no	2
			70010a8aff2e67e44293fa5dc0918ee1ef6e976b		

Warnings:

Information:

Total Files Size (in bytes):	30225

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
14/726,139	05/29/2015	Gregory I. Ostrow	46682-701.201

CONFIRMATION NO. 7389

FORMALITIES LETTER



OC000000075617227

21971
WILSON, SONSINI, GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304-1050

Date Mailed: 06/09/2015

NOTICE TO FILE MISSING PARTS OF NONPROVISIONAL APPLICATION

FILED UNDER 37 CFR 1.53(b)

Filing Date Granted

Items Required To Avoid Abandonment:

An application number and filing date have been accorded to this application. The item(s) indicated below, however, are missing.

Applicant is given **TWO MONTHS** from the date of this Notice within which to file all required items below to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

- Surcharge as set forth in 37 CFR 1.16(f) must be submitted.

The surcharge is due for any one of:

- late submission of the basic filing fee, search fee, or examination fee,
- late submission of inventor's oath or declaration,
- filing an application that does not contain at least one claim on filing, or
- submission of an application filed by reference to a previously filed application.

SUMMARY OF FEES DUE:

The fee(s) required within **TWO MONTHS** from the date of this Notice to avoid abandonment is/are itemized below. Small entity discount is in effect. If applicant is qualified for micro entity status, an acceptable Certification of Micro Entity Status must be submitted to establish micro entity status. (See 37 CFR 1.29 and forms PTO/SB/15A and 15B.)

- \$ **70** surcharge.
- \$(**0**) previous unapplied payment amount.
- \$ **70** TOTAL FEE BALANCE DUE.

Items Required To Avoid Processing Delays:

Applicant is notified that the above-identified application contains the deficiencies noted below. No period for reply is set forth in this notice for correction of these deficiencies. However, if a deficiency relates to the inventor's oath or declaration, the applicant must file an oath or declaration in compliance with 37 CFR 1.63, or a substitute statement in compliance with 37 CFR 1.64, executed by or with respect to each actual inventor no later than the expiration of the time period set in the "Notice of Allowability" to avoid abandonment. See 37 CFR 1.53(f).

- A properly executed inventor's oath or declaration has not been received for the following inventor(s):

Gregory I. Ostrow
Kenneth J. Widder
David S. Baker

Replies must be received in the USPTO within the set time period or must include a proper Certificate of Mailing or Transmission under 37 CFR 1.8 with a mailing or transmission date within the set time period. For more information and a suggested format, see Form PTO/SB/92 and MPEP 512.

Replies should be mailed to:

Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

Registered users of EFS-Web may alternatively submit their reply to this notice via EFS-Web, including a copy of this Notice and selecting the document description "Applicant response to Pre-Exam Formalities Notice".
<https://sportal.uspto.gov/authenticate/AuthenticateUserLocalEPF.html>

For more information about EFS-Web please call the USPTO Electronic Business Center at 1-866-217-9197 or visit our website at <http://www.uspto.gov/ebc>.

If you are not using EFS-Web to submit your reply, you must include a copy of this notice.

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/tnguyen/

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875						Application or Docket Number 14/726,139				
APPLICATION AS FILED - PART I										
(Column 1)		(Column 2)		SMALL ENTITY		OR OTHER THAN SMALL ENTITY				
FOR	NUMBER FILED	NUMBER EXTRA	RATE(\$)	FEE(\$)		RATE(\$)	FEE(\$)			
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	70		N/A				
SEARCH FEE (37 CFR 1.16(k), (l), or (m))	N/A	N/A	N/A	300		N/A				
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	360		N/A				
TOTAL CLAIMS (37 CFR 1.16(j))	30	minus 20 = *	10	x 40 =						
INDEPENDENT CLAIMS (37 CFR 1.16(h))	3	minus 3 = *		x 210 =						
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is \$310 (\$155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			0.00						
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				0.00						
				TOTAL		TOTAL				
					1130					
* If the difference in column 1 is less than zero, enter "0" in column 2.										
APPLICATION AS AMENDED - PART II										
(Column 1)		(Column 2)		(Column 3)		SMALL ENTITY		OR OTHER THAN SMALL ENTITY		
AMENDMENT A		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE(\$)	ADDITIONAL FEE(\$)		RATE(\$)	ADDITIONAL FEE(\$)
	Total (37 CFR 1.16(i))	*	Minus	**	=	x	=		x	=
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x	=		x	=
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))									
						TOTAL ADD'L FEE			TOTAL ADD'L FEE	
AMENDMENT B		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE(\$)	ADDITIONAL FEE(\$)		RATE(\$)	ADDITIONAL FEE(\$)
	Total (37 CFR 1.16(i))	*	Minus	**	=	x	=		x	=
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x	=		x	=
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))									
						TOTAL ADD'L FEE			TOTAL ADD'L FEE	
* If the entry in column 1 is less than the entry in column 2, write "0" in column 3. ** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20". *** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3". The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.										



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UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY. DOCKET NO	TOT CLAIMS	IND CLAIMS
14/726,139	05/29/2015	1629	1130	46682-701.201	30	3

CONFIRMATION NO. 7389

FILING RECEIPT

21971
WILSON, SONSINI, GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304-1050



Date Mailed: 06/09/2015

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Inventor(s)

Gregory I. Ostrow, San Diego, CA;
Kenneth J. Widder, Rancho Santa Fe, CA;
David S. Baker, Carlsbad, CA;

Applicant(s)

Sydnexis, Inc., San Diego, CA;

Power of Attorney: None

Domestic Priority data as claimed by applicant

This appln claims benefit of 62/151,926 04/23/2015
and claims benefit of 62/096,433 12/23/2014
and claims benefit of 62/016,502 06/24/2014

Foreign Applications for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see <http://www.uspto.gov> for more information.) - None.

Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

Permission to Access - A proper **Authorization to Permit Access to Application by Participating Offices** (PTO/SB/39 or its equivalent) has been received by the USPTO.

If Required, Foreign Filing License Granted: 06/08/2015

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 14/726,139**

Projected Publication Date: 12/24/2015

Non-Publication Request: No

Early Publication Request: No

**** SMALL ENTITY ****

Title

OPHTHALMIC COMPOSITION

Preliminary Class

514

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at <http://www.uspto.gov/web/offices/pac/doc/general/index.html>.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, <http://www.stopfakes.gov>. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

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Title 35, United States Code, Section 184
Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign Assets Control, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

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The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The U.S. offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to promote and facilitate business investment. SelectUSA provides information assistance to the international investor community; serves as an ombudsman for existing and potential investors; advocates on behalf of U.S. cities, states, and regions competing for global investment; and counsels U.S. economic development organizations on investment attraction best practices. To learn more about why the United States is the best country in the world to develop technology, manufacture products, deliver services, and grow your business, visit <http://www.SelectUSA.gov> or call +1-202-482-6800.

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UTILITY PATENT APPLICATION TRANSMITTAL <i>(Only for new nonprovisional applications under 37 CFR 1.53(b))</i>		Attorney Docket No. 46682-701.201	
		First Named Inventor Gregory I. Ostrow	
		Title OPHTHALMIC COMPOSITION	
		Express Mail Label No. Electronically Filed 05/29/15	

APPLICATION ELEMENTS <i>See MPEP chapter 600 concerning utility patent application contents.</i>	Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450		
1. ☐ Fee Transmittal Form (PTO/SB/17 or equivalent) 2. ☒ Applicant asserts small entity status. See 37 CFR 1.27 3. ☐ Applicant certifies micro entity status. See 37 CFR 1.29. Applicant must attach form PTO/SB/15A or B or equivalent. 4. ☒ Specification [Total Pages <u>108</u>] Both the claims and abstract must start on a new page. (See MPEP § 608.01(a) for information on the preferred arrangement) 5. ☒ Drawing(s) (35 U.S.C. 113) [Total Sheets <u>10</u>] 6. Inventor's Oath or Declaration [Total Pages _____] (including substitute statements under 37 CFR 1.64 and assignments serving as an oath or declaration under 37 CFR 1.63(e)) a. ☐ Newly executed (original or copy) b. ☐ A copy from a prior application (37 CFR 1.63(d)) 7. ☒ Application Data Sheet * See note below. See 37 CFR 1.76 (PTO/AIA/14 or equivalent) 8. CD-ROM or CD-R in duplicate, large table, or Computer Program (Appendix) ☐ Landscape Table on CD 9. Nucleotide and/or Amino Acid Sequence Submission (if applicable, items a. – c. are required) a. ☐ Computer Readable Form (CRF) b. ☐ Specification Sequence Listing on: i. ☐ CD-ROM or CD-R (2 copies); or ii. ☐ Paper c. ☐ Statements verifying identity of above copies	ACCOMPANYING APPLICATION PAPERS 10. ☐ Assignment Papers (cover sheet & document(s)) Name of Assignee _____ 11. ☐ 37 CFR 3.73(c) Statement ☐ Power of Attorney (when there is an assignee) 12. ☐ English Translation Document (if applicable) 13. ☐ Information Disclosure Statement (PTO/SB/08 or PTO-1449) ☐ Copies of citations attached 14. ☐ Preliminary Amendment 15. ☐ Return Receipt Postcard (MPEP § 503) (Should be specifically itemized) 16. ☐ Certified Copy of Priority Document(s) (if foreign priority is claimed) 17. ☐ Nonpublication Request Under 35 U.S.C. 122(b)(2)(B)(i). Applicant must attach form PTO/SB/35 or equivalent. 18. ☒ Other: <u>Track One Request</u> _____ _____ _____ _____		
*Note: (1) Benefit claims under 37 CFR 1.78 and foreign priority claims under 1.55 must be included in an Application Data Sheet (ADS). (2) For applications filed under 35 U.S.C. 111, the application must contain an ADS specifying the applicant if the applicant is an assignee, person to whom the inventor is under an obligation to assign, or person who otherwise shows sufficient proprietary interest in the matter. See 37 CFR 1.46(b).			
19. CORRESPONDENCE ADDRESS			
☒ The address associated with Customer Number: <u>21971</u> OR ☐ Correspondence address below			
Name			
Address			
City	State	Zip Code	
Country	Telephone	Email	
Signature	<u>/Xiaofan Yang/</u>		Date
Name (Print/Type)	<u>Xiaofan Yang</u>		Registration No. (Attorney/Agent)
			<u>05/29/2015</u>
			<u>61,459</u>

This collection of information is required by 37 CFR 1.53(b). The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

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Application Data Sheet 37 CFR 1.76		Attorney Docket Number	46682-701.201
		Application Number	
Title of Invention	OPHTHALMIC COMPOSITION		
The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76. This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.			

Secrecy Order 37 CFR 5.2

☐	Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor 1					Remove
Legal Name					
Prefix	Given Name	Middle Name	Family Name	Suffix	
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Residence Information (Select One) ☒ US Residency ☐ Non US Residency ☐ Active US Military Service					
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Inventor 3					Remove
Legal Name					
Prefix	Given Name	Middle Name	Family Name	Suffix	
	David	S.	Baker		
Residence Information (Select One) ☒ US Residency ☐ Non US Residency ☐ Active US Military Service					

Application Data Sheet 37 CFR 1.76		Attorney Docket Number		46682-701.201	
		Application Number			
Title of Invention		OPHTHALMIC COMPOSITION			
City	Carlsbad	State/Province	CA	Country of Residence i	US
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City	Carlsbad	State/Province	CA		
Postal Code	92011	Country i	US		
All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the Add button.					Add

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☐ An Address is being provided for the correspondence Information of this application.			
Customer Number	21971		
Email Address	patentdocket@wsgr.com	Add Email	Remove Email

Application Information:

Title of the Invention	OPHTHALMIC COMPOSITION		
Attorney Docket Number	46682-701.201	Small Entity Status Claimed	☒
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	10	Suggested Figure for Publication (if any)	

Filing By Reference :

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country i

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)
☐ Request Not to Publish. I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application has not and will not be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	46682-701.201
		Application Number	
Title of Invention	OPHTHALMIC COMPOSITION		

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32). Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

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Customer Number	21971		

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This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) or indicate National Stage entry from a PCT application. Providing this information in the application data sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.

When referring to the current application, please leave the application number blank.

Prior Application Status	Pending	Remove	
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
	Claims benefit of provisional	62151926	2015-04-23
Prior Application Status	Pending	Remove	
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
	Claims benefit of provisional	62096433	2014-12-23
Prior Application Status	Pending	Remove	
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
	Claims benefit of provisional	62016502	2014-06-24
Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the Add button.			Add

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55(d). When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX) the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(h)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

Remove			
Application Number	Country ⁱ	Filing Date (YYYY-MM-DD)	Access Code ^j (if applicable)

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	46682-701.201
		Application Number	
Title of Invention	OPHTHALMIC COMPOSITION		

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Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.

NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

Authorization to Permit Access:

☒ Authorization to Permit Access to the Instant Application by the Participating Offices

If checked, the undersigned hereby grants the USPTO authority to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the World Intellectual Property Office (WIPO), and any other intellectual property offices in which a foreign application claiming priority to the instant patent application is filed access to the instant patent application. See 37 CFR 1.14(c) and (h). This box should not be checked if the applicant does not wish the EPO, JPO, KIPO, WIPO, or other intellectual property office in which a foreign application claiming priority to the instant patent application is filed to have access to the instant patent application.

In accordance with 37 CFR 1.14(h)(3), access will be provided to a copy of the instant patent application with respect to: 1) the instant patent application-as-filed; 2) any foreign application to which the instant patent application claims priority under 35 U.S.C. 119(a)-(d) if a copy of the foreign application that satisfies the certified copy requirement of 37 CFR 1.55 has been filed in the instant patent application; and 3) any U.S. application-as-filed from which benefit is sought in the instant patent application.

In accordance with 37 CFR 1.14(c), access may be provided to information concerning the date of filing this Authorization.

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	46682-701.201
		Application Number	
Title of Invention	OPHTHALMIC COMPOSITION		

Applicant 1				Remove	
If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.					
Clear					
☒ Assignee		☐ Legal Representative under 35 U.S.C. 117		☐ Joint Inventor	
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If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:					
Name of the Deceased or Legally Incapacitated Inventor :					
If the Applicant is an Organization check here. ☒					
Organization Name		Sydnexis, Inc.			
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Address 1		14051 Caminito Vistana			
Address 2					
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Assignee Information including Non-Applicant Assignee Information:

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Assignee 1	
Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.	
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Application Data Sheet 37 CFR 1.76		Attorney Docket Number	46682-701.201
		Application Number	
Title of Invention	OPHTHALMIC COMPOSITION		

Prefix	Given Name	Middle Name	Family Name	Suffix

Mailing Address Information For Assignee including Non-Applicant Assignee:

Address 1			
Address 2			
City		State/Province	
Country i		Postal Code	
Phone Number		Fax Number	
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Signature:

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Signature	/Xiaofan Yang/		Date (YYYY-MM-DD)	2015-05-29	
First Name	Xiaofan	Last Name	Yang	Registration Number	61459

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This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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The information provided by you in this form will be subject to the following routine uses:

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3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
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9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

U.S. UTILITY APPLICATION
OPHTHALMIC COMPOSITION

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ELECTRONICALLY FILED ON MAY 29, 2015

OPHTHALMIC COMPOSITION

BACKGROUND OF THE DISCLOSURE

[0001] Pharmaceutical formulations have an expiration date which is based on the degradation of the active ingredient.

SUMMARY OF THE DISCLOSURE

[0002] Provided herein are ophthalmic compositions. In some embodiments, disclosed herein is an ophthalmic composition, comprising from about 0.001 wt% to about 0.05 wt% of a muscarinic antagonist and deuterated water, at a pD of from about 4.2 to about 7.9.

[0003] In some embodiments, the muscarinic antagonist comprises atropine, atropine sulfate, noratropine, atropine-N-oxide, tropine, tropic acid, hyoscine, scopolomine, tropicamide, cyclopentolate, pirenzapine, homatropine, or a combination thereof. In some embodiments, the muscarinic antagonist is atropine, or atropine sulfate.

[0004] In some embodiments, the ophthalmic composition has a pD of one of: less than about 7.3, less than about 7.2, less than about 7.1, less than about 7, less than about 6.8, less than about 6.5, less than about 6.4, less than about 6.3, less than about 6.2, less than about 6.1, less than about 6, less than about 5.9, less than about 5.8, less than about 5.2, or less than about 4.8 after extended period of time under storage condition.

[0005] In some embodiments, the ophthalmic composition comprises one of: at least about 80%, at least about 85%, at least about 90%, at least about 93%, at least about 95%, at least about 97%, at least about 98%, or at least about 99% of the muscarinic antagonist based on initial concentration after extended period of time under storage condition. As described in this disclosure, the percentage of the ophthalmic agent in the composition after storage is based on the amount of ophthalmic agent that is initially present in the composition (i.e. prior to the storage condition).

[0006] In some embodiments, the ophthalmic composition further has a potency of one of: at least 80%, at least 85%, at least 90%, at least 93%, at least 95%, at least 97%, at least 98%, or at least 99% after extended period of time under storage condition. As described in this disclosure, the potency of the ophthalmic agent in the composition after storage is based on the potency of ophthalmic agent that is initially present in the composition (i.e. prior to the storage condition).

[0007] In some embodiments, the extended period of time is one of: about 1 week, about 2 weeks, about 3 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 8 months, about 10 months, about 12 months, about 18 months, about 24 months, about 36 months, about 4 years, or about 5 years.

[0008] In some embodiments, the storage condition has a storage temperature of from about 2°C to about 10°C or from about 16°C to about 26°C. In some embodiments, the storage condition has a storage temperature of about 25°C. In some embodiments, the storage condition has a storage temperature of about 40°C. In some embodiments, the storage condition has a storage temperature of about 60°C.

[0009] In some embodiments, the storage condition has a relative humidity of about 60%. In some embodiments, the storage condition has a relative humidity of about 75%.

[0010] In some embodiments, the muscarinic antagonist is present in the composition at a concentration of one of: from about 0.001 wt% to about 0.04 wt%, from about 0.001 wt% to about 0.03 wt%, from about 0.001 wt% to about 0.025 wt%, from about 0.001 wt% to about 0.02 wt%, from about 0.001 wt% to about 0.01 wt%, from about 0.001 wt% to about 0.008 wt%, or from about 0.001 wt% to about 0.005 wt%.

[0011] In some embodiments, the composition comprises less than 20% of major degradant based on the concentration of the ophthalmic agent after extended period of time under storage condition. In some embodiments, the composition comprises less than 15% of major degradant based on the concentration of the ophthalmic agent after extended period of time under storage condition.

[0012] In some embodiments, the composition comprises less than 10% of major degradant based on the concentration of the ophthalmic agent after extended period of time under storage condition. In some embodiments, the composition comprises less than 5% of major degradant based on the concentration of the ophthalmic agent after extended period of time under storage condition. In some embodiments, the composition comprises less than 2.5% of major degradant based on the concentration of the ophthalmic agent after extended period of time under storage condition. In some embodiments, the composition comprises less than 2.0% of major degradant based on the concentration of the ophthalmic agent after extended period of time under storage condition. In some embodiments, the composition comprises less than 1.5% of major degradant based on the concentration of the ophthalmic agent after extended period of time under storage condition. In some embodiments, the composition comprises less than 1.0% of major degradant based on the concentration of the ophthalmic agent after extended period of time under storage condition. In some embodiments, the composition comprises less than 0.5% of major degradant based on the concentration of the ophthalmic agent after extended period of time under storage condition. In some embodiments, the composition comprises less than 0.4% of major degradant based on the concentration of the ophthalmic agent after extended period of time under storage condition. In some embodiments, the composition comprises less than 0.3% of major degradant based on the concentration of the ophthalmic agent after extended period of time under storage condition. In some embodiments, the

composition comprises less than 0.2% of major degradant based on the concentration of the ophthalmic agent after extended period of time under storage condition. In some embodiments, the composition comprises less than 0.1% of major degradant based on the concentration of the ophthalmic agent after extended period of time under storage condition. In some embodiments, the major degradant is tropic acid. As described in this disclosure, the percentage of the primary degradant in the composition after storage is based on the amount of ophthalmic agent that is initially present in the composition (i.e. prior to the storage condition).

[0013] In some embodiments, the composition is in a form of an aqueous solution.

[0014] In some embodiments, the composition further comprises an osmolarity adjusting agent. In some embodiments, the osmolarity adjusting agent is sodium chloride.

[0015] In some embodiments, the ophthalmic composition further comprises a preservative. In some embodiments, the preservative is selected from benzalkonium chloride, cetrimonium, sodium perborate, stabilized oxychloro complex, SofZia, polyquaternium-1, chlorobutanol, edetate disodium, polyhexamethylene biguanide, or combinations thereof.

[0016] In some embodiments, the ophthalmic composition further comprises a buffer agent. In some embodiments, the buffer agent is selected from borates, borate-polyol complexes, phosphate buffering agents, citrate buffering agents, acetate buffering agents, carbonate buffering agents, organic buffering agents, amino acid buffering agents, or combinations thereof.

[0017] In some embodiments, the ophthalmic composition further comprises a tonicity adjusting agent. In some embodiments, the tonicity adjusting agent is selected from sodium chloride, sodium nitrate, sodium sulfate, sodium bisulfate, potassium chloride, calcium chloride, magnesium chloride, zinc chloride, potassium acetate, sodium acetate, sodium bicarbonate, sodium carbonate, sodium thiosulfate, magnesium sulfate, disodium hydrogen phosphate, sodium dihydrogen phosphate, potassium dihydrogen phosphate, dextrose, mannitol, sorbitol, dextrose, sucrose, urea, propylene glycol, glycerin, or a combination thereof.

[0018] In some embodiments, the composition is stored in a plastic container. In some embodiments, the material of the plastic container comprises low-density polyethylene (LDPE).

[0019] In some embodiments, the ophthalmic composition is essentially free of procaine and benactyzine, or pharmaceutically acceptable salts thereof.

[0020] In some embodiments, the composition has a dose-to-dose ophthalmic agent concentration variation of less than 50%. In some embodiments, the composition has a dose-to-dose ophthalmic agent concentration variation of less than 40%. In some embodiments, the composition has a dose-to-dose ophthalmic agent concentration variation of less than 30%. In some embodiments, the

composition has a dose-to-dose ophthalmic agent concentration variation of less than 20%. In some embodiments, the composition has a dose-to-dose ophthalmic agent concentration variation of less than 10%. In some embodiments, the composition has a dose-to-dose ophthalmic agent concentration variation of less than 5%. In some embodiments, the dose-to-dose ophthalmic agent concentration variation is based on 10 consecutive doses. In some embodiments, the dose-to-dose ophthalmic agent concentration variation is based on 8 consecutive doses. In some embodiments, the dose-to-dose ophthalmic agent concentration variation is based on 5 consecutive doses. In some embodiments, the dose-to-dose ophthalmic agent concentration variation is based on 3 consecutive doses. In some embodiments, the dose-to-dose ophthalmic agent concentration variation is based on 2 consecutive doses.

[0021] In some embodiments, the composition further comprises a pD adjusting agent. In some embodiments, the pD adjusting agent comprises DCl, NaOD, CD_3COOD , or $\text{C}_6\text{D}_8\text{O}_7$.

[0022] In some embodiments, the ophthalmically acceptable carrier further comprises at least one viscosity-enhancing agent. In some embodiments, the viscosity-enhancing agent is selected from cellulose-based polymers, polyoxyethylene-polyoxypropylene triblock copolymers, dextran-based polymers, polyvinyl alcohol, dextrin, polyvinylpyrrolidone, polyalkylene glycols, chitosan, collagen, gelatin, hyaluronic acid, or combinations thereof.

[0023] In some embodiments, the ophthalmic composition comprises one of: less than 60% of H_2O , less than 55% of H_2O , less than 50% of H_2O , less than 45% of H_2O , less than 40% of H_2O , less than 35% of H_2O , less than 30% of H_2O , less than 25% of H_2O , less than 20% of H_2O , less than 15% of H_2O , or less than 10% of H_2O .

[0024] In some embodiments, the ophthalmic composition comprises one of: less than 5% of H_2O , less than 4% of H_2O , less than 3% of H_2O , less than 2% of H_2O , less than 1% of H_2O , less than 0.5% of H_2O , less than 0.1% of H_2O , or 0% of H_2O .

[0025] In some embodiments, the ophthalmic composition is stored below room temperature prior to first use. In some embodiments, the ophthalmic composition is stored at between about 2 °C to about 10 °C prior to first use. In some embodiments, the ophthalmic composition is stored at between about 4 °C to about 8 °C prior to first use.

[0026] In some embodiments, the ophthalmic composition is stored at room temperature after first use. In some embodiments, the ophthalmic composition is stored at between about 16 °C to about 26 °C after first use.

[0027] In some embodiments, the ophthalmic composition is not formulated as an injectable formulation.

[0028] In some embodiments, the ophthalmic composition is formulated as an ophthalmic solution for the treatment of pre-myopia, myopia, or progression of myopia.

[0029] In some embodiments, disclosed herein is a method of arresting myopia development that comprises administering to an eye of an individual in need thereof an effective amount of an ophthalmic composition described herein. Also described herein is a method of preventing myopia development that comprises administering to an eye of an individual in need thereof an effective amount of an ophthalmic composition described herein. In some embodiments, described herein is a method of arresting or preventing myopia development, comprising administering to an eye of an individual in need thereof an effective amount of an ophthalmic composition comprising from about 0.001 wt% to about 0.05 wt% of a muscarinic antagonist and deuterated water, at a pD of from about 4.2 to about 7.9. In some embodiments, the ophthalmic composition is administered at predetermined time intervals over an extended period of time. In some embodiments, the ophthalmic composition is administered once every day. In some embodiments, the ophthalmic composition is administered every other day. In some embodiments, the ophthalmic composition is administered over 1 week, 2 weeks, 1 month, 2 months, 3 months, 6 months, 1 year, 2 years, 3 years, 4 years, 5 years, 6 years, 7 years, 8 years, 9 years, 10 years, 11 years, or 12-15 years. In some embodiments, the ophthalmic composition is stored at between about 2 °C to about 10 °C prior to first use. In some embodiments, the ophthalmic composition is stored at between about 16 °C to about 26 °C after first use.

[0030] In some embodiments, disclosed herein is an ophthalmic solution that comprises from about 0.001 wt% to about 0.05 wt% of a muscarinic antagonist and deuterated water, at a pD of from about 4.2 to about 7.9. In some embodiments, the ophthalmic solution has a pD of one of: less than about 7.3, less than about 7.2, less than about 7.1, less than about 7, less than about 6.8, less than about 6.5, less than about 6.4, less than about 6.3, less than about 6.2, less than about 6.1, less than about 6, less than about 5.9, less than about 5.8, less than about 5.2, or less than about 4.8 after extended period of time under storage condition. In some embodiments, the muscarinic antagonist comprises atropine, atropine sulfate, noratropine, atropine-N-oxide, tropine, tropic acid, hyoscine, scopolamine, tropicamide, cyclopentolate, pirenzapine, homatropine, or a combination thereof. In some embodiments, the ophthalmic solution comprises one of: less than 5% of H₂O, less than 4% of H₂O, less than 3% of H₂O, less than 2% of H₂O, less than 1% of H₂O, less than 0.5% of H₂O, less than 0.1% of H₂O, or 0% of H₂O. In some embodiments, the ophthalmic composition comprises one of: at least about 80%, at least about 85%, at least about 90%, at least about 93%, at least about 95%, at least about 97%, at least about 98%, or at least about 99% of the muscarinic antagonist based on initial concentration after extended period of time under storage condition. In some embodiments, the ophthalmic composition further has a potency of

one of: at least 80%, at least 85%, at least 90%, at least 93%, at least 95%, at least 97%, at least 98%, or at least 99% after extended period of time under storage condition. In some embodiments, the extended period of time is one of: about 1 week, about 2 weeks, about 3 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 8 months, about 10 months, about 12 months, about 18 months, about 24 months, about 36 months, about 4 years, or about 5 years. In some embodiments, the muscarinic antagonist is present in the composition at a concentration of one of: from about 0.001 wt% to about 0.04 wt%, from about 0.001 wt% to about 0.03 wt%, from about 0.001 wt% to about 0.025 wt%, from about 0.001 wt% to about 0.02 wt%, from about 0.001 wt% to about 0.01 wt%, from about 0.001 wt% to about 0.008 wt%, or from about 0.001 wt% to about 0.005 wt%. In some embodiments, the storage condition has a storage temperature of from about 2°C to about 10°C or from about 16°C to about 26°C. In some embodiments, the ophthalmic composition has a dose-to-dose muscarinic antagonist concentration variation of one of: less than 50%, less than 40%, less than 30%, less than 20%, less than 10%, or less than 5%. In some embodiments, the dose-to-dose muscarinic antagonist concentration variation is based on one of: 10 consecutive doses, 8 consecutive doses, 5 consecutive doses, 3 consecutive doses, or 2 consecutive doses.

[0031] Other features and technical effects of the methods and compositions described herein will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating specific embodiments, are given by way of illustration only.

BRIEF DESCRIPTION OF THE DRAWINGS

[0032] The novel features of the disclosure are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present disclosure will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the disclosure are utilized, and the accompanying drawings of which:

[0033] Fig. 1A-Fig. 1C show the shelf life prediction of 0.01% atropine sulfate solution with a primary degradant RRT 0.87-0.89, and a n.m.t. of 0.5% area, based on data obtained from samples stored at 25°C and 40°C. The pH range of the atropine sulfate solution is from 5.9-6.2.

[0034] Fig. 2A-Fig. 2C show the shelf life prediction of 0.01% atropine sulfate solution with a primary degradant RRT 0.87-0.89, and a n.m.t. of 0.5% area, based on data obtained from samples stored at 25°C and 60°C. The pH range of the atropine sulfate solution is from 5.9-6.2.

[0035] Fig. 3 illustrates mass balance at 4 weeks and at 60°C condition for atropine sulfate formulations disclosed in Example 9.

[0036] Fig. 4 illustrates atropine sulfate (0.010%) formulation stability in acetic acid. The atropine sulfate formulation is formulated with acetic acid and either with H₂O (top panel, Formulation 3) or D₂O (bottom panel, Formulation 7). Formulation 3 has a pH of 4.8 and Formulation 7 has a pD of 5.2. Both formulations are stored at 60 °C for 4 weeks prior to analysis.

[0037] Fig. 5 illustrates atropine sulfate (0.01%) formulation stability in citric acid. The atropine sulfate formulation is formulated with citric acid and either with H₂O (top panel, Formulation 5) or D₂O (bottom panel, Formulation 8). Formulation 5 has a pH of 5.8 and Formulation 8 has a pD of 6.2. Both formulations are stored at 60 °C for 4 weeks prior to analysis.

[0038] Fig. 6 illustrates comparison of total RS and tropic acid for atropine sulfate (0.025%) formulation (Formulation 4) at pH 4.8 in H₂O.

[0039] Fig. 7 illustrates comparison of total RS and tropic acid for atropine sulfate (0.01%) formulation (Formulation 7) at pD 5.2 in D₂O.

[0040] Fig. 8 illustrates comparison of total RS and tropic acid for atropine sulfate (0.01%) formulation (Formulation 5) at pH 5.8 in H₂O.

[0041] Fig. 9 illustrates comparison of total RS and tropic acid for atropine sulfate (0.025%) formulation (Formulation 6) at pH 5.8 in H₂O.

[0042] Fig. 10 illustrates estimated shelf lives for D₂O and H₂O formulations disclosed in Examples 11 and 12.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0043] The present disclosure recognizes that there is a need for a stabilized ophthalmic composition with extended shelf life upon storage. The present disclosure also recognizes that there is a need for stabilizing an ophthalmic composition through arresting or reducing hydrolysis of at least some of its active agents. The present disclosure further recognizes that there is a need for an ophthalmic composition that provides convenient and effective delivery of a muscarinic antagonist such as atropine in the eye of a patient.

[0044] The present disclosure recognizes that muscarinic antagonist (e.g. atropine or its pharmaceutically acceptable salts) prevents or arrests the development of myopia in humans, for example as evidenced by reduction of the rate of increase of myopia in young people. The present disclosure also recognizes the effects of muscarinic antagonist (e.g. atropine or its pharmaceutically acceptable salts) on reduction of axial elongation and myopia in visually impaired chick eyes, and on ocular growth and muscarinic cholinergic receptors in young rhesus monkeys.

[0045] In addition, the present disclosure recognizes that systemic absorption of muscarinic antagonist (e.g. atropine) sometimes leads to undesirable side effect, and that localized delivery of muscarinic antagonist (e.g. atropine or its pharmaceutically acceptable salts) reduces or prevents the aforementioned systemic exposure.

[0046] Further, the present disclosure recognizes that some liquid muscarinic antagonist (e.g. atropine) compositions are formulated at a relatively lower pH range (e.g. less than 4.5) for stability of muscarinic antagonist (e.g. atropine or its pharmaceutically acceptable salts). For some individuals, the lower pH range in some instances causes discomfort or other side effects such as pain or burning sensation in the eye, which is prevented or alleviated by formulating muscarinic antagonist (e.g. atropine) compositions at higher pH ranges. For some individuals, the lower pH in some instances elicits a tear response which reduces the absorption of the drug in the eye and therefore the effectiveness.

[0047] Still further, the present disclosure recognizes that some muscarinic antagonist (e.g. atropine) liquid compositions formulated at lower concentrations (e.g. 0.001% to 0.05%) present stability challenges that are less so in higher concentrations (e.g. 0.1-1%). Without wishing to be bound by any particular theory, it is contemplated that the some muscarinic antagonist (e.g. atropine) contributes to the stability of an ophthalmic composition, such as an aqueous solution. For example, the concentration of the muscarinic antagonist (e.g. atropine) in some embodiments affects the pH or pD of the ophthalmic composition, such as with the muscarinic antagonist acting as a buffering agent. Furthermore, the concentration of the muscarinic antagonist (e.g. atropine) in some embodiments affects the interaction between the muscarinic antagonist and other ingredients of the ophthalmic composition, which in turn affects the stability of the ophthalmic composition.

[0048] Finally, the present disclosure recognizes that deuterated water stabilizes ophthalmic compositions. In some cases, the deuterated water is a weak acid as compared to H₂O, as such deuterated water comprises a lower concentration of the reactive species (e.g., -OD) which in some instances leads to base catalyzed hydrolysis of an active agent in the ophthalmic composition. As such, in some instances compositions comprising deuterated water leads to reduced base catalyzed hydrolysis when compared to compositions comprising H₂O. In some instances, deuterated water further lowers the buffering capacity of an ophthalmic composition, leading to less tear reflex in the eye.

[0049] Myopia, axial elongation of the eye, affects a large proportion of the population. The onset of myopia is generally during the grade school years and progresses until growth of the eye is completed. The present disclosure recognizes the importance of compositions and treatments for preventing or arresting the development of myopia, especially compositions and treatments that allow

convenient administration, reduce potential side effects, has suitable stability, and/or provide relatively consistent therapeutic effects.

[0050] Ophthalmic Muscarinic Antagonist Composition

[0051] Provided herein is an ophthalmic composition containing low concentrations of an ophthalmic agent. In some embodiments, the ophthalmic composition includes from about 0.001 wt% to about 0.05 wt% of an ophthalmic agent for treatment of an ophthalmic disorder or condition; and an ophthalmically acceptable carrier, wherein the ophthalmic agent is distributed with substantial uniformity throughout the ophthalmically acceptable carrier. In some instances, the ophthalmic agent is a muscarinic antagonist.

[0052] Provided herein is an ophthalmic composition containing low concentrations of a muscarinic antagonist. In some embodiments, the ophthalmic composition includes from about 0.001 wt% to about 0.05 wt% of a muscarinic antagonist for treatment of an ophthalmic disorder or condition; and an ophthalmically acceptable carrier, wherein the muscarinic antagonist is distributed with substantial uniformity throughout the ophthalmically acceptable carrier.

[0053] In some instances, the muscarinic antagonist includes atropine, atropine sulfate, noratropine, atropine-N-oxide, tropine, tropic acid, atropine methonitrate, diphenhydramine, dimenhydrinate, dicyclomine, flavoxate, oxybutynin, tiotropium, hyoscine, scopolomine (L-hyoscine), hydroxyzine, ipratropium, tropicamide, cyclopentolate, pirenzapine, homatropine, solifenacin, darifenacin, benzatropine, mebeverine, procyclidine, acridinium bromide, trihexyphenidyl/benzhexol, tolterodine, or a combination thereof. In some instances, the muscarinic antagonist includes atropine, atropine sulfate, noratropine, atropine-N-oxide, tropine, tropic acid, hyoscine, scopolomine, tropicamide, cyclopentolate, pirenzapine, homatropine, or a combination thereof. In some embodiments, the muscarinic antagonist is atropine, or a pharmaceutically acceptable salt or prodrug thereof. In some embodiments, the muscarinic antagonist is atropine sulfate.

[0054] In some embodiments, the ophthalmic composition comprise a muscarinic antagonist selected from atropine, atropine sulfate, noratropine, atropine-N-oxide, tropine, tropic acid, atropine methonitrate, diphenhydramine, dimenhydrinate, dicyclomine, flavoxate, oxybutynin, tiotropium, hyoscine, scopolomine (L-hyoscine), hydroxyzine, ipratropium, tropicamide, cyclopentolate, pirenzapine, homatropine, solifenacin, darifenacin, benzatropine, mebeverine, procyclidine, acridinium bromide, trihexyphenidyl/benzhexol, tolterodine, or a combination thereof. In some instances, the muscarinic antagonist includes atropine, atropine sulfate, noratropine, atropine-N-oxide, tropine, tropic acid, hyoscine, scopolomine, tropicamide, cyclopentolate, pirenzapine, or homatropine.

[0055] In some embodiments, the ophthalmic composition comprise two or more muscarinic antagonists in which the two or more muscarinic antagonists comprises atropine, atropine sulfate, noratropine, atropine-N-oxide, tropine, tropic acid, atropine methonitrate, diphenhydramine, dimenhydrinate, dicyclomine, flavoxate, oxybutynin, tiotropium, hyoscine, scopolomine (L-hyoscine), hydroxyzine, ipratropium, tropicamide, cyclopentolate, pirenzapine, homatropine, solifenacin, darifenacin, benzatropine, mebeverine, procyclidine, acridinium bromide, trihexyphenidyl/benzhexol, tolterodine, or a combination thereof. In some instances, the muscarinic antagonist includes atropine, atropine sulfate, noratropine, atropine-N-oxide, tropine, tropic acid, hyoscine, scopolomine, tropicamide, cyclopentolate, pirenzapine, homatropine, or any combination thereof.

[0056] In some embodiments, the ophthalmic composition comprises one or more muscarinic antagonist in combination with one or more sympathetic agonists. In some embodiments, the sympathetic agonist is selected from phenylephrine or hydroxyamphetamine. In some embodiments, the ophthalmic composition comprises one or more of muscarinic antagonist: atropine, atropine sulfate, noratropine, atropine-N-oxide, tropine, tropic acid, atropine methonitrate, diphenhydramine, dimenhydrinate, dicyclomine, flavoxate, oxybutynin, tiotropium, hyoscine, scopolomine (L-hyoscine), hydroxyzine, ipratropium, tropicamide, cyclopentolate, pirenzapine, homatropine, solifenacin, darifenacin, benzatropine, mebeverine, procyclidine, acridinium bromide, trihexyphenidyl/benzhexol, or tolterodine; in combination with one or more of sympathetic agonists: phenylephrine or hydroxyamphetamine.

[0057] Provided herein is an ophthalmic composition containing low concentrations of atropine or its pharmaceutically acceptable salts. In some embodiments, the ophthalmic composition includes from about 0.001 wt% to about 0.05 wt% of atropine or its pharmaceutically acceptable salts for treatment of an ophthalmic disorder or condition; and an ophthalmically acceptable carrier, wherein the ophthalmic agent is distributed with substantial uniformity throughout the ophthalmically acceptable carrier.

[0058] Provided herein is an ophthalmic composition containing low concentrations of atropine sulfate. In some embodiments, the ophthalmic composition includes from about 0.001 wt% to about 0.05 wt% of atropine sulfate for treatment of an ophthalmic disorder or condition; and an ophthalmically acceptable carrier, wherein the ophthalmic agent is distributed with substantial uniformity throughout the ophthalmically acceptable carrier.

[0059] In some embodiments, the ophthalmic disorder or condition is pre-myopia, myopia or progression of myopia.

[0060] The present disclosure further recognizes that the clinical use of atropine as a therapy has been limited due to its ocular side effects including glare from pupillary dilation and blurred vision due to loss of accommodation. Without wishing to be bound by any particular theory, it is contemplated that the

limited use of atropine against myopia development, include its ocular side effects, is attributable to the concentration of atropine used in known ophthalmic formulations (e.g. 1wt% or higher).

[0061] The present disclosure further recognizes the challenges present in formulation of compositions that contain low concentrations, especially very low concentrations (e.g. from about 0.001 wt% to about 0.5 wt%), of ophthalmic agents, such as muscarinic antagonist (e.g. atropine or its pharmaceutically acceptable salts). In particular, pharmaceutical compositions with ophthalmic agent at such low concentrations are difficult to maintain dose-to-dose uniformity in term of ophthalmic agent content and/or distribution.

[0062] In some aspects, described herein are formulations or solutions of muscarinic antagonist (e.g., atropine) formulated in deuterated water. In some aspects, formulations or solutions of muscarinic antagonist (e.g., atropine) formulated in deuterated water are stable at different temperatures, at different relative humidity, with an acidic pD, and with a potency of at least 80% relative to the ophthalmic agent. In additional aspects, formulations or solutions of muscarinic antagonist (e.g., atropine) formulated in deuterated water has a lowered buffering capacity. In such instances, the lowered buffering capacity of the ophthalmic formulations or solutions when administered into the eye allows the ophthalmic formulation or solution to reach physiological pH at a faster rate than compared to an equivalent ophthalmic formulation or solution formulated in H₂O.

[0063] In some aspects, described herein are formulations of muscarinic antagonist (e.g. atropine) at low concentrations that does not have a dose-to-dose variation. In some aspects, described herein are formulations of muscarinic antagonist (e.g. atropine) at low concentrations that are stable at different temperatures, at different relative humidity, with an acidic pD, and with a potency of at least 80% relative to the ophthalmic agent.

[0064] In other aspects, described herein include formulating the ophthalmic composition as an ophthalmic gel or an ophthalmic ointment. For example, some ophthalmic gel or an ophthalmic ointment described herein allows desirable dose-to-dose uniformity, reduced or limited systemic exposure, or combinations thereof.

[0065] Ophthalmic Solution Muscarinic Antagonist Composition

[0066] Disclosed herein, in certain embodiments, is an ophthalmic composition formulated as an aqueous solution. In some embodiments, the ophthalmic composition comprises from about 0.001 wt% to about 0.05 wt% of a muscarinic antagonist and deuterated water. As used herein, deuterated water refers to D₂O, DHO, heavy water, and/or deuterium oxide.

[0067] In some embodiments, the composition comprises at least about 80% of the ophthalmic agent (e.g. muscarinic antagonist) for an extended period of time under storage condition. In some

[illegible]

[illegible]

[0069] In some embodiments, the extended period of time is at least 1 week. In some embodiments, the extended period of time is at least 2 weeks. In some embodiments, the extended period of time is at least 3 weeks. In some embodiments, the extended period of time is at least 1 month. In some embodiments, the extended period of time is at least 2 months. In some embodiments, the extended period of time is at least 3 months. In some embodiments, the extended period of time is at least 4 months. In some embodiments, the extended period of time is at least 5 months. In some embodiments, the extended period of time is at least 6 months. In some embodiments, the extended period of time is at least 7 months. In some embodiments, the extended period of time is at least 8 months. In some embodiments, the extended period of time is at least 9 months. In some embodiments, the extended period

of time is at least 10 months. In some embodiments, the extended period of time is at least 11 months. In some embodiments, the extended period of time is at least 12 months (i.e. 1 year). In some embodiments, the extended period of time is at least 18 months (i.e. 1.5 years). In some embodiments, the extended period of time is at least 24 months (i.e. 2 years). In some embodiments, the extended period of time is at least 36 months (i.e. 3 years). In some embodiments, the extended period of time is at least 3 years. In some embodiments, the extended period of time is at least 5 years, or more.

[0070] In some embodiments, the temperature of the storage condition is between about 20°C and about 70°C. In some embodiments, the temperature of the storage condition is between about 25°C and about 65°C, about 30°C and about 60°C, about 35°C and about 55°C, or about 40°C and about 50°C. In some embodiments, the temperature of the storage condition is about 25°C. In some embodiments, the temperature of the storage condition is about 40°C. In some embodiments, the temperature of the storage condition is about 60°C.

[0071] In some embodiments, the relative humidity of the storage condition is between about 50% and about 80%, or between about 60% and about 75%. In some embodiments, the relative humidity of the storage condition is about 60%. In some embodiments, the relative humidity of the storage condition is about 75%.

[0072] In some embodiments, the composition comprises less than 60% of H₂O. In some embodiments, the composition comprises less than 55% of H₂O. In some embodiments, the composition comprises less than 50% of H₂O. In some embodiments, the composition comprises less than 45% of H₂O. In some embodiments, the composition comprises less than 40% of H₂O. In some embodiments, the composition comprises less than 35% of H₂O. In some embodiments, the composition comprises less than 30% of H₂O. In some embodiments, the composition comprises less than 25% of H₂O. In some embodiments, the composition comprises less than 20% of H₂O. In some embodiments, the composition comprises less than 15% of H₂O. In some embodiments, the composition comprises less than 10% of H₂O.

[0073] In some embodiments, the composition comprises from less than 5% of H₂O to 0% of H₂O. In some embodiments, the composition comprises less than 5% of H₂O. In some embodiments, the composition comprises less than 4.5% of H₂O. In some embodiments, the composition comprises less than 4% of H₂O. In some embodiments, the composition comprises less than 3.5% of H₂O. In some embodiments, the composition comprises less than 3% of H₂O. In some embodiments, the composition comprises less than 2.5% of H₂O. In some embodiments, the composition comprises less than 2% of H₂O. In some embodiments, the composition comprises less than 1.5% of H₂O. In some embodiments, the composition comprises less than 1% of H₂O. In some embodiments, the composition comprises less than

0.5% of H₂O. In some embodiments, the composition comprises less than 0.4% of H₂O. In some embodiments, the composition comprises less than 0.3% of H₂O. In some embodiments, the composition comprises less than 0.2% of H₂O. In some embodiments, the composition comprises less than 0.1% of H₂O. In some embodiments, the composition comprises 0% of H₂O.

[0074] In some embodiments, the composition has a pD of between about 4 and about 8, about 4.5 and about 7.8, about 5 and about 7.5, or about 5.5 and about 7. In some embodiments, the composition has a pD of less than about 7.5. In some embodiments, the composition has a pD of less than about 7.4. In some embodiments, the composition has a pD of less than about 7.3. In some embodiments, the composition has a pD of less than about 7.2. In some embodiments, the composition has a pD of less than about 7.1. In some embodiments, the composition has a pD of less than about 7. In some embodiments, the composition has a pD of less than about 6.9. In some embodiments, the composition has a pD of less than about 6.8. In some embodiments, the composition has a pD of less than about 6.7. In some embodiments, the composition has a pD of less than about 6.6. In some embodiments, the composition has a pD of less than about 6.5. In some embodiments, the composition has a pD of less than about 6.4. In some embodiments, the composition has a pD of less than about 6.3. In some embodiments, the composition has a pD of less than about 6.2. In some embodiments, the composition has a pD of less than about 6.1. In some embodiments, the composition has a pD of less than about 6. In some embodiments, the composition has a pD of less than about 5.9. In some embodiments, the composition has a pD of less than about 5.8. In some embodiments, the composition has a pD of less than about 5.7. In some embodiments, the composition has a pD of less than about 5.6. In some embodiments, the composition has a pD of less than about 5.5. In some embodiments, the composition has a pD of less than about 5.4. In some embodiments, the composition has a pD of less than about 5.3. In some embodiments, the composition has a pD of less than about 5.2. In some embodiments, the composition has a pD of less than about 5.1. In some embodiments, the composition has a pD of less than about 5. In some embodiments, the composition has a pD of less than about 4.9. In some embodiments, the composition has a pD of less than about 4.8. In some embodiments, the composition has a pD of less than about 4.7. In some embodiments, the composition has a pD of less than about 4.6. In some embodiments, the composition has a pD of less than about 4.5. In some embodiments, the composition has a pD of less than about 4.4. In some embodiments, the composition has a pD of less than about 4.3. In some embodiments, the composition has a pD of less than about 4.2. In some embodiments, the composition has a pD of less than about 4.1. In some embodiments, the composition has a pD of less than about 4.

[0075] In some embodiments, the composition comprising deuterated water has a lowered buffering capacity than an equivalent composition comprising H₂O. As described elsewhere herein, in some embodiments, the lowered buffering capacity allows the composition comprising deuterated water

to normalize to physiological pH at a faster rate than a composition comprising H₂O. In some embodiments, the lowered buffering capacity allows the composition to induce less tear reflex than an equivalent composition comprising H₂O.

[0076] In some instances, the composition comprising deuterated water stabilizes muscarinic antagonist (e.g., atropine). In some embodiments, this is due to a lower concentration of the reactive species (e.g., -OD) in the D₂O aqueous system compared to the concentration of the reactive species (e.g., -OH) in an equivalent H₂O aqueous system. In some cases, base catalyzed hydrolysis leads to the presence of tropine degradant from atropine. In some cases, with a lower concentration of the reactive species that causes tropine degradant formation, atropine solution is more stable in a D₂O aqueous system than compared to an equivalent H₂O aqueous system. In some embodiments, the ophthalmic composition formulated with deuterated water allows for a more stable ophthalmic composition relative to the ophthalmic composition formulated with H₂O.

[0077] In some embodiments, the composition comprises less than 20% of major degradant based on the concentration of the ophthalmic agent after extended period of time under storage condition. In some embodiments, the composition comprises less than 15% of major degradant based on the concentration of the ophthalmic agent after extended period of time under storage condition.

[0078] In some embodiments, the composition comprises less than 10% of major degradant based on the concentration of the ophthalmic agent after extended period of time under storage condition. In some embodiments, the composition comprises less than 5% of major degradant based on the concentration of the ophthalmic agent after extended period of time under storage condition. In some embodiments, the composition comprises less than 2.0% of major degradant based on the concentration of the ophthalmic agent after extended period of time under storage condition. In some embodiments, the composition comprises less than 1.5% of major degradant based on the concentration of the ophthalmic agent after extended period of time under storage condition. In some embodiments, the composition comprises less than 1.0% of major degradant based on the concentration of the ophthalmic agent after extended period of time under storage condition. In some embodiments, the composition comprises less than 0.5% of major degradant based on the concentration of the ophthalmic agent after extended period of time under storage condition. In some embodiments, the composition comprises less than 0.4% of major degradant based on the concentration of the ophthalmic agent after extended period of time under storage condition. In some embodiments, the composition comprises less than 0.3% of major degradant based on the concentration of the ophthalmic agent after extended period of time under storage condition. In some embodiments, the composition comprises less than 0.2% of major degradant based on the concentration of the ophthalmic agent after extended period of time under storage condition. In some embodiments, the

composition comprises less than 0.1% of major degradant based on the concentration of the ophthalmic agent after extended period of time under storage condition. In some embodiments, the major degradant is tropic acid.

[0079] In some embodiments, the primary degradant is an early eluting related substance at RRT of 0.87-0.89 according to the UPLC method described herein (Table 10). In some instances, the early eluting related substance is referred to as RRT 0.87-0.89. In some embodiments, the primary degradant is RRT 0.87-0.89.

[0080] Ophthalmic Muscarinic Antagonist Concentration

[0081] In some embodiments, the compositions described herein have a concentration of ophthalmic agent between about 0.001% to about 0.050%, between about 0.005% to about 0.050%, between about 0.010% to about 0.050%, between about 0.015% to about 0.050%, between about 0.020% to about 0.050%, between about 0.025% to about 0.050%, between about 0.030% to about 0.050%, between about 0.035% to about 0.050%, between about 0.040% to about 0.050%, or between about 0.045% to about 0.050% of the ophthalmic agent, or pharmaceutically acceptable prodrug or salt thereof, by weight of the composition. In some instances, the prodrug of the ophthalmic agent (e.g. muscarinic antagonist) is chemically converted into the ophthalmic agent (e.g. muscarinic antagonist) after the administration of the ophthalmic composition. In a non-limiting example, the muscarinic antagonist prodrug has a chemical bond that is cleavable by one or more enzymes in tears. In some embodiments, the ophthalmic agent is a muscarinic antagonist. In some embodiments, the muscarinic antagonist comprises atropine, atropine sulfate, noratropine, atropine-N-oxide, tropine, tropic acid, hyoscyne, scopolomine, tropicamide, cyclopentolate, pirenzapine, homatropine, or a combination thereof. In some embodiments, the muscarinic antagonist is atropine, or a pharmaceutically acceptable salt thereof. In some embodiments, the muscarinic antagonist is atropine sulfate. As described herein, the ophthalmic agent includes optically pure stereoisomers, optically enriched stereoisomers, and a racemic mixture of stereoisomers. For example, some ophthalmic compositions disclosed herein includes atropine or atropine sulfate in which the atropine is a racemic mixture of D- and L-isomers; and some ophthalmic compositions disclosed herein includes atropine or atropine sulfate in which the atropine is a optically enriched in favor of the more ophthalmically active L-isomer.

[0082] In some embodiments, the compositions described herein have a concentration of ophthalmic agent between about 0.001% to about 0.045%, between about 0.005% to about 0.045%, between about 0.010% to about 0.045%, between about 0.015% to about 0.045%, between about 0.020% to about 0.045%, between about 0.025% to about 0.045%, between about 0.030% to about 0.045%, between about 0.035% to about 0.045%, or between about 0.040% to about 0.045% of the ophthalmic

agent, or pharmaceutically acceptable prodrug or salt thereof, by weight of the composition. In some embodiments, the ophthalmic agent is a muscarinic antagonist. In some embodiments, the muscarinic antagonist comprises atropine, atropine sulfate, noratropine, atropine-N-oxide, tropine, tropic acid, hyoscine, scopolomine, tropicamide, cyclopentolate, pirenzapine, homatropine, or a combination thereof. In some embodiments, the muscarinic antagonist is atropine, or a pharmaceutically acceptable salt thereof. In some embodiments, the muscarinic antagonist is atropine sulfate.

[0083] In some embodiments, the compositions described herein have a concentration of ophthalmic agent between about 0.001% to about 0.040%, between about 0.005% to about 0.040%, between about 0.010% to about 0.040%, between about 0.015% to about 0.040%, between about 0.020% to about 0.040%, between about 0.025% to about 0.040%, between about 0.030% to about 0.040%, between about 0.035% to about 0.040% of the active ingredient, or pharmaceutically acceptable prodrug or salt thereof, by weight of the composition. In some embodiments, the ophthalmic agent is a muscarinic antagonist. In some embodiments, the muscarinic antagonist comprises atropine, atropine sulfate, noratropine, atropine-N-oxide, tropine, tropic acid, hyoscine, scopolomine, tropicamide, cyclopentolate, pirenzapine, homatropine, or a combination thereof. In some embodiments, the muscarinic antagonist is atropine, or a pharmaceutically acceptable salt thereof. In some embodiments, the muscarinic antagonist is atropine sulfate.

[0084] In some embodiments, the compositions described herein have a concentration of ophthalmic agent between about 0.001% to about 0.035%, between about 0.005% to about 0.035%, between about 0.010% to about 0.035%, between about 0.015% to about 0.035%, between about 0.020% to about 0.035%, between about 0.025% to about 0.035%, or between about 0.030% to about 0.035% of the ophthalmic agent, or pharmaceutically acceptable prodrug or salt thereof, by weight of the composition. In some embodiments, the ophthalmic agent is a muscarinic antagonist. In some embodiments, the muscarinic antagonist comprises atropine, atropine sulfate, noratropine, atropine-N-oxide, tropine, tropic acid, hyoscine, scopolomine, tropicamide, cyclopentolate, pirenzapine, homatropine, or a combination thereof. In some embodiments, the muscarinic antagonist is atropine, or a pharmaceutically acceptable salt thereof. In some embodiments, the muscarinic antagonist is atropine sulfate.

[0085] In some embodiments, the compositions described herein have a concentration of ophthalmic agent between about 0.001% to about 0.030%, between about 0.005% to about 0.030%, between about 0.010% to about 0.030%, between about 0.015% to about 0.030%, between about 0.020% to about 0.030%, or between about 0.025% to about 0.030% of the active ingredient, or pharmaceutically acceptable prodrug or salt thereof, by weight of the composition. In some embodiments, the ophthalmic

agent is a muscarinic antagonist. In some embodiments, the muscarinic antagonist comprises atropine, atropine sulfate, noratropine, atropine-N-oxide, tropine, tropic acid, hyoscine, scopolomine, tropicamide, cyclopentolate, pirenzapine, homatropine, or a combination thereof. In some embodiments, the muscarinic antagonist is atropine, or a pharmaceutically acceptable salt thereof. In some embodiments, the muscarinic antagonist is atropine sulfate.

[0086] In some embodiments, the compositions described herein have a concentration of ophthalmic agent between about 0.001% to about 0.025%, between about 0.005% to about 0.025%, between about 0.010% to about 0.025%, between about 0.015% to about 0.025%, or between about 0.020% to about 0.025% of the ophthalmic agent, or pharmaceutically acceptable prodrug or salt thereof, by weight of the composition. In some embodiments, the ophthalmic agent is a muscarinic antagonist. In some embodiments, the muscarinic antagonist comprises atropine, atropine sulfate, noratropine, atropine-N-oxide, tropine, tropic acid, hyoscine, scopolomine, tropicamide, cyclopentolate, pirenzapine, homatropine, or a combination thereof. In some embodiments, the muscarinic antagonist is atropine, or a pharmaceutically acceptable salt thereof. In some embodiments, the muscarinic antagonist is atropine sulfate.

[0087] In some embodiments, the compositions described herein have a concentration of ophthalmic agent between about 0.001% to about 0.020%, between about 0.005% to about 0.020%, between about 0.010% to about 0.020%, or between about 0.015% to about 0.020% of the active ingredient, or pharmaceutically acceptable prodrug or salt thereof, by weight of the composition. In some embodiments, the ophthalmic agent is a muscarinic antagonist. In some embodiments, the muscarinic antagonist comprises atropine, atropine sulfate, noratropine, atropine-N-oxide, tropine, tropic acid, hyoscine, scopolomine, tropicamide, cyclopentolate, pirenzapine, homatropine, or a combination thereof. In some embodiments, the muscarinic antagonist is atropine, or a pharmaceutically acceptable salt thereof. In some embodiments, the muscarinic antagonist is atropine sulfate.

[0088] In some embodiments, the compositions described herein have a concentration of ophthalmic agent between about 0.001% to about 0.015%, between about 0.005% to about 0.015%, or between about 0.010% to about 0.015% of the ophthalmic agent, or pharmaceutically acceptable prodrug or salt thereof, by weight of the composition. In some embodiments, the ophthalmic agent is a muscarinic antagonist. In some embodiments, the muscarinic antagonist comprises atropine, atropine sulfate, noratropine, atropine-N-oxide, tropine, tropic acid, hyoscine, scopolomine, tropicamide, cyclopentolate, pirenzapine, homatropine, or a combination thereof. In some embodiments, the muscarinic antagonist is atropine, or a pharmaceutically acceptable salt thereof. In some embodiments, the muscarinic antagonist is atropine sulfate.

[0089] In some embodiments, the compositions described herein have a concentration of ophthalmic agent between about 0.001% to about 0.010%, between about 0.005% to about 0.010%, or between about 0.008% to about 0.010% of the ophthalmic agent, or pharmaceutically acceptable prodrug or salt thereof, by weight of the composition. In some embodiments, the ophthalmic agent is a muscarinic antagonist. In some embodiments, the muscarinic antagonist comprises atropine, atropine sulfate, noratropine, atropine-N-oxide, tropine, tropic acid, hyoscine, scopolomine, tropicamide, cyclopentolate, pirenzapine, homatropine, or a combination thereof. In some embodiments, the muscarinic antagonist is atropine, or a pharmaceutically acceptable salt thereof. In some embodiments, the muscarinic antagonist is atropine sulfate.

[0090] In some embodiments, the compositions described herein have a concentration of ophthalmic agent about 0.001%, 0.005%, 0.010%, 0.015%, 0.020%, 0.025%, 0.030%, 0.035%, 0.040%, 0.045%, or 0.050% of the ophthalmic agent, or pharmaceutically acceptable prodrug or salt thereof, by weight of the composition. In some embodiments, the ophthalmic agent is a muscarinic antagonist. In some embodiments, the muscarinic antagonist comprises atropine, atropine sulfate, noratropine, atropine-N-oxide, tropine, tropic acid, hyoscine, scopolomine, tropicamide, cyclopentolate, pirenzapine, homatropine, or a combination thereof. In some embodiments, the muscarinic antagonist is atropine, or a pharmaceutically acceptable salt thereof. In some embodiments, the muscarinic antagonist is atropine sulfate.

[0091] Without wishing to be bound by any particular theory, it is contemplated herein that the low concentration of the ophthalmic agent (e.g. muscarinic antagonist such as atropine or atropine sulfate) in the disclosed ophthalmic composition provides sufficient and consistent therapeutic benefits to an individual in need thereof, while reducing or avoiding the ocular side effects including glare from pupillary dilation and blurred vision due to loss of accommodation that are associated with ophthalmic formulations containing higher concentrations of the ophthalmic agent (e.g. muscarinic antagonist such as atropine or atropine sulfate).

[0092] Aqueous Solution Stability

[0093] In some embodiments, the composition described herein comprises a buffer. In some embodiments, a buffer is selected from borates, borate-polyol complexes, phosphate buffering agents, citrate buffering agents, acetate buffering agents, carbonate buffering agents, organic buffering agents, amino acid buffering agents, or combinations thereof. In some embodiments, the composition described herein comprises buffer comprising deuterated water. In some embodiments, a deuterated buffer is selected from borates, borate-polyol complexes, phosphate buffering agents, citrate buffering agents,

acetate buffering agents, carbonate buffering agents, organic buffering agents, amino acid buffering agents, or combinations thereof, formulated in deuterated water.

[0094] In some instances, borates include boric acid, salts of boric acid, other pharmaceutically acceptable borates, and combinations thereof. In some cases, borates include boric acid, sodium borate, potassium borate, calcium borate, magnesium borate, manganese borate, and other such borate salts.

[0095] As used herein, the term polyol includes any compound having at least one hydroxyl group on each of two adjacent carbon atoms that are not in trans configuration relative to each other. In some embodiments, the polyols is linear or cyclic, substituted or unsubstituted, or mixtures thereof, so long as the resultant complex is water soluble and pharmaceutically acceptable. In some instances, examples of polyol include: sugars, sugar alcohols, sugar acids and uronic acids. In some cases, polyols include, but are not limited to: mannitol, glycerin, xylitol and sorbitol.

[0096] In some embodiments, phosphate buffering agents include phosphoric acid; alkali metal phosphates such as disodium hydrogen phosphate, sodium dihydrogen phosphate, trisodium phosphate, dipotassium hydrogen phosphate, potassium dihydrogen phosphate, and tripotassium phosphate; alkaline earth metal phosphates such as calcium phosphate, calcium hydrogen phosphate, calcium dihydrogen phosphate, monomagnesium phosphate, dimagnesium phosphate (magnesium hydrogen phosphate), and trimagnesium phosphate; ammonium phosphates such as diammonium hydrogen phosphate and ammonium dihydrogen phosphate; or a combination thereof. In some instances, the phosphate buffering agent is an anhydride. In some instances, the phosphate buffering agent is a hydrate.

[0097] In some embodiments, borate-polyol complexes include those described in U.S. Pat. No. 6,503,497. In some instances, the borate-polyol complexes comprise borates in an amount of from about 0.01 to about 2.0% w/v, and one or more polyols in an amount of from about 0.01% to about 5.0% w/v.

[0098] In some cases, citrate buffering agents include citric acid and sodium citrate.

[0099] In some instances, acetate buffering agents include acetic acid, potassium acetate, and sodium acetate.

[00100] In some instances, carbonate buffering agents include sodium bicarbonate and sodium carbonate.

[00101] In some cases, organic buffering agents include Good's Buffer, such as for example 2-(N-morpholino)ethanesulfonic acid (MES), *N*-(2-Acetamido)iminodiacetic acid, *N*-(Carbamoylmethyl)iminodiacetic acid (ADA), piperazine-*N,N'*-bis(2-ethanesulfonic acid (PIPES), *N*-(2-acetamido)-2-aminoethanesulfonic acid (ACES), β -Hydroxy-4-morpholinepropanesulfonic acid, 3-Morpholino-2-hydroxypropanesulfonic acid (MOPSO), choline chloride, 3-(*N*-morpholino)propanesulfonic acid (MOPS), *N,N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES),

2-[(2-Hydroxy-1,1-bis(hydroxymethyl)ethyl)amino]ethanesulfonic acid (TES), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 3-(N,N-Bis[2-hydroxyethyl]amino)-2-hydroxypropanesulfonic acid (DIPSO), acetamidoglycine, 3-{{1,3-Dihydroxy-2-(hydroxymethyl)-2-propanyl}amino}-2-hydroxy-1-propanesulfonic acid (TAPSO), piperazine-1,4,-bis (2-hydroxypropanesulphonic acid) (POPSO), 4-(2-hydroxyethyl)piperazine-1-(2-hydroxypropanesulfonic acid) hydrate (HEPPSO), 3-[4-(2-hydroxyethyl)-1-piperazinyl]propanesulfonic acid (HEPPS), tricine, glycine, bicine or N-tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid sodium (TAPS); glycine; and diethanolamine (DEA).

[00102] In some cases, amino acid buffering agents include taurine, aspartic acid and its salts (e.g., potassium salts, etc), E-aminocaproic acid, and the like.

[00103] In some instances, the composition described herein further comprises a tonicity adjusting agent. Tonicity adjusting agent is an agent introduced into a preparation such as an ophthalmic composition to reduce local irritation by preventing osmotic shock at the site of application. In some instances, buffer solution and/or a pH adjusting agent that broadly maintains the ophthalmic solution at a particular ion concentration and pH are considered as tonicity adjusting agents. In some cases, tonicity adjusting agents include various salts, such as halide salts of a monovalent cation. In some cases, tonicity adjusting agents include mannitol, sorbitol, dextrose, sucrose, urea, and glycerin. In some instances, suitable tonicity adjusters comprise sodium chloride, sodium nitrate, sodium sulfate, sodium bisulfate, potassium chloride, calcium chloride, magnesium chloride, zinc chloride, potassium acetate, sodium acetate, sodium bicarbonate, sodium carbonate, sodium thiosulfate, magnesium sulfate, disodium hydrogen phosphate, sodium dihydrogen phosphate, potassium dihydrogen phosphate, dextrose, mannitol, sorbitol, dextrose, sucrose, urea, propylene glycol, glycerin, or a combination thereof.

[00104] In some instances, the concentration of the tonicity adjusting agent in a composition described herein is between about 0.5% and about 2.0%. In some instances, the concentration of the tonicity adjusting agent in a composition described herein is between about 0.7% and about 1.8%, about 0.8% and about 1.5%, or about 1% and about 1.3%. In some instances, the concentration of the tonicity adjusting agent is about 0.6%, 0.7%, 0.8%, 0.9%, 1.0%, 1.1%, 1.2%, 1.3%, 1.4%, 1.5%, 1.6%, 1.7%, 1.8%, or 1.9%. In some cases, the percentage is a weight percentage.

[00105] In some cases, the composition described herein further comprises a pH adjusting agent. In some embodiments, the pH adjusting agent used is an acid or a base. In some embodiments, the base is oxides, hydroxides, carbonates, bicarbonates and the likes. In some instances, the oxides are metal oxides such as calcium oxide, magnesium oxide and the likes; hydroxides are of alkali metals and alkaline earth metals such as sodium hydroxide, potassium hydroxide, calcium hydroxide and the likes or their

deuterated equivalents, and carbonates are sodium carbonate, sodium bicarbonates, potassium bicarbonates and the likes. In some instances, the acid is mineral acid and organic acids such as hydrochloric acid, nitric acid, phosphoric acid, acetic acid, citric acid, fumaric acid, malic acid tartaric acid and the likes or their deuterated equivalents. In some instances, the pD adjusting agent includes, but is not limited to, acetate, bicarbonate, ammonium chloride, citrate, phosphate, pharmaceutically acceptable salts thereof and combinations or mixtures thereof. In some embodiments, the pD adjusting agent comprises DCl and NaOD.

[00106] In some instances, the composition has a pD of between about 4 and about 8, about 4.5 and about 7.8, about 5 and about 7.5, or about 5.5 and about 7. In some embodiments, the composition has a pD of less than about 7.5. In some embodiments, the composition has a pD of less than about 7.4. In some embodiments, the composition has a pD of less than about 7.3. In some embodiments, the composition has a pD of less than about 7.2. In some embodiments, the composition has a pD of less than about 7.1. In some embodiments, the composition has a pD of less than about 7. In some embodiments, the composition has a pD of less than about 6.9. In some embodiments, the composition has a pD of less than about 6.8. In some embodiments, the composition has a pD of less than about 6.7. In some embodiments, the composition has a pD of less than about 6.6. In some embodiments, the composition has a pD of less than about 6.5. In some embodiments, the composition has a pD of less than about 6.4. In some embodiments, the composition has a pD of less than about 6.3. In some embodiments, the composition has a pD of less than about 6.2. In some embodiments, the composition has a pD of less than about 6.1. In some embodiments, the composition has a pD of less than about 6. In some embodiments, the composition has a pD of less than about 5.9. In some embodiments, the composition has a pD of less than about 5.8. In some embodiments, the composition has a pD of less than about 5.7. In some embodiments, the composition has a pD of less than about 5.6. In some embodiments, the composition has a pD of less than about 5.5. In some embodiments, the composition has a pD of less than about 5.4. In some embodiments, the composition has a pD of less than about 5.3. In some embodiments, the composition has a pD of less than about 5.2. In some embodiments, the composition has a pD of less than about 5.1. In some embodiments, the composition has a pD of less than about 5. In some embodiments, the composition has a pD of less than about 4.9. In some embodiments, the composition has a pD of less than about 4.8. In some embodiments, the composition has a pD of less than about 4.7. In some embodiments, the composition has a pD of less than about 4.6. In some embodiments, the composition has a pD of less than about 4.5. In some embodiments, the composition has a pD of less than about 4.4. In some embodiments, the composition has a pD of less than about 4.3. In some embodiments, the composition has a pD of less than about 4.2. In some embodiments, the composition has a pD of less than

about 4.1. In some embodiments, the composition has a pD of less than about 4. In some embodiments, the pD is the pD of the composition after extended period of time under storage condition.

[00107] In some instances, the composition has an initial pD of between about 4 and about 8, about 4.5 and about 7.8, about 5 and about 7.5, or about 5.5 and about 7. In some embodiments, the composition has an initial pD of about 7.5. In some embodiments, the composition has an initial pD of about 7.4. In some embodiments, the composition has an initial pD of about 7.3. In some embodiments, the composition has an initial pD of about 7.2. In some embodiments, the composition has an initial pD of about 7.1. In some embodiments, the composition has an initial pD of about 7. In some embodiments, the composition has an initial pD of about 6.9. In some embodiments, the composition has an initial pD of about 6.8. In some embodiments, the composition has an initial pD of about 6.7. In some embodiments, the composition has an initial pD of about 6.6. In some embodiments, the composition has an initial pD of about 6.5. In some embodiments, the composition has an initial pD of about 6.4. In some embodiments, the composition has an initial pD of about 6.3. In some embodiments, the composition has an initial pD of about 6.2. In some embodiments, the composition has an initial pD of about 6.1. In some embodiments, the composition has an initial pD of about 6. In some embodiments, the composition has an initial pD of about 5.9. In some embodiments, the composition has an initial pD of about 5.8. In some embodiments, the composition has an initial pD of about 5.7. In some embodiments, the composition has an initial pD of about 5.6. In some embodiments, the composition has an initial pD of about 5.5. In some embodiments, the composition has an initial pD of about 5.4. In some embodiments, the composition has an initial pD of about 5.3. In some embodiments, the composition has an initial pD of about 5.2. In some embodiments, the composition has an initial pD of about 5.1. In some embodiments, the composition has an initial pD of about 5. In some embodiments, the composition has an initial pD of about 4.9. In some embodiments, the composition has an initial pD of about 4.8. In some embodiments, the composition has an initial pD of about 4.7. In some embodiments, the composition has an initial pD of about 4.6. In some embodiments, the composition has an initial pD of about 4.5. In some embodiments, the composition has an initial pD of about 4.4. In some embodiments, the composition has an initial pD of about 4.3. In some embodiments, the composition has an initial pD of about 4.2. In some embodiments, the composition has an initial pD of about 4.1. In some embodiments, the composition has an initial pD of about 4.

[00108] In some embodiments, the pD of the composition described herein is associated with the stability of the composition. In some embodiments, a stable composition comprises a pD of between about 4 and about 8, about 4.5 and about 7.8, about 5 and about 7.5, or about 5.5 and about 7. In some embodiments, a stable composition comprises a pD of less than about 7.5. In some embodiments, a stable composition comprises a pD of less than about 7.4. In some embodiments, a stable composition comprises a pD of less than about 7.3. In some embodiments, a stable composition comprises a pD of less

than about 7.2. In some embodiments, a stable composition comprises a pD of less than about 7.1. In some embodiments, a stable composition comprises a pD of less than about 7. In some embodiments, a stable composition comprises a pD of less than about 6.9. In some embodiments, a stable composition comprises a pD of less than about 6.8. In some embodiments, a stable composition comprises a pD of less than about 6.7. In some embodiments, a stable composition comprises a pD of less than about 6.6. In some embodiments, a stable composition comprises a pD of less than about 6.5. In some embodiments, a stable composition comprises a pD of less than about 6.4. In some embodiments, a stable composition comprises a pD of less than about 6.3. In some embodiments, a stable composition comprises a pD of less than about 6.2. In some embodiments, a stable composition comprises a pD of less than about 6.1. In some embodiments, a stable composition comprises a pD of less than about 6. In some embodiments, a stable composition comprises a pD of less than about 5.9. In some embodiments, a stable composition comprises a pD of less than about 5.8. In some embodiments, a stable composition comprises a pD of less than about 5.7. In some embodiments, a stable composition comprises a pD of less than about 5.6. In some embodiments, a stable composition comprises a pD of less than about 5.5. In some embodiments, a stable composition comprises a pD of less than about 5.4. In some embodiments, a stable composition comprises a pD of less than about 5.3. In some embodiments, a stable composition comprises a pD of less than about 5.2. In some embodiments, a stable composition comprises a pD of less than about 5.1. In some embodiments, a stable composition comprises a pD of less than about 5. In some embodiments, a stable composition comprises a pD of less than about 4.9. In some embodiments, a stable composition comprises a pD of less than about 4.8. In some embodiments, a stable composition comprises a pD of less than about 4.7. In some embodiments, a stable composition comprises a pD of less than about 4.6. In some embodiments, a stable composition comprises a pD of less than about 4.5. In some embodiments, a stable composition comprises a pD of less than about 4.4. In some embodiments, a stable composition comprises a pD of less than about 4.3. In some embodiments, a stable composition comprises a pD of less than about 4.2. In some embodiments, a stable composition comprises a pD of less than about 4.1. In some embodiments, a stable composition comprises a pD of less than about 4.

[00109] As described elsewhere herein, in some instances, the D₂O aqueous system stabilizes a muscarinic antagonist (e.g., atropine). In some embodiments, this is due to a lower concentration of the reactive species (e.g., -OD) in the D₂O aqueous system compared to the concentration of the reactive species (e.g., -OH) in an equivalent H₂O aqueous system. In some instances, the concentration of the reactive species (e.g., -OD) in the D₂O aqueous system is about one third less than the concentration of the reactive species (e.g., -OH) in the equivalent H₂O aqueous system. In some cases, this is due to a lower or smaller dissociation constant of D₂O than H₂O. For example, the K_a(H₂O) is 1×10^{-14} , whereas the K_a(D₂O) is 1×10^{-15} . As such, D₂O is a weaker acid than H₂O. In some cases, base catalyzed hydrolysis

leads to the presence of tropine degradant from atropine. In some cases, with a lower concentration of the reactive species that causes tropine degradant formation, atropine solution is more stable in a D₂O aqueous system than compared to an equivalent H₂O aqueous system. In some embodiments, the ophthalmic composition formulated with deuterated water allows for a more stable ophthalmic composition relative to the ophthalmic composition formulated with H₂O.

[00110] In some embodiments, the presence of deuterated water shifts the pKa of the buffer. In some embodiments, the presence of deuterated water allows for the ophthalmic composition to simulate the stability of a lower pH system. In some instances, the buffer capacity of the ophthalmic composition is lowered, thereby allowing a faster shift in pH. In some instances, the lowered buffering capacity of the ophthalmic composition when administered into the eye allows the ophthalmic composition to reach physiological pH at a faster rate than compared to an ophthalmic composition formulated in H₂O. In some instances, the ophthalmic composition formulated with deuterated water allows for a lower tear production, or less tear reflex in the eye, in comparison with an ophthalmic composition formulated with H₂O.

[00111] In some instances, the composition described herein further comprises a disinfecting agent. In some cases, disinfecting agents include polymeric biguanides, polymeric quarternary ammonium compounds, chlorites, bisbiguanides, chlorite compounds (e.g. potassium chlorite, sodium chlorite, calcium chlorite, magnesium chlorite, or mixtures thereof), and a combination thereof.

[00112] In some instances, the composition described herein further comprises a preservative. In some cases, a preservative is added at a concentration to a composition described herein to prevent the growth of or to destroy a microorganism introduced into the composition. In some instances, microorganisms refer to bacteria (e.g. *Proteus mirabilis*, *Serratia marcesens*), virus (e.g. Herpes simplex virus, herpes zoster virus), fungus (e.g. fungi from the genus *Fusarium*), yeast (e.g. *Candida albicans*), parasites (e.g. *Plasmodium* spp., *Gnathostoma* spp.), protozoan (e.g. *Giardia lamblia*), nematodes (e.g. *Onchocercus volvulus*), worm (e.g. *Dirofilaria immitis*), and/or amoeba (e.g. *Acanthameoba*).

[00113] In some instances, the concentration of the preservative is between about 0.0001% and about 1%, about 0.001% and about 0.8%, about 0.004% and about 0.5%, about 0.008 % and about 0.1%, and about 0.01% and about 0.08%. In some cases, the concentration of the preservatives is about 0.001%, 0.002%, 0.003%, 0.004%, 0.005%, 0.006%, 0.008%, 0.009%, 0.009%, 0.01%, 0.015%, 0.02%, 0.025%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9% or 1.0%.

[00114] In some embodiments, the preservative is selected from benzalkonium chloride, cetrimonium, sodium perborate, stabilized oxychloro complex, SofZia (Alcon), polyquaternium-1, chlorobutanol, edetate disodium, and polyhexamethylene biguanide.

[00115] In some embodiments, the composition described herein is stored in a plastic container. In some embodiments, the material of the plastic container comprises high density polyethylene (HDPE), low density polyethylene (LDPE), polyethylene terephthalate (PET), polyvinyl chloride (PVC), polypropylene (PP), polystyrene (PS), fluorine treated HDPE, post-consumer resin (PCR), K-resine (SBC), or bioplastic. In some embodiments, the material of the plastic container comprises LDPE.

[00116] In some embodiments, the composition described herein is stored in a plastic container. In some embodiments, the composition stored in a plastic container has a pD of between about 4 and about 8, about 4.5 and about 7.9, or about 4.9 and about 7.5. In some embodiments, the composition stored in a plastic container has a pD of less than about 7.4. In some embodiments, the composition stored in a plastic container has a pD of less than about 7.3. In some embodiments, the composition stored in a plastic container has a pD of less than about 7.2. In some embodiments, the composition stored in a plastic container has a pD of less than about 7.1. In some embodiments, the composition stored in a plastic container has a pD of less than about 7. In some embodiments, the composition stored in a plastic container has a pD of less than about 6.9. In some embodiments, the composition stored in a plastic container has a pD of less than about 6.8. In some embodiments, the composition stored in a plastic container has a pD of less than about 6.7. In some embodiments, the composition stored in a plastic container has a pD of less than about 6.6. In some embodiments, the composition stored in a plastic container has a pD of less than about 6.5. In some embodiments, the composition stored in a plastic container has a pD of less than about 6.4. In some embodiments, the composition stored in a plastic container has a pD of less than about 6.3. In some embodiments, the composition stored in a plastic container has a pD of less than about 6.2. In some embodiments, the composition stored in a plastic container has a pD of less than about 6.1. In some embodiments, the composition stored in a plastic container has a pD of less than about 6. In some embodiments, the composition stored in a plastic container has a pD of less than about 5.9. In some embodiments, the composition stored in a plastic container has a pD of less than about 5.8. In some embodiments, the composition stored in a plastic container has a pD of less than about 5.7. In some embodiments, the composition stored in a plastic container has a pD of less than about 5.6. In some embodiments, the composition stored in a plastic container has a pD of less than about 5.5. In some embodiments, the composition stored in a plastic container has a pD of less than about 5.4. In some embodiments, the composition stored in a plastic container has a pD of less than about 5.3. In some embodiments, the composition stored in a plastic

container has a pD of less than about 5.2. In some embodiments, the composition stored in a plastic container has a pD of less than about 5.1. In some embodiments, the composition stored in a plastic container has a pD of less than about 5. In some embodiments, the composition stored in a plastic container has a pD of less than about 4.9. In some embodiments, the composition stored in a plastic container has a pD of less than about 4.8. In some embodiments, the composition stored in a plastic container has a pD of less than about 4.7. In some embodiments, the composition stored in a plastic container has a pD of less than about 4.6. In some embodiments, the composition stored in a plastic container has a pD of less than about 4.5. In some embodiments, the composition stored in a plastic container has a pD of less than about 4.4. In some embodiments, the composition stored in a plastic container has a pD of less than about 4.3. In some embodiments, the composition stored in a plastic container has a pD of less than about 4.2. In some embodiments, the composition stored in a plastic container has a pD of less than about 4.1. In some embodiments, the composition stored in a plastic container has a pD of less than about 4.

[00117] In some embodiments, the composition stored in a plastic container has a potency of at least 80% after extended period of time under storage condition. In some embodiments, the composition stored in a plastic container has a potency of at least 85% after extended period of time under storage condition. In some embodiments, the composition stored in a plastic container has a potency of at least 90% after extended period of time under storage condition. In some embodiments, the composition stored in a plastic container has a potency of at least 93% after extended period of time under storage condition. In some embodiments, the composition stored in a plastic container has a potency of at least 95% after extended period of time under storage condition. In some embodiments, the composition stored in a plastic container has a potency of at least 97% after extended period of time under storage condition. In some embodiments, the composition stored in a plastic container has a potency of at least 98% after extended period of time under storage condition. In some embodiments, the composition stored in a plastic container has a potency of at least 99% after extended period of time under storage condition. In some instances, the storage condition comprises a temperature of about 25°C, about 40°C, or about 60°C. In some instances, the extended period of time is at least 1 week, at least 2 weeks, at least 3 weeks, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 8 months, at least 10 months, at least 12 months, at least 18 months, or at least 24 months.

[00118] In some embodiments, the composition stored in a plastic container has a potency of at least 80% at a temperature of about 25°C, about 40°C, or about 60°C. In some embodiments, the composition stored in a plastic container has a potency of at least 85% at a temperature of about 25°C, about 40°C, or about 60°C. In some embodiments, the composition stored in a plastic container has a potency of at least 90% at a temperature of about 25°C, about 40°C, or about 60°C. In some

embodiments, the composition stored in a plastic container has a potency of at least 93% at a temperature of about 25°C, about 40°C, or about 60°C. In some embodiments, the composition stored in a plastic container has a potency of at least 95% at a temperature of about 25°C, about 40°C, or about 60°C. In some embodiments, the composition stored in a plastic container has a potency of at least 97% at a temperature of about 25°C, about 40°C, or about 60°C. In some embodiments, the composition stored in a plastic container has a potency of at least 98% at a temperature of about 25°C, about 40°C, or about 60°C. In some embodiments, the composition stored in a plastic container has a potency of at least 99% at a temperature of about 25°C, about 40°C, or about 60°C.

[illegible]

99% for a period of at least 1 week, at least 2 weeks, at least 3 weeks, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 8 months, at least 10 months, at least 12 months, at least 18 months, or at least 24 months.

[00120] In some embodiments, the composition stored in a plastic container comprises less than 20% of primary degradant based on the concentration of the ophthalmic agent after extended period of time under storage condition. In some embodiments, the composition stored in a plastic container comprises less than 15% of primary degradant based on the concentration of the ophthalmic agent after extended period of time under storage condition. In some embodiments, the composition stored in a plastic container comprises less than 10% of primary degradant based on the concentration of the ophthalmic agent after extended period of time under storage condition. In some embodiments, the composition stored in a plastic container comprises less than 5% of primary degradant based on the concentration of the ophthalmic agent after extended period of time under storage condition.

[00121] In some embodiments, the composition stored in a plastic container comprises from less than 2.5% of primary degradant to less than 0.1% of primary degradant based on the concentration of the ophthalmic agent after extended period of time under storage condition. In some embodiments, the composition stored in a plastic container comprises less than 2.5% of primary degradant based on the concentration of the ophthalmic agent after extended period of time under storage condition. In some embodiments, the composition stored in a plastic container comprises less than 2.0% of primary degradant based on the concentration of the ophthalmic agent after extended period of time under storage condition. In some embodiments, the composition stored in a plastic container comprises less than 1.5% of primary degradant based on the concentration of the ophthalmic agent after extended period of time under storage condition. In some embodiments, the composition stored in a plastic container comprises less than 1.0% of primary degradant based on the concentration of the ophthalmic agent after extended period of time under storage condition. In some embodiments, the composition stored in a plastic container comprises less than 0.5% of primary degradant based on the concentration of the ophthalmic agent after extended period of time under storage condition. In some embodiments, the composition stored in a plastic container comprises less than 0.4% of primary degradant based on the concentration of the ophthalmic agent after extended period of time under storage condition. In some embodiments, the composition stored in a plastic container comprises less than 0.3% of primary degradant based on the concentration of the ophthalmic agent after extended period of time under storage condition. In some embodiments, the composition stored in a plastic container comprises less than 0.2% of primary degradant based on the concentration of the ophthalmic agent after extended period of time under storage condition. In some embodiments, the composition stored in a plastic container comprises less than 0.1% of primary degradant based on the concentration of the ophthalmic agent after extended period of time

under storage condition. In some instances, the storage condition comprises a temperature of about 25°C, about 40°C, or about 60°C. In some instances, the extended period of time is at least 1 week, at least 2 weeks, at least 3 weeks, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 8 months, at least 10 months, at least 12 months, at least 18 months, or at least 24 months.

[00122] In some embodiments, the composition stored in a plastic container comprises less than 20% of primary degradant based on the concentration of the ophthalmic agent at a temperature of about 25°C, about 40°C, or about 60°C. In some embodiments, the composition stored in a plastic container comprises less than 15% of primary degradant based on the concentration of the ophthalmic agent at a temperature of about 25°C, about 40°C, or about 60°C. In some embodiments, the composition stored in a plastic container comprises less than 10% of primary degradant based on the concentration of the ophthalmic agent at a temperature of about 25°C, about 40°C, or about 60°C. In some embodiments, the composition stored in a plastic container comprises less than 5% of primary degradant based on the concentration of the ophthalmic agent at a temperature of about 25°C, about 40°C, or about 60°C.

[00123] In some embodiments, the composition stored in a plastic container comprises from less than 2.5% of primary degradant to less than 0.1% of primary degradant based on the concentration of the ophthalmic agent at a temperature of about 25°C, about 40°C, or about 60°C. In some embodiments, the composition stored in a plastic container comprises less than 2.5% of primary degradant based on the concentration of the ophthalmic agent at a temperature of about 25°C, about 40°C, or about 60°C. In some embodiments, the composition stored in a plastic container comprises less than 2.0% of primary degradant based on the concentration of the ophthalmic agent at a temperature of about 25°C, about 40°C, or about 60°C. In some embodiments, the composition stored in a plastic container comprises less than 1.5% of primary degradant based on the concentration of the ophthalmic agent at a temperature of about 25°C, about 40°C, or about 60°C. In some embodiments, the composition stored in a plastic container comprises less than 1.0% of primary degradant based on the concentration of the ophthalmic agent at a temperature of about 25°C, about 40°C, or about 60°C. In some embodiments, the composition stored in a plastic container comprises less than 0.5% of primary degradant based on the concentration of the ophthalmic agent at a temperature of about 25°C, about 40°C, or about 60°C. In some embodiments, the composition stored in a plastic container comprises less than 0.4% of primary degradant based on the concentration of the ophthalmic agent at a temperature of about 25°C, about 40°C, or about 60°C. In some embodiments, the composition stored in a plastic container comprises less than 0.3% of primary degradant based on the concentration of the ophthalmic agent at a temperature of about 25°C, about 40°C, or about 60°C. In some embodiments, the composition stored in a plastic container comprises less than 0.2% of primary degradant based on the concentration of the ophthalmic agent at a temperature of about

25°C, about 40°C, or about 60°C. In some embodiments, the composition stored in a plastic container comprises less than 0.1% of primary degradant based on the concentration of the ophthalmic agent at a temperature of about 25°C, about 40°C, or about 60°C.

[00124] In some embodiments, the composition stored in a plastic container comprises less than 20% of primary degradant based on the concentration of the ophthalmic agent for a period of at least 1 week, at least 2 weeks, at least 3 weeks, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 8 months, at least 10 months, at least 12 months, at least 18 months, or at least 24 months. In some embodiments, the composition stored in a plastic container comprises less than 15% of primary degradant based on the concentration of the ophthalmic agent for a period of at least 1 week, at least 2 weeks, at least 3 weeks, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 8 months, at least 10 months, at least 12 months, at least 18 months, or at least 24 months. In some embodiments, the composition stored in a plastic container comprises less than 10% of primary degradant based on the concentration of the ophthalmic agent for a period of at least 1 week, at least 2 weeks, at least 3 weeks, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 8 months, at least 10 months, at least 12 months, at least 18 months, or at least 24 months. In some embodiments, the composition stored in a plastic container comprises less than 5% of primary degradant based on the concentration of the ophthalmic agent for a period of at least 1 week, at least 2 weeks, at least 3 weeks, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 8 months, at least 10 months, at least 12 months, at least 18 months, or at least 24 months.

[00125] In some embodiments, the composition stored in a plastic container comprises from less than 2.5% of primary degradant to less than 0.1% of primary degradant based on the concentration of the ophthalmic agent for a period of at least 1 week, at least 2 weeks, at least 3 weeks, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 8 months, at least 10 months, at least 12 months, at least 18 months, or at least 24 months. In some embodiments, the composition stored in a plastic container comprises less than 2.5% of primary degradant based on the concentration of the ophthalmic agent for a period of at least 1 week, at least 2 weeks, at least 3 weeks, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 8 months, at least 10 months, at least 12 months, at least 18 months, or at least 24 months. In some embodiments, the composition stored in a plastic container comprises less than 2.0% of primary degradant based on the concentration of the ophthalmic agent for a period of at least 1 week, at least 2 weeks, at least 3 weeks, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 8 months, at least 10 months, at least 12 months, at least 18 months, or

at least 24 months. In some embodiments, the composition stored in a plastic container comprises less than 1.5% of primary degradant based on the concentration of the ophthalmic agent for a period of at least 1 week, at least 2 weeks, at least 3 weeks, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 8 months, at least 10 months, at least 12 months, at least 18 months, or at least 24 months. In some embodiments, the composition stored in a plastic container comprises less than 1.0% of primary degradant based on the concentration of the ophthalmic agent for a period of at least 1 week, at least 2 weeks, at least 3 weeks, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 8 months, at least 10 months, at least 12 months, at least 18 months, or at least 24 months. In some embodiments, the composition stored in a plastic container comprises less than 0.5% of primary degradant based on the concentration of the ophthalmic agent for a period of at least 1 week, at least 2 weeks, at least 3 weeks, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 8 months, at least 10 months, at least 12 months, at least 18 months, or at least 24 months. In some embodiments, the composition stored in a plastic container comprises less than 0.4% of primary degradant based on the concentration of the ophthalmic agent for a period of at least 1 week, at least 2 weeks, at least 3 weeks, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 8 months, at least 10 months, at least 12 months, at least 18 months, or at least 24 months. In some embodiments, the composition stored in a plastic container comprises less than 0.3% of primary degradant based on the concentration of the ophthalmic agent for a period of at least 1 week, at least 2 weeks, at least 3 weeks, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 8 months, at least 10 months, at least 12 months, at least 18 months, or at least 24 months. In some embodiments, the composition stored in a plastic container comprises less than 0.2% of primary degradant based on the concentration of the ophthalmic agent for a period of at least 1 week, at least 2 weeks, at least 3 weeks, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 8 months, at least 10 months, at least 12 months, at least 18 months, or at least 24 months. In some embodiments, the composition stored in a plastic container comprises less than 0.1% of primary degradant based on the concentration of the ophthalmic agent for a period of at least 1 week, at least 2 weeks, at least 3 weeks, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 8 months, at least 10 months, at least 12 months, at least 18 months, or at least 24 months.

[00126] In some embodiments, the composition described herein is stored in a glass container. In some embodiments, the glass container is a glass vial, such as for example, a type I, type II or type III glass vial. In some embodiments, the glass container is a type I glass vial. In some embodiments, the type I glass vial is a borasilicate glass vial.

[00127] In some embodiments, the composition stored in a glass container has a pD of higher than about 7. In some embodiments, the composition stored in a glass container has a pD of higher than about 7.5. In some embodiments, the composition stored in a glass container has a pD of higher than about 8. In some embodiments, the composition stored in a glass container has a pD of higher than about 8.5. In some embodiments, the composition stored in a glass container has a pD of higher than about 9.

[00128] In some embodiments, the composition stored in a glass container has a potency of less than 60% at a temperature of about 25°C, about 40°C, or about 60°C. In some embodiments, the composition stored in a glass container has a potency of less than 60% for a period of at least 1 week, at least 2 weeks, at least 3 weeks, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 8 months, at least 10 months, at least 12 months, at least 18 months, or at least 24 months.

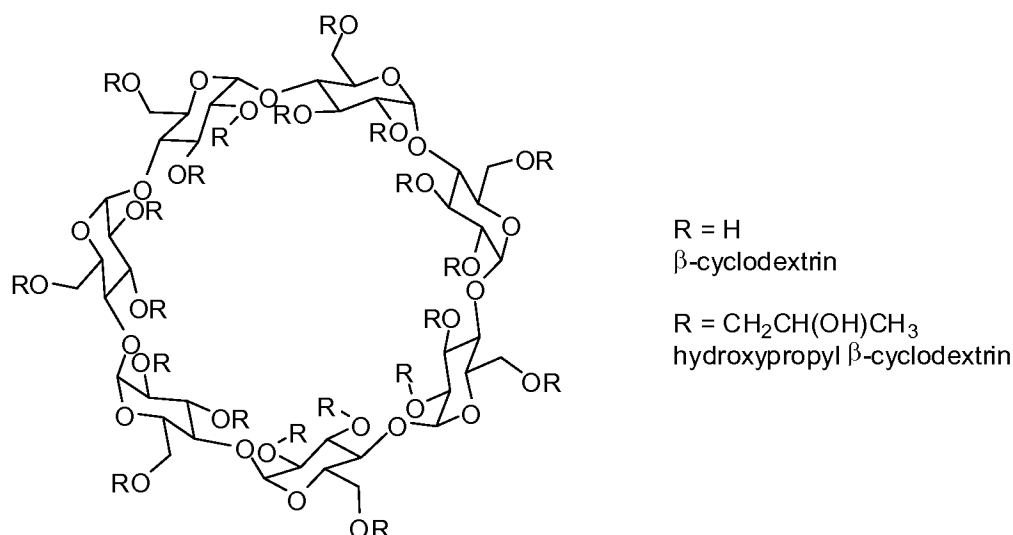
[00129] In some embodiments, the composition stored in a glass container is less stable than a composition stored in a plastic container.

[00130] In some embodiments, the composition is stored under in the dark. In some instances, the composition is stored in the presence of light. In some instances, the light is indoor light, room light, or sun light. In some instances, the composition is stable while stored in the presence of light.

[00131] In some embodiments, the composition described herein is formulated as an aqueous solution. In some embodiments, the aqueous solution is a stable aqueous solution. In some instances, the aqueous solution is stored in a plastic container as described above. In some instances, the aqueous solution is not stored in a glass container. In some instances, the aqueous solution is stored in the dark. In some instances, the aqueous solution is stored in the presence of light. In some instances, the aqueous solution is stable in the presence of light.

[00132] In a specific embodiment, the ophthalmically acceptable formulations alternatively comprise a cyclodextrin. Cyclodextrins are cyclic oligosaccharides containing 6, 7, or 8 glucopyranose units, referred to as α -cyclodextrin, β -cyclodextrin, or γ -cyclodextrin respectively. Cyclodextrins have a hydrophilic exterior, which enhances water-soluble, and a hydrophobic interior which forms a cavity. In an aqueous environment, hydrophobic portions of other molecules often enter the hydrophobic cavity of cyclodextrin to form inclusion compounds. Additionally, cyclodextrins are also capable of other types of nonbonding interactions with molecules that are not inside the hydrophobic cavity. Cyclodextrins have three free hydroxyl groups for each glucopyranose unit, or 18 hydroxyl groups on α -cyclodextrin, 21 hydroxyl groups on β -cyclodextrin, and 24 hydroxyl groups on γ -cyclodextrin. In some embodiments, one or more of these hydroxyl groups are reacted with any of a number of reagents to form a large variety of

cyclodextrin derivatives, including hydroxypropyl ethers, sulfonates, and sulfoalkylethers. Shown below is the structure of β -cyclodextrin and the hydroxypropyl- β -cyclodextrin (HP β CD).



[00133] In some embodiments, the use of cyclodextrins in the pharmaceutical compositions described herein improves the solubility of the drug. Inclusion compounds are involved in many cases of enhanced solubility; however other interactions between cyclodextrins and insoluble compounds also improves solubility. Hydroxypropyl- β -cyclodextrin (HP β CD) is commercially available as a pyrogen free product. It is a nonhygroscopic white powder that readily dissolves in water. HP β CD is thermally stable and does not degrade at neutral pH. Thus, cyclodextrins improve the solubility of a therapeutic agent in a composition or formulation. Accordingly, in some embodiments, cyclodextrins are included to increase the solubility of the ophthalmically acceptable ophthalmic agents within the formulations described herein. In other embodiments, cyclodextrins in addition serve as controlled release excipients within the formulations described herein.

[00134] By way of example only, cyclodextrin derivatives for use include α -cyclodextrin, β -cyclodextrin, γ -cyclodextrin, hydroxyethyl- β -cyclodextrin, hydroxypropyl- γ -cyclodextrin, sulfated β -cyclodextrin, sulfated α -cyclodextrin, sulfobutyl ether β -cyclodextrin.

[00135] The concentration of the cyclodextrin used in the compositions and methods disclosed herein varies according to the physiochemical properties, pharmacokinetic properties, side effect or adverse events, formulation considerations, or other factors associated with the therapeutically ophthalmic agent, or a salt or prodrug thereof, or with the properties of other excipients in the composition. Thus, in certain circumstances, the concentration or amount of cyclodextrin used in accordance with the compositions and methods disclosed herein will vary, depending on the need. When used, the amount of cyclodextrins needed to increase solubility of the ophthalmic agent and/or function as a controlled release

excipient in any of the formulations described herein is selected using the principles, examples, and teachings described herein.

[00136] Other stabilizers that are useful in the ophthalmically acceptable formulations disclosed herein include, for example, fatty acids, fatty alcohols, alcohols, long chain fatty acid esters, long chain ethers, hydrophilic derivatives of fatty acids, polyvinyl pyrrolidones, polyvinyl ethers, polyvinyl alcohols, hydrocarbons, hydrophobic polymers, moisture-absorbing polymers, and combinations thereof. In some embodiments, amide analogues of stabilizers are also used. In further embodiments, the chosen stabilizer changes the hydrophobicity of the formulation, improves the mixing of various components in the formulation, controls the moisture level in the formula, or controls the mobility of the phase.

[00137] In other embodiments, stabilizers are present in sufficient amounts to inhibit the degradation of the ophthalmic agent. Examples of such stabilizing agents, include, but are not limited to: glycerol, methionine, monothioglycerol, EDTA, ascorbic acid, polysorbate 80, polysorbate 20, arginine, heparin, dextran sulfate, cyclodextrins, pentosan polysulfate and other heparinoids, divalent cations such as magnesium and zinc, or combinations thereof.

[00138] Additional useful stabilization agents for ophthalmically acceptable formulations include one or more anti-aggregation additives to enhance stability of ophthalmic formulations by reducing the rate of protein aggregation. The anti-aggregation additive selected depends upon the nature of the conditions to which the ophthalmic agents, for example a muscarinic antagonist (e.g. atropine or its pharmaceutically acceptable salts), are exposed. For example, certain formulations undergoing agitation and thermal stress require a different anti-aggregation additive than a formulation undergoing lyophilization and reconstitution. Useful anti-aggregation additives include, by way of example only, urea, guanidinium chloride, simple amino acids such as glycine or arginine, sugars, polyalcohols, polysorbates, polymers such as polyethylene glycol and dextrans, alkyl saccharides, such as alkyl glycoside, and surfactants.

[00139] Other useful formulations optionally include one or more ophthalmically acceptable antioxidants to enhance chemical stability where required. Suitable antioxidants include, by way of example only, ascorbic acid, methionine, sodium thiosulfate and sodium metabisulfite. In one embodiment, antioxidants are selected from metal chelating agents, thiol containing compounds and other general stabilizing agents.

[00140] Still other useful compositions include one or more ophthalmically acceptable surfactants to enhance physical stability or for other purposes. Suitable nonionic surfactants include, but are not limited to, polyoxyethylene fatty acid glycerides and vegetable oils, e.g., polyoxyethylene (60)

hydrogenated castor oil; and polyoxyethylene alkylethers and alkylphenyl ethers, e.g., octoxynol 10, octoxynol 40.

[00141] In some embodiments, the ophthalmically acceptable pharmaceutical formulations described herein are stable with respect to compound degradation (e.g. less than 30% degradation, less than 25% degradation, less than 20% degradation, less than 15% degradation, less than 10% degradation, less than 8% degradation, less than 5% degradation, less than 3% degradation, less than 2% degradation, or less than 5% degradation) over a period of any of at least about 1 day, at least about 2 days, at least about 3 days, at least about 4 days, at least about 5 days, at least about 6 days, at least about 1 week, at least about 2 weeks, at least about 3 weeks, at least about 4 weeks, at least about 5 weeks, at least about 6 weeks, at least about 7 weeks, at least about 8 weeks, at least about 3 months, at least about 4 months, at least about 5 months, or at least about 6 months under storage conditions (e.g. room temperature). In other embodiments, the formulations described herein are stable with respect to compound degradation over a period of at least about 1 week. Also described herein are formulations that are stable with respect to compound degradation over a period of at least about 1 month.

[00142] In other embodiments, an additional surfactant (co-surfactant) and/or buffering agent is combined with one or more of the pharmaceutically acceptable vehicles previously described herein so that the surfactant and/or buffering agent maintains the product at an optimal pD for stability. Suitable co-surfactants include, but are not limited to: a) natural and synthetic lipophilic agents, e.g., phospholipids, cholesterol, and cholesterol fatty acid esters and derivatives thereof; b) nonionic surfactants, which include for example, polyoxyethylene fatty alcohol esters, sorbitan fatty acid esters (Spans), polyoxyethylene sorbitan fatty acid esters (e.g., polyoxyethylene (20) sorbitan monooleate (Tween 80), polyoxyethylene (20) sorbitan monostearate (Tween 60), polyoxyethylene (20) sorbitan monolaurate (Tween 20) and other Tweens, sorbitan esters, glycerol esters, e.g., Myrj and glycerol triacetate (triacetin), polyethylene glycols, cetyl alcohol, cetostearyl alcohol, stearyl alcohol, polysorbate 80, poloxamers, poloxamines, polyoxyethylene castor oil derivatives (e.g., Cremophor[®] RH40, Cremphor A25, Cremphor A20, Cremophor[®] EL) and other Cremophors, sulfosuccinates, alkyl sulphates (SLS); PEG glyceryl fatty acid esters such as PEG-8 glyceryl caprylate/caprate (Labrasol), PEG-4 glyceryl caprylate/caprate (Labrafac Hydro WL 1219), PEG-32 glyceryl laurate (Gelucire 444/14), PEG-6 glyceryl mono oleate (Labrafil M 1944 CS), PEG-6 glyceryl linoleate (Labrafil M 2125 CS); propylene glycol mono- and di-fatty acid esters, such as propylene glycol laurate, propylene glycol caprylate/caprate; Brij[®] 700, ascorbyl-6-palmitate, stearylamine, sodium lauryl sulfate, polyoxethyleneglycerol triiricinoleate, and any combinations or mixtures thereof; c) anionic surfactants include, but are not limited to, calcium carboxymethylcellulose, sodium carboxymethylcellulose, sodium sulfosuccinate, dioctyl, sodium alginate, alkyl polyoxyethylene sulfates, sodium lauryl sulfate, triethanolamine stearate, potassium

laurate, bile salts, and any combinations or mixtures thereof; and d) cationic surfactants such as cetyltrimethylammonium bromide, and lauryldimethylbenzyl-ammonium chloride.

[00143] In a further embodiment, when one or more co-surfactants are utilized in the ophthalmically acceptable formulations of the present disclosure, they are combined, e.g., with a pharmaceutically acceptable vehicle and is present in the final formulation, e.g., in an amount ranging from about 0.1% to about 20%, from about 0.5% to about 10%.

[00144] In one embodiment, the surfactant has an HLB value of 0 to 20. In additional embodiments, the surfactant has an HLB value of 0 to 3, of 4 to 6, of 7 to 9, of 8 to 18, of 13 to 15, of 10 to 18.

[00145] pD

[00146] In some embodiments, the pD of a composition described herein is adjusted (e.g., by use of a buffer and/or a pD adjusting agent) to an ophthalmically compatible pD range of from about 4 to about 8, about 4.5 to about 7.5, or about 5 to about 7. In some embodiments, the ophthalmic composition has a pD of from about 5.0 to about 7.0. In some embodiments, the ophthalmic composition has a pD of from about 5.5 to about 7.0. In some embodiments, the ophthalmic composition has a pD of from about 6.0 to about 7.0.

[00147] In some embodiments, useful formulations include one or more pD adjusting agents or buffering agents. Suitable pD adjusting agents or buffers include, but are not limited to acetate, bicarbonate, ammonium chloride, citrate, phosphate, deuterated forms of acetate, bicarbonate, ammonium chloride, citrate, phosphate, pharmaceutically acceptable salts thereof and combinations or mixtures thereof. In some embodiments, the pD adjusting agents or buffers include deuterated hydrochloric acid (DCl), deuterated sodium hydroxide (NaOD), deuterated acetic acid (CD₃COOD), or deuterated citric acid (C₆D₈O₇).

[00148] In one embodiment, when one or more buffers are utilized in the formulations of the present disclosure, they are combined, e.g., with a pharmaceutically acceptable vehicle and are present in the final formulation, e.g., in an amount ranging from about 0.1% to about 20%, from about 0.5% to about 10%. In certain embodiments of the present disclosure, the amount of buffer included in the gel formulations are an amount such that the pD of the gel formulation does not interfere with the body's natural buffering system.

[00149] In one embodiment, diluents are also used to stabilize compounds because they provide a more stable environment. In some instances, salts dissolved in buffered solutions (which also provides pD control or maintenance) are utilized as diluents in the art, including, but not limited to a phosphate buffered saline solution.

[00150] In some embodiments, the pD is calculated according to the formula disclosed in Glasoe *et al.*, “Use of glass electrodes to measure acidities in deuterium oxide,” J. Physical Chem. 64(1): 188-190 (1960). In some embodiment, the pD is calculated as $pD = pH^* + 0.4$, in which pH^* is the measured or observed pH of the ophthalmic composition formulated in a solution comprising deuterated water (e.g., D_2O).

[00151] In some embodiments, the ophthalmic aqueous, gel, or ointment composition described herein has a pD of between about 4 and about 8, between about 4.5 and about 8, between about 4.9 and about 7.9, between about 5.4 and about 7.9, between about 5.9 and about 7.9, between about 6.4 and about 7.9, or between about 7.4 and about 7.9. In some embodiments, the ophthalmic aqueous, gel, or ointment composition described herein has a pD of between about 4.5-7.5, between about 5.0 and about 7.5, between about 5.5 and about 7.5, between about 6.0 and about 7.5, or between about 7.0 and about 7.5. In some embodiments, the ophthalmic aqueous, gel, or ointment composition described herein has a pD of between about 4.5-7.0, between about 5.0 and about 7.0, between about 5.5 and about 7.0, between about 6.0 and about 7.0, or between about 6.5 and about 7.0. In some embodiments, the ophthalmic aqueous, gel, or ointment composition described herein has a pD of between about 4.9-7.4, between about 5.4 and about 7.4, between about 5.9 and about 7.4, between about 6.4 and about 7.4, or between about 6.9 and about 7.4. In some embodiments, the ophthalmic aqueous, gel, or ointment composition described herein has a pD of between about 4.5-6.5, between about 5.0 and about 6.5, between about 5.5 and about 6.5, or between about 6.0 and about 6.5. In some embodiments, the ophthalmic aqueous, gel, or ointment composition described herein has a pD of between about 4.9-6.9, between about 5.4 and about 6.9, between about 5.9 and about 6.9, or between about 6.4 and about 6.9. In some embodiments, the ophthalmic aqueous, gel, or ointment composition described herein has a pD of between about 4.5-6.0, between about 5.0 and about 6.0, or between about 5.5 and about 6.0. In some embodiments, the ophthalmic aqueous, gel, or ointment composition described herein has a pD of between about 4.9-6.4, between about 5.4 and about 6.4, or between about 5.9 and about 6.4. In some embodiments, the ophthalmic aqueous, gel, or ointment composition described herein has a pD of between about 4.5-5.5, or between about 5.0 and about 5.5. In some embodiments, the ophthalmic aqueous, gel, or ointment composition described herein has a pD of between about 4.9-5.9, or between about 5.4 and about 5.9. In some embodiments, the ophthalmic aqueous, gel, or ointment composition described herein has a pD of between about 4.5-5.0. In some embodiments, the ophthalmic aqueous, gel, or ointment composition described herein has a pD of between about 4.9-5.4.

[00152] In some embodiments, the ophthalmic composition is an ophthalmic aqueous composition. In some instances, the ophthalmic aqueous composition has a pD of between about 4 and

about 8, about 4.5 and about 7.8, about 5 and about 7.5, or about 5.5 and about 7. In some embodiments, the ophthalmic aqueous composition has a pD of about 7.5. In some embodiments, the ophthalmic aqueous composition has a pD of about 7.4. In some embodiments, the ophthalmic aqueous composition has a pD of about 7.3. In some embodiments, the ophthalmic aqueous composition has a pD of about 7.2. In some embodiments, the ophthalmic aqueous composition has a pD of about 7.1. In some embodiments, the ophthalmic aqueous composition has a pD of about 7. In some embodiments, the ophthalmic aqueous composition has a pD of about 6.9. In some embodiments, the ophthalmic aqueous composition has a pD of about 6.8. In some embodiments, the ophthalmic aqueous composition has a pD of about 6.7. In some embodiments, the ophthalmic aqueous composition has a pD of about 6.6. In some embodiments, the ophthalmic aqueous composition has a pD of about 6.5. In some embodiments, the ophthalmic aqueous composition has a pD of about 6.4. In some embodiments, the ophthalmic aqueous composition has a pD of about 6.3. In some embodiments, the ophthalmic aqueous composition has a pD of about 6.2. In some embodiments, the ophthalmic aqueous composition has a pD of about 6.1. In some embodiments, the ophthalmic aqueous composition has a pD of about 6. In some embodiments, the ophthalmic aqueous composition has a pD of about 5.9. In some embodiments, the ophthalmic aqueous composition has a pD of about 5.8. In some embodiments, the ophthalmic aqueous composition has a pD of about 5.7. In some embodiments, the ophthalmic aqueous composition has a pD of about 5.6. In some embodiments, the ophthalmic aqueous composition has a pD of about 5.5. In some embodiments, the ophthalmic aqueous composition has a pD of about 5.4. In some embodiments, the ophthalmic aqueous composition has a pD of about 5.3. In some embodiments, the ophthalmic aqueous composition has a pD of about 5.2. In some embodiments, the ophthalmic aqueous composition has a pD of about 5.1. In some embodiments, the ophthalmic aqueous composition has a pD of about 5. In some embodiments, the ophthalmic aqueous composition has a pD of about 4.9. In some embodiments, the ophthalmic aqueous composition has a pD of about 4.8. In some embodiments, the ophthalmic aqueous composition has a pD of about 4.7. In some embodiments, the ophthalmic aqueous composition has a pD of about 4.6. In some embodiments, the ophthalmic aqueous composition has a pD of about 4.5. In some embodiments, the ophthalmic aqueous composition has a pD of about 4.4. In some embodiments, the ophthalmic aqueous composition has a pD of about 4.3. In some embodiments, the ophthalmic aqueous composition has a pD of about 4.2. In some embodiments, the ophthalmic aqueous composition has a pD of about 4.1. In some embodiments, the ophthalmic aqueous composition has a pD of about 4. In some embodiments, the pD is an initial pD of the ophthalmic aqueous composition. In some embodiments, the pD is the pD of the ophthalmic aqueous composition after extended period of time under storage condition.

[00153] In some instances, the ophthalmic aqueous composition has an initial pD of between about 4 and about 8, about 4.5 and about 7.8, about 5 and about 7.5, or about 5.5 and about 7. In some

[illegible]

[00154] In some instances, the ophthalmic aqueous composition has a pD of between about 4 and about 8, about 4.5 and about 7.8, about 5 and about 7.5, or about 5.5 and about 7. In some embodiments,

[illegible]

the pD is the pD of the ophthalmic aqueous composition after extended period of time under storage condition.

[00155] In some embodiments, the pD of the ophthalmic aqueous composition described herein is associated with the stability of the ophthalmic aqueous composition. In some embodiments, a stable composition comprises a pD of between about 4 and about 8, about 4.5 and about 7.8, about 5 and about 7.5, or about 5.5 and about 7. In some embodiments, a stable composition comprises a pD of less than about 7.5. In some embodiments, a stable composition comprises a pD of less than about 7.4. In some embodiments, a stable composition comprises a pD of less than about 7.3. In some embodiments, a stable composition comprises a pD of less than about 7.2. In some embodiments, a stable composition comprises a pD of less than about 7.1. In some embodiments, a stable composition comprises a pD of less than about 7. In some embodiments, a stable composition comprises a pD of less than about 6.9. In some embodiments, a stable composition comprises a pD of less than about 6.8. In some embodiments, a stable composition comprises a pD of less than about 6.7. In some embodiments, a stable composition comprises a pD of less than about 6.6. In some embodiments, a stable composition comprises a pD of less than about 6.5. In some embodiments, a stable composition comprises a pD of less than about 6.4. In some embodiments, a stable composition comprises a pD of less than about 6.3. In some embodiments, a stable composition comprises a pD of less than about 6.2. In some embodiments, a stable composition comprises a pD of less than about 6.1. In some embodiments, a stable composition comprises a pD of less than about 6. In some embodiments, a stable composition comprises a pD of less than about 5.9. In some embodiments, a stable composition comprises a pD of less than about 5.8. In some embodiments, a stable composition comprises a pD of less than about 5.7. In some embodiments, a stable composition comprises a pD of less than about 5.6. In some embodiments, a stable composition comprises a pD of less than about 5.5. In some embodiments, a stable composition comprises a pD of less than about 5.4. In some embodiments, a stable composition comprises a pD of less than about 5.3. In some embodiments, a stable composition comprises a pD of less than about 5.2. In some embodiments, a stable composition comprises a pD of less than about 5.1. In some embodiments, a stable composition comprises a pD of less than about 5. In some embodiments, a stable composition comprises a pD of less than about 4.9. In some embodiments, a stable composition comprises a pD of less than about 4.8. In some embodiments, a stable composition comprises a pD of less than about 4.7. In some embodiments, a stable composition comprises a pD of less than about 4.6. In some embodiments, a stable composition comprises a pD of less than about 4.5. In some embodiments, a stable composition comprises a pD of less than about 4.4. In some embodiments, a stable composition comprises a pD of less than about 4.3. In some embodiments, a stable composition comprises a pD of less than about 4.2. In some embodiments, a

stable composition comprises a pD of less than about 4.1. In some embodiments, a stable composition comprises a pD of less than about 4.

[00156] In some embodiments, the D₂O aqueous system stabilizes a muscarinic antagonist (e.g., atropine). In some embodiments, this is due to a lower concentration of the reactive species (e.g., -OD) in the D₂O aqueous system compared to the concentration of the reactive species (e.g., -OH) in an equivalent H₂O aqueous system. In some instances, the concentration of the reactive species (e.g., -OD) in the D₂O aqueous system is about one third less than the concentration of the reactive species (e.g., -OH) in the equivalent H₂O aqueous system. In some cases, this is due to a lower or smaller dissociation constant of D₂O than H₂O. For example, the K_a(H₂O) is 1×10^{-14} , whereas the K_a(D₂O) is 1×10^{-15} . As such, D₂O is a weaker acid than H₂O. In some cases, base catalyzed hydrolysis leads to the presence of tropine degradant from atropine. In some cases, with a lower concentration of the reactive species that causes tropine degradant formation, atropine solution is more stable in a D₂O aqueous system than compared to an equivalent H₂O aqueous system. In some embodiments, the ophthalmic composition formulated with deuterated water allows for a more stable ophthalmic composition relative to the ophthalmic composition formulated with H₂O.

[00157] In some embodiments, the presence of deuterated water shifts the pK_a of the buffer. In some embodiments, the presence of deuterated water allows for the ophthalmic composition to simulate the stability of a lower pH system. In some instances, the buffer capacity of the ophthalmic composition is lowered, thereby allowing a faster shift in pH. In some instances, the lowered buffering capacity of the ophthalmic composition when administered into the eye allows the ophthalmic composition to reach physiological pH at a faster rate than compared to an ophthalmic composition formulated in H₂O. In some instances, the ophthalmic composition formulated with deuterated water allows for a lower tear production, or less tear reflex in the eye, in comparison with an ophthalmic composition formulated with H₂O.

[00158] In some embodiment, the ophthalmic gel or ointment composition described herein has a pD of about 4, about 4.1, about 4.2, about 4.3, about 4.4, about 4.5, about 4.6, about 4.7, about 4.8, about 4.9, about 5.0, about 5.1, about 5.2, about 5.3, about 5.4, about 5.5, about 5.6, about 5.7, about 5.8, about 5.9, about 6.0, about 6.1, about 6.2, about 6.3, about 6.4, about 6.5, about 6.6, about 6.7, about 6.8, about 6.9, about 7.0, about 7.1, about 7.2, about 7.3, about 7.4, about 7.5, about 7.6, about 7.7, about 7.8, or about 7.9.

[00159] In some embodiment, the pD of the ophthalmic aqueous, gel, or ointment composition described herein is suitable for sterilization (e.g., by filtration or aseptic mixing or heat treatment and/or

autoclaving (e.g., terminal sterilization)) of ophthalmic formulations described herein. As used in the present disclosure, the term “aqueous composition” includes compositions that are based on D₂O.

[00160] In some embodiments, the pharmaceutical formulations described herein are stable with respect to pD over a period of any of at least about 1 day, at least about 2 days, at least about 3 days, at least about 4 days, at least about 5 days, at least about 6 days, at least about 1 week, at least about 2 weeks, at least about 3 weeks, at least about 4 weeks, at least about 5 weeks, at least about 6 weeks, at least about 7 weeks, at least about 8 weeks, at least about 1 month, at least about 2 months, at least about 3 months, at least about 4 months, at least about 5 months, at least about 6 months, at least about 7 months, at least about 8 months, at least about 9 months, at least about 10 months, at least about 11 months, at least about 12 months, at least about 18 months, at least about 24 months, at least about 3 years, at least about 4 years, at least about 5 years, at least about 6 years, at least about 7 years, at least about 8 years, at least about 9 years, at least about 10 years, or more. In other embodiments, the formulations described herein are stable with respect to pD over a period of at least about 1 week. In other embodiments, the formulations described herein are stable with respect to pD over a period of at least about 2 weeks. In other embodiments, the formulations described herein are stable with respect to pD over a period of at least about 3 weeks. In other embodiments, the formulations described herein are stable with respect to pD over a period of at least about 1 month. Also described herein are formulations that are stable with respect to pD over a period of at least about 2 months, at least about 3 months, at least about 4 months, at least about 5 months, at least about 6 months, at least about 12 months, at least about 18 months, at least about 2 years, or more.

[00161] Aqueous Solution Dose-To-Dose Uniformity

[00162] Typical ophthalmic aqueous solutions are packaged in eye drop bottles and administered as drops. For example, a single administration (i.e. a single dose) of an ophthalmic aqueous solution includes a single drop, two drops, three drops or more into the eyes of the patient. In some embodiments, one dose of the ophthalmic aqueous solution described herein is one drop of the aqueous solution composition from the eye drop bottle.

[00163] In some cases, described herein include ophthalmic aqueous compositions which provide a dose-to-dose uniform concentrations. In some instances, the dose-to-dose uniform concentration does not present significant variations of drug content from one dose to another. In some instances, the dose-to-dose uniform concentration does provide consistent drug content from one dose to another.

[00164] In some embodiments, the composition has a dose-to-dose ophthalmic agent concentration variation of less than 50%. In some embodiments, the composition has a dose-to-dose ophthalmic agent concentration variation of less than 40%. In some embodiments, the composition has a

dose-to-dose ophthalmic agent concentration variation of less than 30%. In some embodiments, the composition has a dose-to-dose ophthalmic agent concentration variation of less than 20%. In some embodiments, the composition has a dose-to-dose ophthalmic agent concentration variation of less than 10%. In some embodiments, the composition has a dose-to-dose ophthalmic agent concentration variation of less than 5%.

[00165] In some embodiments, the dose-to-dose ophthalmic agent concentration variation is based on 10 consecutive doses. In some embodiments, the dose-to-dose ophthalmic agent concentration variation is based on 8 consecutive doses. In some embodiments, the dose-to-dose ophthalmic agent concentration variation is based on 5 consecutive doses. In some embodiments, the dose-to-dose ophthalmic agent concentration variation is based on 3 consecutive doses. In some embodiments, the dose-to-dose ophthalmic agent concentration variation is based on 2 consecutive doses.

[00166] A nonsettling formulation should not require shaking to disperse drug uniformly. A “no-shake” formulation is potentially advantageous over formulations that require shaking for the simple reason that patients’ shaking behavior is a major source of variability in the amount of drug dosed. It has been reported that patients often times do not or forget to shake their ophthalmic compositions that requires shaking before administering a dose, despite the instructions to shake that were clearly marked on the label. On the other hand, even for those patients who do shake the product, it is normally not possible to determine whether the shaking is adequate in intensity and/or duration to render the product uniform. In some embodiments, the ophthalmic gel compositions and ophthalmic ointment compositions described herein are “no-shake” formulations that maintained the dose-to-dose uniformity described herein.

[00167] To evaluate the dose-to-dose uniformity, drop bottles or tubes containing the ophthalmic aqueous compositions, the ophthalmic gel compositions, or ophthalmic ointment compositions are stored upright for a minimum of 12 hours prior to the start of the test. To simulate the recommended dosing of these products, predetermined number of drops or strips are dispensed from each commercial bottles or tubes at predetermined time intervals for an extended period of time or until no product was left in the bottle or tube. All drops and strips are dispensed into tared glass vials, capped, and stored at room temperature until analysis. Concentrations of a muscarinic antagonist such as atropine in the expressed drops were determined using a reverse-phase HPLC method.

[00168] **Aqueous Solution Viscosity**

[00169] In some embodiments, the composition has a Brookfield RVDV viscosity of from about 10 to about 50,000 cps at about 20°C and sheer rate of 1s^{-1} . In some embodiments, the composition has a Brookfield RVDV viscosity of from about 100 to about 40,000 cps at about 20°C and sheer rate of 1s^{-1} .

In some embodiments, the composition has a Brookfield RVDV viscosity of from about 500 to about 30,000 cps at about 20°C and sheer rate of 1s^{-1} . In some embodiments, the composition has a Brookfield RVDV viscosity of from about 1000 to about 20,000 cps at about 20°C and sheer rate of 1s^{-1} . In some embodiments, the composition has a Brookfield RVDV viscosity of from about 2000 to about 10,000 cps at about 20°C and sheer rate of 1s^{-1} . In some embodiments, the composition has a Brookfield RVDV viscosity of from about 4000 to about 8000 cps at about 20°C and sheer rate of 1s^{-1} .

[00170] In some embodiments, the ophthalmic aqueous formulation contains a viscosity enhancing agent sufficient to provide a viscosity of between about 500 and 50,000 centipoise, between about 750 and 50,000 centipoise; between about 1000 and 50,000 centipoise; between about 1000 and 40,000 centipoise; between about 2000 and 30,000 centipoise; between about 3000 and 20,000 centipoise; between about 4000 and 10,000 centipoise, or between about 5000 and 8000 centipoise.

[00171] In some embodiments, the compositions described herein are low viscosity compositions at body temperature. In some embodiments, low viscosity compositions contain from about 1% to about 10% of a viscosity enhancing agent (e.g., gelling components such as polyoxyethylene-polyoxypropylene copolymers). In some embodiments, low viscosity compositions contain from about 2% to about 10% of a viscosity enhancing agent (e.g., gelling components such as polyoxyethylene-polyoxypropylene copolymers). In some embodiments, low viscosity compositions contain from about 5% to about 10% of a viscosity enhancing agent (e.g., gelling components such as polyoxyethylene-polyoxypropylene copolymers). In some embodiments, low viscosity compositions are substantially free of a viscosity enhancing agent (e.g., gelling components such as polyoxyethylene-polyoxypropylene copolymers). In some embodiments, a low viscosity ophthalmic agent composition described herein provides an apparent viscosity of from about 100 cP to about 10,000 cP. In some embodiments, a low viscosity ophthalmic agent composition described herein provides an apparent viscosity of from about 500 cP to about 10,000 cP. In some embodiments, a low viscosity ophthalmic agent composition described herein provides an apparent viscosity of from about 1000 cP to about 10,000 cP.

[00172] **Osmolarity**

[00173] In some embodiments, a composition disclosed herein is formulated in order to not disrupt the ionic balance of the eye. In some embodiments, a composition disclosed herein has an ionic balance that is the same as or substantially the same as the eye. In some embodiments, a composition disclosed herein does not does not disrupt the ionic balance of the eye.

[00174] As used herein, “practical osmolarity/osmolality” or “deliverable osmolarity/osmolality” means the osmolarity/osmolality of a composition as determined by measuring the osmolarity/osmolality of the ophthalmic agent and all excipients except the gelling and/or the thickening agent (e.g.,

polyoxyethylene-polyoxypropylene copolymers, carboxymethylcellulose or the like). The practical osmolarity of a composition disclosed herein is measured by a suitable method, e.g., a freezing point depression method as described in Viegas et. al., Int. J. Pharm., 1998, 160, 157-162. In some instances, the practical osmolarity of a composition disclosed herein is measured by vapor pressure osmometry (e.g., vapor pressure depression method) that allows for determination of the osmolarity of a composition at higher temperatures. In some instances, vapor pressure depression method allows for determination of the osmolarity of a composition comprising a gelling agent (e.g., a thermoreversible polymer) at a higher temperature wherein the gelling agent is in the form of a gel.

[00175] In some embodiments, the osmolarity at a target site of action (e.g., the eye) is about the same as the delivered osmolarity of a composition described herein. In some embodiments, a composition described herein has a deliverable osmolarity of about 150 mOsm/L to about 500 mOsm/L, about 250 mOsm/L to about 500 mOsm/L, about 250 mOsm/L to about 350 mOsm/L, about 280 mOsm/L to about 370 mOsm/L or about 250 mOsm/L to about 320 mOsm/L.

[00176] The practical osmolality of an ophthalmic composition disclosed herein is from about 100 mOsm/kg to about 1000 mOsm/kg, from about 200 mOsm/kg to about 800 mOsm/kg, from about 250 mOsm/kg to about 500 mOsm/kg, or from about 250 mOsm/kg to about 320 mOsm/kg, or from about 250 mOsm/kg to about 350 mOsm/kg or from about 280 mOsm/kg to about 320 mOsm/kg. In some embodiments, a composition described herein has a practical osmolarity of about 100 mOsm/L to about 1000 mOsm/L, about 200 mOsm/L to about 800 mOsm/L, about 250 mOsm/L to about 500 mOsm/L, about 250 mOsm/L to about 350 mOsm/L, about 250 mOsm/L to about 320 mOsm/L, or about 280 mOsm/L to about 320 mOsm/L.

[00177] In some embodiments, suitable tonicity adjusting agents include, but are not limited to any pharmaceutically acceptable sugar, salt or any combinations or mixtures thereof, such as, but not limited to dextrose, glycerin, mannitol, sorbitol, sodium chloride, and other electrolytes. In some instances, the tonicity adjusting agent is selected from sodium chloride, sodium nitrate, sodium sulfate, sodium bisulfate, potassium chloride, calcium chloride, magnesium chloride, zinc chloride, potassium acetate, sodium acetate, sodium bicarbonate, sodium carbonate, sodium thiosulfate, magnesium sulfate, disodium hydrogen phosphate, sodium dihydrogen phosphate, potassium dihydrogen phosphate, dextrose, mannitol, sorbitol, dextrose, sucrose, urea, propylene glycol, glycerin, or a combination thereof.

[00178] In some embodiment, the ophthalmic compositions described herein include one or more salts in an amount required to bring osmolality of the composition into an acceptable range. Such salts include those having sodium, potassium or ammonium cations and chloride, citrate, ascorbate, borate,

phosphate, bicarbonate, sulfate, thiosulfate or bisulfite anions; suitable salts include sodium chloride, potassium chloride, sodium thiosulfate, sodium bisulfite and ammonium sulfate.

[00179] **Sterility**

[00180] In some embodiments, the compositions are sterilized. Included within the embodiments disclosed herein are means and processes for sterilization of a pharmaceutical composition disclosed herein for use in humans. The goal is to provide a safe pharmaceutical product, relatively free of infection causing micro-organisms. The U. S. Food and Drug Administration has provided regulatory guidance in the publication “Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing” available at: <http://www.fda.gov/cder/guidance/5882fnl.htm>, which is incorporated herein by reference in its entirety.

[00181] As used herein, sterilization means a process used to destroy or remove microorganisms that are present in a product or packaging. Any suitable method available for sterilization of objects and compositions is used. Available methods for the inactivation of microorganisms include, but are not limited to, the application of extreme heat, lethal chemicals, or gamma radiation. In some embodiments, a process for the preparation of an ophthalmic formulation comprises subjecting the formulation to a sterilization method selected from heat sterilization, chemical sterilization, radiation sterilization or filtration sterilization. The method used depends largely upon the nature of the device or composition to be sterilized. Detailed descriptions of many methods of sterilization are given in Chapter 40 of Remington: The Science and Practice of Pharmacy published by Lippincott, Williams & Wilkins, and is incorporated by reference with respect to this subject matter.

Filtration

[00182] Filtration sterilization is a method used to remove but not destroy microorganisms from solutions. Membrane filters are used to filter heat-sensitive solutions. Such filters are thin, strong, homogenous polymers of mixed cellulosic esters (MCE), polyvinylidene fluoride (PVF; also known as PVDF), or polytetrafluoroethylene (PTFE) and have pore sizes ranging from 0.1 to 0.22 μ m. Solutions of various characteristics are optionally filtered using different filter membranes. For example, PVF and PTFE membranes are well suited to filtering organic solvents while aqueous solutions are filtered through PVF or MCE membranes. Filter apparatus are available for use on many scales ranging from the single point-of-use disposable filter attached to a syringe up to commercial scale filters for use in manufacturing plants. The membrane filters are sterilized by autoclave or chemical sterilization. Validation of membrane filtration systems is performed following standardized protocols (Microbiological Evaluation of Filters for Sterilizing Liquids, Vol 4, No. 3. Washington, D.C: Health Industry Manufacturers Association, 1981)

and involve challenging the membrane filter with a known quantity (ca. $10^7/\text{cm}^2$) of unusually small microorganisms, such as *Brevundimonas diminuta* (ATCC 19146).

[00183] Pharmaceutical compositions are optionally sterilized by passing through membrane filters. Formulations comprising nanoparticles (U.S. Pat No. 6,139,870) or multilamellar vesicles (Richard et al., International Journal of Pharmaceutics (2006), 312(1-2):144-50) are amenable to sterilization by filtration through 0.22 μm filters without destroying their organized structure.

[00184] In some embodiments, the methods disclosed herein comprise sterilizing the formulation (or components thereof) by means of filtration sterilization. In ophthalmic gel compositions that includes thermosetting polymers, filtration is carried out below (e.g. about 5°C) the gel temperature (T_{gel}) of a formulation described herein and with viscosity that allows for filtration in a reasonable time using a peristaltic pump (e.g. below a theoretical value of 100cP).

[00185] Accordingly, provided herein are methods for sterilization of ophthalmic formulations that prevent degradation of polymeric components (e.g., thermosetting and/or other viscosity enhancing agents) and/or the ophthalmic agent during the process of sterilization. In some embodiments, degradation of the ophthalmic agent (e.g., a muscarinic antagonist such as atropine or atropine sulfate) is reduced or eliminated through the use of specific pD ranges for buffer components and specific proportions of viscosity enhancing agents in the formulations. In some embodiments, the choice of an appropriate viscosity enhancing agents or thermosetting polymer allows for sterilization of formulations described herein by filtration. In some embodiments, the use of an appropriate thermosetting polymer or other viscosity enhancing agents in combination with a specific pD range for the formulation allows for high temperature sterilization of formulations described with substantially no degradation of the therapeutic agent or the polymeric excipients. An advantage of the methods of sterilization provided herein is that, in certain instances, the formulations are subjected to terminal sterilization via autoclaving without any loss of the ophthalmic agent and/or excipients and/or viscosity enhancing agents during the sterilization step and are rendered substantially free of microbes and/or pyrogens.

Radiation Sterilization

[00186] One advantage of radiation sterilization is the ability to sterilize many types of products without heat degradation or other damage. The radiation commonly employed is beta radiation or alternatively, gamma radiation from a ^{60}Co source. The penetrating ability of gamma radiation allows its use in the sterilization of many product types, including solutions, compositions and heterogeneous mixtures. The germicidal effects of irradiation arise from the interaction of gamma radiation with biological macromolecules. This interaction generates charged species and free-radicals. Subsequent chemical reactions, such as rearrangements and cross-linking processes, result in the loss of normal

function for these biological macromolecules. The formulations described herein are also optionally sterilized using beta irradiation.

Sterilization by Heat

[00187] Many methods are available for sterilization by the application of high heat. One method is through the use of a saturated steam autoclave. In this method, saturated steam at a temperature of at least 121 °C is allowed to contact the object to be sterilized. The transfer of heat is either directly to the microorganism, in the case of an object to be sterilized, or indirectly to the microorganism by heating the bulk of an aqueous solution to be sterilized. This method is widely practiced as it allows flexibility, safety and economy in the sterilization process.

Microorganisms

[00188] In some embodiments, the compositions are substantially free of microorganisms. Acceptable bioburden or sterility levels are based on applicable standards that define therapeutically acceptable compositions, including but not limited to United States Pharmacopeia Chapters <111> et seq. For example, acceptable sterility (e.g., bioburden) levels include about 10 colony forming units (cfu) per gram of formulation, about 50 cfu per gram of formulation, about 100 cfu per gram of formulation, about 500 cfu per gram of formulation or about 1000 cfu per gram of formulation. In some embodiments, acceptable bioburden levels or sterility for formulations include less than 10 cfu/mL, less than 50 cfu/mL, less than 500 cfu/mL or less than 1000 cfu/mL microbial agents. In addition, acceptable bioburden levels or sterility include the exclusion of specified objectionable microbiological agents. By way of example, specified objectionable microbiological agents include but are not limited to *Escherichia coli* (*E. coli*), *Salmonella* sp., *Pseudomonas aeruginosa* (*P. aeruginosa*) and/or other specific microbial agents.

[00189] An important component of the sterility assurance quality control, quality assurance and validation process is the method of sterility testing. Sterility testing, by way of example only, is performed by two methods. The first is direct inoculation wherein a sample of the composition to be tested is added to growth medium and incubated for a period of time up to 21 days. Turbidity of the growth medium indicates contamination. Drawbacks to this method include the small sampling size of bulk materials which reduces sensitivity, and detection of microorganism growth based on a visual observation. An alternative method is membrane filtration sterility testing. In this method, a volume of product is passed through a small membrane filter paper. The filter paper is then placed into media to promote the growth of microorganisms. This method has the advantage of greater sensitivity as the entire bulk product is sampled. The commercially available Millipore Steritest sterility testing system is optionally used for determinations by membrane filtration sterility testing. For the filtration testing of creams or ointments Steritest filter system No. TLHVSL210 are used. For the filtration testing of

emulsions or viscous products Steritest filter system No. TLAREM210 or TDAREM210 are used. For the filtration testing of pre-filled syringes Steritest filter system No. TTHASY210 are used. For the filtration testing of material dispensed as an aerosol or foam Steritest filter system No. TTHVA210 are used. For the filtration testing of soluble powders in ampoules or vials Steritest filter system No. TTHADA210 or TTHADV210 are used.

[00190] Testing for *E. coli* and *Salmonella* includes the use of lactose broths incubated at 30 – 35 °C for 24-72 hours, incubation in MacConkey and/or EMB agars for 18-24 hours, and/or the use of Rappaport medium. Testing for the detection of *P. aeruginosa* includes the use of NAC agar. United States Pharmacopeia Chapter <62> further enumerates testing procedures for specified objectionable microorganisms.

[00191] In certain embodiments, the ophthalmic formulation described herein has less than about 60 colony forming units (CFU), less than about 50 colony forming units, less than about 40 colony forming units, or less than about 30 colony forming units of microbial agents per gram of formulation. In certain embodiments, the ophthalmic formulations described herein are formulated to be isotonic with the eye.

Endotoxins

[00192] An additional aspect of the sterilization process is the removal of by-products from the killing of microorganisms (hereinafter, “Product”). The process of depyrogenation removes pyrogens from the sample. Pyrogens are endotoxins or exotoxins which induce an immune response. An example of an endotoxin is the lipopolysaccharide (LPS) molecule found in the cell wall of gram-negative bacteria. While sterilization procedures such as autoclaving or treatment with ethylene oxide kill the bacteria, the LPS residue induces a proinflammatory immune response, such as septic shock. Because the molecular size of endotoxins varies widely, the presence of endotoxins is expressed in “endotoxin units” (EU). One EU is equivalent to 100 picograms of *E. coli* LPS. In some cases, humans develop a response to as little as 5 EU/kg of body weight. The bioburden (e.g., microbial limit) and/or sterility (e.g., endotoxin level) is expressed in any units as recognized in the art. In certain embodiments, ophthalmic compositions described herein contain lower endotoxin levels (e.g. < 4 EU/kg of body weight of a subject) when compared to conventionally acceptable endotoxin levels (e.g., 5 EU/kg of body weight of a subject). In some embodiments, the ophthalmic formulation has less than about 5 EU/kg of body weight of a subject. In other embodiments, the ophthalmic formulation has less than about 4 EU/kg of body weight of a subject. In additional embodiments, the ophthalmic formulation has less than about 3 EU/kg of body weight of a subject. In additional embodiments, the ophthalmic formulation has less than about 2 EU/kg of body weight of a subject.

[00193] In some embodiments, the ophthalmic formulation has less than about 5 EU/kg of formulation. In other embodiments, the ophthalmic formulation has less than about 4 EU/kg of formulation. In additional embodiments, the ophthalmic formulation has less than about 3 EU/kg of formulation. In some embodiments, the ophthalmic formulation has less than about 5 EU/kg Product. In other embodiments, the ophthalmic formulation has less than about 1 EU/kg Product. In additional embodiments, the ophthalmic formulation has less than about 0.2 EU/kg Product. In some embodiments, the ophthalmic formulation has less than about 5 EU/g of unit or Product. In other embodiments, the ophthalmic formulation has less than about 4 EU/ g of unit or Product. In additional embodiments, the ophthalmic formulation has less than about 3 EU/g of unit or Product. In some embodiments, the ophthalmic formulation has less than about 5 EU/mg of unit or Product. In other embodiments, the ophthalmic formulation has less than about 4 EU/ mg of unit or Product. In additional embodiments, the ophthalmic formulation has less than about 3 EU/mg of unit or Product. In certain embodiments, ophthalmic formulations described herein contain from about 1 to about 5 EU/mL of formulation. In certain embodiments, ophthalmic formulations described herein contain from about 2 to about 5 EU/mL of formulation, from about 3 to about 5 EU/mL of formulation, or from about 4 to about 5 EU/mL of formulation.

[00194] In certain embodiments, ophthalmic compositions described herein contain lower endotoxin levels (e.g. < 0.5 EU/mL of formulation) when compared to conventionally acceptable endotoxin levels (e.g., 0.5 EU/mL of formulation). In some embodiments, the ophthalmic formulation has less than about 0.5 EU/mL of formulation. In other embodiments, the ophthalmic formulation has less than about 0.4 EU/mL of formulation. In additional embodiments, the ophthalmic formulation has less than about 0.2 EU/mL of formulation.

[00195] Pyrogen detection, by way of example only, is performed by several methods. Suitable tests for sterility include tests described in United States Pharmacopoeia (USP) <71> Sterility Tests (23rd edition, 1995). The rabbit pyrogen test and the Limulus amebocyte lysate test are both specified in the United States Pharmacopoeia Chapters <85> and <151> (USP23/NF 18, Biological Tests, The United States Pharmacopoeial Convention, Rockville, MD, 1995). Alternative pyrogen assays have been developed based upon the monocyte activation-cytokine assay. Uniform cell lines suitable for quality control applications have been developed and have demonstrated the ability to detect pyrogenicity in samples that have passed the rabbit pyrogen test and the Limulus amebocyte lysate test (Taktak et al, J. Pharm. Pharmacol. (1990), 43:578-82). In an additional embodiment, the ophthalmic formulation is subject to depyrogenation. In a further embodiment, the process for the manufacture of the ophthalmic

formulation comprises testing the formulation for pyrogenicity. In certain embodiments, the formulations described herein are substantially free of pyrogens.

[00196] Ophthalmic Muscarinic Antagonist-Mucus Penetrating Particle (MPP) Composition

[00197] Mucus-penetrating particles (MPPs) are particles that rapidly traverse mucus (e.g. human mucus). In some cases, MPPs comprise of a nanoparticle with a particle size of between about 200nm and 500nm. In some instances, the nanoparticle is further coated with a mucus penetrating agent. In some instances, a composition described herein is formulated with MPPs for mucus penetration. In some instances, an ophthalmic agent composition described herein is formulated with MPPs for mucus penetration. In some instances, the ophthalmic agent is a muscarinic antagonist. In some instances, a muscarinic antagonist composition described herein is formulated with MPPs for mucus penetration. In some instances, a muscarinic antagonist comprises atropine, atropine sulfate, noratropine, atropine-N-oxide, tropine, tropic acid, atropine methonitrate, diphenhydramine, dimenhydrinate, dicyclomine, flavoxate, oxybutynin, tiotropium, hyoscine, scopolamine (L-hyoscine), hydroxyzine, ipratropium, tropicamide, cyclopentolate, pirenzapine, homatropine, solifenacin, darifenacin, benzatropine, mebeverine, procyclidine, acridinium bromide, trihexyphenidyl/benzhexol, or tolterodine. In some instances, a muscarinic antagonist is atropine or its pharmaceutically acceptable salt thereof. In some instances, a muscarinic antagonist is atropine sulfate. In some instances, an atropine composition described herein is formulated with MPPs for mucus penetration. In some instances, an atropine sulfate composition described herein is formulated with MPPs for mucus penetration. In a non-limiting example, the MPPs for use in the disclosed composition is obtained from Kala Pharmaceuticals, Inc. (100 Beaver Street #201, Waltham, MA 02453).

[00198] In some embodiments, the nanoparticle comprises of any suitable material, such as an organic material, an inorganic material, a polymer, or combinations thereof. In some instances, the nanoparticle comprises of inorganic material, such as for example, a metal (e.g., Ag, Au, Pt, Fe, Cr, Co, Ni, Cu, Zn, and other transition metals), a semiconductor (e.g., silicon, silicon compounds and alloys, cadmium selenide, cadmium sulfide, indium arsenide, and indium phosphide), or an insulator (e.g., ceramics such as silicon oxide). In some instances, the nanoparticle comprises organic materials such as a synthetic polymer and/or a natural polymer. Examples of synthetic polymers include non-degradable polymers such as polymethacrylate and degradable polymers such as polylactic acid, polyglycolic acid and copolymers thereof. Examples of natural polymers include hyaluronic acid, chitosan, and collagen.

[00199] In some embodiments, the nanoparticle is coated with a mucus penetrating agent. In some instances, the mucus penetrating agent comprises any suitable material, such as a hydrophobic material, a hydrophilic material, and/or an amphiphilic material. In some instances, the mucus penetrating agent is a

polymer. In some instances, the polymer is a synthetic polymer (i.e., a polymer not produced in nature). In other embodiments, the polymer is a natural polymer (e.g., a protein, polysaccharide, rubber). In certain embodiments, the polymer is a surface active polymer. In certain embodiments, the polymer is a non-ionic polymer. In certain embodiments, the polymer is a non-ionic block copolymer. In some embodiments, the polymer is a diblock copolymer, a triblock copolymer, e.g., e.g., where one block is a hydrophobic polymer and another block is a hydrophilic polymer. In some embodiments, the polymer is charged or uncharged.

[00200] Additional examples of suitable polymers include, but are not limited to, polyamines, polyethers, polyamides, polyesters, polycarbamates, polyureas, polycarbonates, polystyrenes, polyimides, polysulfones, polyurethanes, polyacetylenes, polyethylenes, polyethyleneimines, polyisocyanates, polyacrylates, polymethacrylates, polyacrylonitriles, and polyarylates. Non-limiting examples of specific polymers include poly(caprolactone) (PCL), ethylene vinyl acetate polymer (EVA), poly(lactic acid) (PLA), poly(L-lactic acid) (PLLA), poly(glycolic acid) (PGA), poly(lactic acid-co-glycolic acid) (PLGA), poly(L-lactic acid-co-glycolic acid) (PLLGA), poly(D,L-lactide) (PDLA), poly(L-lactide) (PLLA), poly(D,L-lactide-co-caprolactone), poly(D,L-lactide-co-caprolactone-co-glycolide), poly(D,L-lactide-co-PEO-co-D,L-lactide), poly(D,L-lactide-co-PPO-co-D,L-lactide), polyalkyl cyanoacrylate, polyurethane, poly-L-lysine (PLL), hydroxypropyl methacrylate (HPMA), poly(ethylene glycol), poly-L-glutamic acid, poly(hydroxy acids), polyanhydrides, polyorthoesters, poly(ester amides), polyamides, poly(ester ethers), polycarbonates, polyalkylenes such as polyethylene and polypropylene, polyalkylene glycols such as poly(ethylene glycol) (PEG), polyalkylene oxides (PEO), polyalkylene terephthalates such as poly(ethylene terephthalate), polyvinyl alcohols (PVA), polyvinyl ethers, polyvinyl esters such as poly(vinyl acetate), polyvinyl halides such as poly(vinyl chloride) (PVC), polyvinylpyrrolidone, polysiloxanes, polystyrene (PS), polyurethanes, derivatized celluloses such as alkyl celluloses, hydroxyalkyl celluloses, cellulose ethers, cellulose esters, nitro celluloses, hydroxypropylcellulose, carboxymethylcellulose, polymers of acrylic acids, such as poly(methyl(meth)acrylate) (PMMA), poly(ethyl(meth)acrylate), poly(butyl(meth)acrylate), poly(isobutyl(meth)acrylate), poly(hexyl(meth)acrylate), poly(isodecyl(meth)acrylate), poly(lauryl(meth)acrylate), poly(phenyl(meth)acrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), poly(octadecyl acrylate) (jointly referred to herein as "polyacrylic acids"), and copolymers and mixtures thereof, polydioxanone and its copolymers, polyhydroxyalkanoates, polypropylene fumarate), polyoxymethylene, poloxamers, poly(ortho)esters, poly(butyric acid), poly(valeric acid), poly(lactide-co-caprolactone), and trimethylene carbonate, polyvinylpyrrolidone.

[00201] In some cases, an ophthalmic agent (e.g. a muscarinic antagonist such as atropine or atropine sulfate) is present in the MPP formulation at a concentration of between about 0.001 wt% and

about 0.05 wt%, between about 0.005% to about 0.050%, between about 0.010% to about 0.050%, between about 0.015% to about 0.050%, between about 0.020% to about 0.050%, between about 0.025% to about 0.050%, between about 0.030% to about 0.050%, between about 0.035% to about 0.050%, between about 0.040% to about 0.050%, or between about 0.045% to about 0.050% of the ophthalmic agent, or pharmaceutically acceptable prodrug or salt thereof, by weight of the composition. In some instances, additional agents such as buffers, pH adjusting agents, and/or preservatives are formulated in the MPP formulation.

[00202] In some instances, ophthalmic agent-MPP composition is formulated using any suitable method. In some embodiments, a milling process is used to reduce the size of a solid material to form particles in the micrometer to nanometer size range. In some cases, dry and wet milling processes such as jet milling, cryo-milling, ball milling, media milling, and homogenization are known and are used in methods described herein. Generally, in a wet milling process, a suspension of the material to be used as the nanoparticle is mixed with milling media with or without excipients to reduce particle size. Dry milling is a process wherein the material to be used as the nanoparticle is mixed with milling media with or without excipients to reduce particle size. In a cryo-milling process, a suspension of the material to be used as the nanoparticle is mixed with milling media with or without excipients under cooled temperatures.

[00203] In some embodiments, any suitable grinding medium is used for milling. In some embodiments, a ceramic and/or polymeric material and/or a metal is used. Examples of suitable materials include zirconium oxide, silicon carbide, silicon oxide, silicon nitride, zirconium silicate, yttrium oxide, glass, alumina, alpha- alumina, aluminum oxide, polystyrene, poly(methyl methacrylate), titanium, steel. In some cases, a grinding medium has any suitable size. For example, the grinding medium has an average diameter of at least about 0.1 mm, at least about 0.2 mm, at least about 0.5 mm, at least about 0.8 mm, at least about 1 mm, at least about 2 mm, or at least about 5 mm. In some cases, the grinding medium has an average diameter of less than or equal to about 5 mm, less than or equal to about 2 mm, less than or equal to about 1 mm, less than or equal to about 0.8, less than or equal to about 0.5 mm, or less than or equal to about 0.2 mm. Combinations of the above-referenced ranges are also possible (e.g., an average diameter of at least about 0.5 millimeters and less than or equal to about 1 mm). Other ranges are also possible.

[00204] In some embodiments, any suitable solvent are used for milling. In some cases, the choice of solvent is depend on factors such as the solid material (e.g., a muscarinic antagonist such as atropine) being milled, the particular type of stabilizer/mucus penetrating agent being used (e.g., one that renders the particle mucus penetrating), the grinding material be used, among other factors. In some

cases, suitable solvents are ones that do not substantially dissolve the solid material or the grinding material, but dissolve the stabilizer/mucus penetrating agent to a suitable degree. Non-limiting examples of solvents include, but are not limited to, water, buffered solutions, other aqueous solutions, alcohols (e.g., ethanol, methanol, butanol), and mixtures thereof that optionally include other components such as pharmaceutical excipients, polymers, pharmaceutical agents, salts, preservative agents, viscosity modifiers, tonicity modifier, taste masking agents, antioxidants, pH modifier, and other pharmaceutical excipients. In other embodiments, an organic solvent is used. In some cases, a pharmaceutical agent (e.g. a muscarinic antagonist such as atropine) has any suitable solubility in these or other solvents, such as a solubility in one or more of the ranges described above for aqueous solubility or for solubility in a coating solution.

[00205] In some instances, a MPP is a MPP as described in WO2013/166385. In some instances, a MPP is a MPP as described in Lai *et al.*, “Rapid transport of large polymeric nanoparticles in fresh undiluted human mucus,” *PNAS* 104(5):1482-1487 (2007). In some instances, an ophthalmic agent-MPP composition is formulated using a method as described in WO2013/166385. In some instances, an ophthalmic agent-MPP composition is formulated using a method as described in Lai *et al.*, “Rapid transport of large polymeric nanoparticles in fresh undiluted human mucus,” *PNAS* 104(5):1482-1487 (2007). In some instances, the ophthalmic agent is a muscarinic antagonist such as atropine or atropine sulfate.

[00206] Ophthalmic Gel Muscarinic Antagonist Composition

[00207] Gels have been defined in various ways. For example, the United States Pharmacopoeia defines gels as semisolid systems consisting of either suspensions made up of small inorganic particles or large organic molecules interpenetrated by a liquid. Gels include a single-phase or a two-phase system. A single-phase gel consists of organic macromolecules distributed uniformly throughout a liquid in such a manner that no apparent boundaries exist between the dispersed macromolecules and the liquid. Some single-phase gels are prepared from synthetic macromolecules (e.g., carbomer) or from natural gums, (e.g., tragacanth). In some embodiments, single-phase gels are generally aqueous, but will also be made using alcohols and oils. Two-phase gels consist of a network of small discrete particles.

[00208] In some embodiments, gels are also classified as being hydrophobic or hydrophilic. In certain embodiments, the base of a non-limiting example of a hydrophobic gel includes a liquid paraffin with polyethylene or fatty oils gelled with colloidal silica, or aluminum or zinc soaps. In contrast, the base of a non-limiting example of a hydrophilic gel includes water, glycerol, or propylene glycol gelled with a suitable gelling agent (e.g., tragacanth, starch, cellulose derivatives, carboxyvinylpolymers, and

magnesium-aluminum silicates). In certain embodiments, the rheology of the compositions disclosed herein is pseudo plastic, plastic, thixotropic, or dilatant.

[00209] In some embodiments, the ophthalmic composition is an ophthalmic gel, and wherein the ophthalmically acceptable carrier comprises water and at least one viscosity-enhancing agent. In some embodiments, the viscosity-enhancing agent is selected from cellulose-based polymers, polyoxyethylene-polyoxypropylene triblock copolymers, dextran-based polymers, polyvinyl alcohol, dextrin, polyvinylpyrrolidone, polyalkylene glycols, chitosan, collagen, gelatin, hyaluronic acid, or combinations thereof.

[00210] In some embodiment, the ophthalmic gel composition described herein is a semi-solid or id in a gelled state before it is topically administered (e.g. at room temperature). For example, suitable viscosity-enhancing agents for such gels include by way of example only, gelling agents and suspending agents. In one embodiment, the enhanced viscosity formulation does not include a buffer. In other embodiments, the enhanced viscosity formulation includes a pharmaceutically acceptable buffer. Sodium chloride or other tonicity agents are optionally used to adjust tonicity, if necessary.

[00211] By way of example only, the ophthalmically acceptable viscosity agent includes hydroxypropyl methylcellulose, hydroxyethyl cellulose, polyvinylpyrrolidone, carboxymethyl cellulose, polyvinyl alcohol, sodium chondroitin sulfate, sodium hyaluronate. Other viscosity enhancing agents compatible with the targeted ocular site include, but are not limited to, acacia (gum arabic), agar, aluminum magnesium silicate, sodium alginate, sodium stearate, bladderwrack, bentonite, carbomer, carrageenan, Carbopol, xanthan, cellulose, microcrystalline cellulose (MCC), ceratonia, chitin, carboxymethylated chitosan, chondrus, dextrose, furcellaran, gelatin, Ghatti gum, guar gum, hectorite, lactose, sucrose, maltodextrin, mannitol, sorbitol, honey, maize starch, wheat starch, rice starch, potato starch, gelatin, sterculia gum, xanthum gum, gum tragacanth, ethyl cellulose, ethylhydroxyethyl cellulose, ethylmethyl cellulose, methyl cellulose, hydroxyethyl cellulose, hydroxyethylmethyl cellulose, hydroxypropyl cellulose, poly(hydroxyethyl methacrylate), oxypolygelatin, pectin, polygeline, povidone, propylene carbonate, methyl vinyl ether/maleic anhydride copolymer (PVM/MA), poly(methoxyethyl methacrylate), poly(methoxyethoxyethyl methacrylate), hydroxypropyl cellulose, hydroxypropylmethyl-cellulose (HPMC), sodium carboxymethyl-cellulose (CMC), silicon dioxide, polyvinylpyrrolidone (PVP: povidone), Splenda® (dextrose, maltodextrin and sucralose) or combinations thereof. In specific embodiments, the viscosity-enhancing excipient is a combination of MCC and CMC. In another embodiment, the viscosity-enhancing agent is a combination of carboxymethylated chitosan, or chitin, and alginate. The combination of chitin and alginate with the ophthalmic agents disclosed herein acts as a controlled release formulation, restricting the diffusion of the ophthalmic agents from the formulation.

Moreover, the combination of carboxymethylated chitosan and alginate is optionally used to assist in increasing the permeability of the ophthalmic agents in the eye.

[00212] In some embodiments is an enhanced viscosity formulation, comprising from about 0.1 mM and about 100 mM of an ophthalmic agent, a pharmaceutically acceptable viscosity agent, and water for injection, the concentration of the viscosity agent in the water being sufficient to provide an enhanced viscosity formulation with a final viscosity from about 100 to about 100,000 cP. In certain embodiments, the viscosity of the gel is in the range from about 100 to about 50,000 cP, about 100 cP to about 1,000 cP, about 500 cP to about 1500 cP, about 1000 cP to about 3000 cP, about 2000 cP to about 8,000 cP, about 4,000 cP to about 50,000 cP, about 10,000 cP to about 500,000 cP, about 15,000 cP to about 1,000,000 cP. In other embodiments, when an even more viscous medium is desired, the biocompatible gel comprises at least about 35%, at least about 45%, at least about 55%, at least about 65%, at least about 70%, at least about 75%, or even at least about 80% or so by weight of the ophthalmic agent. In highly concentrated samples, the biocompatible enhanced viscosity formulation comprises at least about 25%, at least about 35%, at least about 45%, at least about 55%, at least about 65%, at least about 75%, at least about 85%, at least about 90% or at least about 95% or more by weight of the ophthalmic agent.

[00213] In one embodiment, the pharmaceutically acceptable enhanced viscosity ophthalmically acceptable formulation comprises at least one ophthalmic agent and at least one gelling agent. Suitable gelling agents for use in preparation of the gel formulation include, but are not limited to, celluloses, cellulose derivatives, cellulose ethers (e.g., carboxymethylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxymethylcellulose, hydroxypropylmethylcellulose, hydroxypropylcellulose, methylcellulose), guar gum, xanthan gum, locust bean gum, alginates (e.g., alginic acid), silicates, starch, tragacanth, carboxyvinyl polymers, carrageenan, paraffin, petrolatum and any combinations or mixtures thereof. In some other embodiments, hydroxypropylmethylcellulose (Methocel®) is utilized as the gelling agent. In certain embodiments, the viscosity enhancing agents described herein are also utilized as the gelling agent for the gel formulations presented herein.

[00214] In some embodiments, the ophthalmic gel composition described herein is an in situ gel formulation. In some instances, the in situ gel formation is based on increased pre-corneal residence time of the ophthalmic composition which improves ocular bioavailability, corneal mucoadhesion, lysosomal interaction and ionic gelation, improved corneal absorption, thermal gelation, or a combination thereof. In some instances, the in situ gel formulation is activated by pH, temperature, ion, UV, or solvent exchange.

[00215] In some instances, the ophthalmic gel composition comprises a muscarinic antagonist and one or more gelling agents. In some instances, the gelling agent includes, but is not limited to, poloxamer (e.g. Poloxamer 407), tetronics, ethyl (hydroxyethyl) cellulose, cellulose acetate phthalate (CAP),

carbopol (e.g. Carbopol 1342P NF, Carbopol 980 NF), alginates (e.g. low acetyl gellan gum (Gelrite®)), gellan, hyaluronic acid, pluronics (e.g. Pluronic F-127), chitosan, polyvinyl alcohol (PVA), polyvinylpyrrolidone (PVP), dextran, hydroxy propyl methyl cellulose (HPMC), hydroxyethylcellulose (HEC), methylcellulose (MC), thiolated xyloglucan, polymethacrylic acid (PMMA), polyethylene glycol (PEG), pseudolatexes, xyloglucans, or combinations thereof.

[00216] In some instances, the in situ gel formation further comprises a permeation enhancer. In some instances, the permeation enhancer includes surfactants (e.g. non-ionic surfactants), benzalkonium chloride, EDTA, surface-active heteroglycosides, calcium chelators, hydroxyl propyl beta cyclodextrin (HP beta CD), bile salts, and the like.

[00217] In some embodiments, other gel formulations are useful depending upon the particular ophthalmic agent, other pharmaceutical agent or excipients/additives used, and as such are considered to fall within the scope of the present disclosure. For example, other commercially-available glycerin-based gels, glycerin-derived compounds, conjugated, or crosslinked gels, matrices, hydrogels, and polymers, as well as gelatins and their derivatives, alginates, and alginate-based gels, and even various native and synthetic hydrogel and hydrogel-derived compounds are all expected to be useful in the ophthalmic agent formulations described herein. In some embodiments, ophthalmically acceptable gels include, but are not limited to, alginate hydrogels SAF®-Gel (ConvaTec, Princeton, N.J.), Duoderm® Hydroactive Gel (ConvaTec), Nu-gel ®(Johnson & Johnson Medical, Arlington, Tex.); Carrasyn®(V) Acemannan Hydrogel (Carrington Laboratories, Inc., Irving, Tex.); glycerin gels Elta® Hydrogel (Swiss-American Products, Inc., Dallas, Tex.) and K-Y® Sterile (Johnson & Johnson). In further embodiments, biodegradable biocompatible gels also represent compounds present in ophthalmically acceptable formulations disclosed and described herein.

[00218] In some embodiments, the viscosity-enhancing agent is a cellulose-based polymer selected from cellulose gum, alkylcellulose, hydroxyl-alkyl cellulose, hydroxyl-alkyl alkylcellulose, carboxy-alkyl cellulose, or combinations thereof. In some embodiments, the viscosity-enhancing agent is hydroxyl-alkyl alkylcellulose. In some embodiment, the viscosity-enhancing agent is hydroxypropyl methylcellulose.

[00219] In certain embodiments, the enhanced viscosity formulation is characterized by a phase transition between room temperature and body temperature (including an individual with a serious fever, e.g., up to about 42 °C). In some embodiments, the phase transition occurs at 1 °C below body temperature, at 2 °C below body temperature, at 3 °C below body temperature, at 4 °C below body temperature, at 6 °C below body temperature, at 8 °C below body temperature, or at 10 °C below body temperature. In some embodiments, the phase transition occurs at about 15 °C below body temperature, at

about 20 °C below body temperature or at about 25 °C below body temperature. In specific embodiments, the gelation temperature (Tgel) of a formulation described herein is about 20 °C, about 25 °C, or about 30 °C. In certain embodiments, the gelation temperature (Tgel) of a formulation described herein is about 35 °C, or about 40 °C. Included within the definition of body temperature is the body temperature of a healthy individual, or an unhealthy individual, including an individual with a fever (up to ~42 °C). In some embodiments, the pharmaceutical compositions described herein are liquids at about room temperature and are administered at or about room temperature.

[00220] Copolymers polyoxypropylene and polyoxyethylene (e.g. polyoxyethylene-polyoxypropylene triblock copolymers) form thermosetting gels when incorporated into aqueous solutions. These polymers have the ability to change from the liquid state to the gel state at temperatures close to body temperature, therefore allowing useful formulations that are applied to the targeted ocular site. The liquid state-to-gel state phase transition is dependent on the polymer concentration and the ingredients in the solution.

[00221] In some embodiments, the amount of thermosetting polymer in any formulation described herein is about 10%, about 15%, about 20%, about 25%, about 30%, about 35% or about 40% of the total weight of the formulation. In some embodiments, the amount of thermosetting polymer in any formulation described herein is about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24% or about 25% of the total weight of the formulation. In some embodiments, the amount of thermosetting polymer (e.g., Poloxamer 407) in any formulation described herein is about 7.5% of the total weight of the formulation. In some embodiments, the amount of thermosetting polymer (e.g., Poloxamer 407) in any formulation described herein is about 10% of the total weight of the formulation. In some embodiments, the amount of thermosetting polymer (e.g., Poloxamer 407) in any formulation described herein is about 11% of the total weight of the formulation. In some embodiments, the amount of thermosetting polymer (e.g., Poloxamer 407) in any formulation described herein is about 12% of the total weight of the formulation. In some embodiments, the amount of thermosetting polymer (e.g., Poloxamer 407) in any formulation described herein is about 13% of the total weight of the formulation. In some embodiments, the amount of thermosetting polymer (e.g., Poloxamer 407) in any formulation described herein is about 14% of the total weight of the formulation. In some embodiments, the amount of thermosetting polymer (e.g., Poloxamer 407) in any formulation described herein is about 15% of the total weight of the formulation. In some embodiments, the amount of thermosetting polymer (e.g., Poloxamer 407) in any formulation described herein is about 16% of the total weight of the formulation. In some embodiments, the amount of thermosetting polymer (e.g., Poloxamer 407) in any formulation described herein is about 17% of the total weight of the formulation. In some embodiments, the amount of

thermosetting polymer (e.g., Poloxamer 407) in any formulation described herein is about 18% of the total weight of the formulation. In some embodiments, the amount of thermosetting polymer (e.g., Poloxamer 407) in any formulation described herein is about 19% of the total weight of the formulation. In some embodiments, the amount of thermosetting polymer (e.g., Poloxamer 407) in any formulation described herein is about 20% of the total weight of the formulation. In some embodiments, the amount of thermosetting polymer (e.g., Poloxamer 407) in any formulation described herein is about 21% of the total weight of the formulation. In some embodiments, the amount of thermosetting polymer (e.g., Poloxamer 407) in any formulation described herein is about 23% of the total weight of the formulation. In some embodiments, the amount of thermosetting polymer (e.g., Poloxamer 407) in any formulation described herein is about 25% of the total weight of the formulation. In some embodiments, the amount of thickening agent (e.g., a gelling agent) in any formulation described herein is about 1%, about 5%, about 10%, or about 15% of the total weight of the formulation. In some embodiments, the amount of thickening agent (e.g., a gelling agent) in any formulation described herein is about 0.5%, about 1%, about 1.5%, about 2%, about 2.5%, about 3%, about 3.5%, about 4%, about 4.5%, or about 5% of the total weight of the formulation.

[00222] In an alternative embodiment, the thermogel is a PEG-PLGA-PEG triblock copolymer (Jeong et al, Nature (1997), 388:860-2; Jeong et al, J. Control. Release (2000), 63:155-63; Jeong et al, Adv. Drug Delivery Rev. (2002), 54:37-51). The polymer exhibits sol-gel behavior over a concentration of about 5% w/w to about 40% w/w. Depending on the properties desired, the lactide/glycolide molar ratio in the PLGA copolymer ranges from about 1:1 to about 20:1. The resulting copolymers are soluble in water and form a free-flowing liquid at room temperature, but form a hydrogel at body temperature. A commercially available PEG-PLGA-PEG triblock copolymer is RESOMER RGP t50106 manufactured by Boehringer Ingelheim. This material is composed of a PLGA copolymer of 50:50 poly(DL-lactide-co-glycolide) and is 10% w/w of PEG and has a molecular weight of about 6000.

[00223] Additional biodegradable thermoplastic polyesters include AtriGel® (provided by Atrix Laboratories, Inc.) and/or those disclosed, e.g., in U.S. Patent Nos. 5,324,519; 4,938,763; 5,702,716; 5,744,153; and 5,990,194; wherein the suitable biodegradable thermoplastic polyester is disclosed as a thermoplastic polymer. Examples of suitable biodegradable thermoplastic polyesters include polylactides, polyglycolides, polycaprolactones, copolymers thereof, terpolymers thereof, and any combinations thereof. In some such embodiments, the suitable biodegradable thermoplastic polyester is a polylactide, a polyglycolide, a copolymer thereof, a terpolymer thereof, or a combination thereof. In one embodiment, the biodegradable thermoplastic polyester is 50/50 poly(DL-lactide-co-glycolide) having a carboxy terminal group; is present in about 30 wt. % to about 40 wt. % of the composition; and has an average molecular weight of about 23,000 to about 45,000. Alternatively, in another embodiment, the

biodegradable thermoplastic polyester is 75/25 poly (DL-lactide-co-glycolide) without a carboxy terminal group; is present in about 40 wt. % to about 50 wt. % of the composition; and has an average molecular weight of about 15,000 to about 24,000. In further or alternative embodiments, the terminal groups of the poly(DL-lactide-co-glycolide) are either hydroxyl, carboxyl, or ester depending upon the method of polymerization. Polycondensation of lactic or glycolic acid provides a polymer with terminal hydroxyl and carboxyl groups. Ring-opening polymerization of the cyclic lactide or glycolide monomers with water, lactic acid, or glycolic acid provides polymers with the same terminal groups. However, ring-opening of the cyclic monomers with a monofunctional alcohol such as methanol, ethanol, or 1-dodecanol provides a polymer with one hydroxyl group and one ester terminal groups. Ring-opening polymerization of the cyclic monomers with a diol such as 1,6-hexanediol or polyethylene glycol provides a polymer with only hydroxyl terminal groups.

[00224] Since the polymer systems of thermosetting gels dissolve more completely at reduced temperatures, methods of solubilization include adding the required amount of polymer to the amount of water to be used at reduced temperatures. Generally after wetting the polymer by shaking, the mixture is capped and placed in a cold chamber or in a thermostatic container at about 0-10 °C in order to dissolve the polymer. The mixture is stirred or shaken to bring about a more rapid dissolution of the thermosetting gel polymer. The ophthalmic agent and various additives such as buffers, salts, and preservatives are subsequently added and dissolved. In some instances the pharmaceutically agent is suspended if it is insoluble in water. The pD is modulated by the addition of appropriate buffering agents.

[00225] Ophthalmic Ointment Muscarinic Antagonist Composition

[00226] An ointment is a homogeneous, viscous, semi-solid preparation, most commonly a greasy, thick oil (e.g. oil 80% - water 20%) with a high viscosity, intended for external application to the skin or mucous membranes. Ointments have a water number that defines the maximum amount of water that it contains. They are used as emollients or for the application of active ingredients to the skin for protective, therapeutic, or prophylactic purposes and where a degree of occlusion is desired. Ointments are used topically on a variety of body surfaces. These include the skin and the mucous membranes of the eye (an eye ointment), vulva, anus, and nose

[00227] The vehicle of an ointment is known as the ointment base. The choice of a base depends upon the clinical indication for the ointment. The different types of ointment bases are: hydrocarbon bases, e.g. hard paraffin, soft paraffin, microcrystalline wax and ceresine; absorption bases, e.g. wool fat, beeswax; water soluble bases, e.g. macrogols 200, 300, 400; emulsifying bases, e.g. emulsifying wax, cetrimide; vegetable oils, e.g. olive oil, coconut oil, sesame oil, almond oil and peanut oil.

[00228] Ointments are formulated using hydrophobic, hydrophilic, or water-emulsifying bases to provide preparations that are immiscible, miscible, or emulsifiable with skin secretions. In some embodiments, they are also derived from hydrocarbon (fatty), absorption, water-removable, or water-soluble bases. The active agents are dispersed in the base, and later they get divided after the drug penetration into the target sites (e.g. membranes, skins, etc.).

[00229] The present disclosure recognizes that it is sometimes difficult to incorporate into the ointment a drug of low concentration with sufficient dose-to-dose uniformity for effectively treating a disorder or disease. In some embodiments, poly(ethylene-glycols), polyethoxylated castor oils (Cremophor®EL), alcohols having 12 to 20 carbon atoms or a mixture of two or more of said components are effective excipients for dispersing and/or dissolving effective amounts of ophthalmic drugs, in particular of ascomycins and staurosporine derivatives, in an ointment base, in particular in an ointment base substantially comprising oleaginous and hydrocarbon components, and that the resulting ointments are excellently tolerated by the skin and by ocular tissue.

[00230] The present disclosure further recognizes that ophthalmic drugs, such as a muscarinic antagonist (e.g. atropine or its pharmaceutically acceptable salts), incorporated in the ointment compositions describes herein target the choroid and/or retina in a patient when the compositions are topically administered to the ocular surface, in particular to the sclera of said patient. In some embodiments, an ophthalmic ointment composition includes an ophthalmic drug, an ointment base and an agent for dispersing and/or dissolving said drug in the ointment base, selected from a poly(ethylene-glycol), a polyethoxylated castor oil, an alcohol having 12 to 20 carbon atoms and a mixture of two or more of said components.

[00231] In some embodiments, the ointment bases include ophthalmically acceptable oil and fat bases, such as natural wax e.g. white and yellow bees wax, carnauba wax, wool wax (wool fat), purified lanolin, anhydrous lanolin; petroleum wax e.g. hard paraffin, microcrystalline wax; hydrocarbons e.g. liquid paraffin, white and yellow soft paraffin, white petrolatum, yellow petrolatum; or combinations thereof.

[00232] The above mentioned oil and fat bases are described in more detail, for instance, in the British Pharmacopoeia, Edition 2001, or the European Pharmacopoeia, 3rd Edition.

[00233] In some embodiments, the ointment base is present in amounts of about 50 to about 95, preferably of 70 to 90% by weight based on the total weight of the composition.

[00234] A preferred ointment base comprises a combination of one or more of one or more natural waxes like those indicated above, preferably wool wax (wool fat), and one or more hydrocarbons

like those indicated above, preferably a soft paraffin or a petrolatum, more preferably in combination with liquid paraffin.

[00235] A special embodiment of the aforementioned ointment base comprises e.g. 5 to 17 parts by weight of wool fat, and 50 to 65 parts by weight of white petrolatum as well as 20 to 30 parts by weight of liquid paraffin.

[00236] In some embodiments, the agent for dispersing and/or dissolving the ophthalmic drug in the ointment base is selected from a poly(ethylene-glycol), a polyethoxylated castor oil, an alcohol having 12 to 20 carbon atoms and a mixture of two or more of said components. The agent is preferably used in amounts of 1 to 20 percent, more preferably 1 to 10 percent by weight of the entire semisolid ophthalmic composition.

[00237] Alcohols having 12 to 20 carbon atoms include particularly stearyl alcohol (C₁₈H₃₇OH), cetyl alcohol (C₁₆H₃₃OH) and mixtures thereof. Preferred are so-called cetostearyl alcohols, mixtures of solid alcohols substantially consisting of stearyl and cetyl alcohol and preferably comprising not less than 40 percent by weight of stearyl alcohol and a sum of stearyl alcohol and cetyl alcohol amounting to at least 90 percent by weight, and compositions comprising not less than 80 percent by weight of cetylstearyl alcohol and an emulsifier, in particular sodium cetostearyl sulfate and/or sodium lauryl sulfate, preferably in amounts not less than 7 percent by weight of emulsifier.

[00238] Polyethoxylated castor oils are reaction products of natural or hydrogenated castor oils and ethylene glycol. In some instances, such products are obtained in known manner, e.g. by reaction of a natural or hydrogenated castor oil or fractions thereof with ethylene oxide, e.g. in a molar ratio of from about 1:30 to about 1:60, with optional removal of free polyethylene glycol components from the product, e.g. in accordance with the methods disclosed in German Auslegeschriften 1,182,388 and 1,518,819. Especially suitable and preferred is a product commercially available under the trade name Cremophor®EL having a molecular weight (by steam osmometry)=ca. 1630, a saponification no.=ca. 65-70, an acid no.=ca. 2, an iodine no.=ca. 28-32 and an nD₂₅=ca.1.471. Also suitable for use in this category is, for instance, Nikkol®HCO-60, a reaction product of hydrogenated castor oil and ethylene oxide exhibiting the following characteristics: acid no.=ca. 0.3; saponification no.=ca. 47.4; hydroxy value=ca. 42.5. pH (5%)=ca. 4.6; Color APHA=ca. 40; m.p.=ca. 36.0° C.; Freezing point=ca. 32.4° C.; H₂O content (% , KF)=ca. 0.03.

[00239] Poly(ethylene-glycols) are used in some embodiments as the agent for dispersing and/or dissolving the ophthalmic drug in the ointment base according to the present disclosure. Suitable poly(ethylene-glycol)s are typically mixtures of polymeric compounds of the general formula H—(OCH₂—CH₂)_nOH, wherein the index n typically range from 4 to 230 and the mean molecular weight

from about 200 to about 10000. Preferably n is a number from about 6 to about 22 and the mean molecular weight between about 300 and about 1000, more preferably n ranges from about 6 to about 13 and the mean molecular weight from about 300 to about 600, most preferably n has a value of about 8.5 to about 9 and the relative molecular weight is about 400. Suitable poly(ethylene-glycols) are readily available commercially, for example poly(ethylene-glycols) having a mean molecular weight of about 200, 300, 400, 600, 1000, 1500, 2000, 3000, 4000, 6000, 8000 and 10000.

[00240] The poly(ethylene-glycols), in particular the preferred types described in the foregoing paragraph, are preferably used in amounts of 1 to 10, more preferably 1 to 5 percent by weight of the entire semisolid ophthalmic composition.

[00241] An especially preferred embodiment of the compositions according to the instant disclosure comprises an agent for dispersing and/or dissolving of the drug in the ointment base which is selected from a poly(ethylene-glycol), a polyethoxylated castor oil and preferably a mixture of said components.

[00242] Gel/Ointment Viscosity

[00243] In some embodiments, the composition has a Brookfield RVDV viscosity of from about 10,000 to about 300,000 cps at about 20°C and sheer rate of $1s^{-1}$. In some embodiments, the composition has a Brookfield RVDV viscosity of from about 15,000 to about 200,000 cps at about 20°C and sheer rate of $1s^{-1}$. In some embodiments, the composition has a Brookfield RVDV viscosity of from about 50,000 to about 150,000 cps at about 20°C and sheer rate of $1s^{-1}$. In some embodiments, the composition has a Brookfield RVDV viscosity of from about 70,000 to about 130,000 cps at about 20°C and sheer rate of $1s^{-1}$. In some embodiments, the composition has a Brookfield RVDV viscosity of from about 90,000 to about 110,000 cps at about 20°C and sheer rate of $1s^{-1}$.

[00244] In some embodiments, the ophthalmic gel formulation contains a viscosity enhancing agent sufficient to provide a viscosity of between about 500 and 1,000,000 centipoise, between about 750 and 1,000,000 centipoise; between about 1000 and 1,000,000 centipoise; between about 1000 and 400,000 centipoise; between about 2000 and 100,000 centipoise; between about 3000 and 50,000 centipoise; between about 4000 and 25,000 centipoise; between about 5000 and 20,000 centipoise; or between about 6000 and 15,000 centipoise. In some embodiments, the ophthalmic gel formulation contains a viscosity enhancing agent sufficient to provide a viscosity of between about 50,000 and 1,000,000 centipoise.

[00245] In some embodiments, the compositions described herein are low viscosity compositions at body temperature. In some embodiments, low viscosity compositions contain from about 1% to about 10% of a viscosity enhancing agent (e.g., gelling components such as polyoxyethylene-polyoxypropylene

copolymers). In some embodiments, low viscosity compositions contain from about 2% to about 10% of a viscosity enhancing agent (e.g., gelling components such as polyoxyethylene-polyoxypropylene copolymers). In some embodiments, low viscosity compositions contain from about 5% to about 10% of a viscosity enhancing agent (e.g., gelling components such as polyoxyethylene-polyoxypropylene copolymers). In some embodiments, low viscosity compositions are substantially free of a viscosity enhancing agent (e.g., gelling components such as polyoxyethylene-polyoxypropylene copolymers). In some embodiments, a low viscosity ophthalmic agent composition described herein provides an apparent viscosity of from about 100 cP to about 10,000 cP. In some embodiments, a low viscosity ophthalmic agent composition described herein provides an apparent viscosity of from about 500 cP to about 10,000 cP. In some embodiments, a low viscosity ophthalmic agent composition described herein provides an apparent viscosity of from about 1000 cP to about 10,000 cP.

[00246] In some embodiments, the compositions described herein are viscous compositions at body temperature. In some embodiments, viscous compositions contain from about 10% to about 25% of a viscosity enhancing agent (e.g., gelling components such as polyoxyethylene-polyoxypropylene copolymers). In some embodiments, the viscous compositions contain from about 14% to about 22% of a viscosity enhancing agent (e.g., gelling components such as polyoxyethylene-polyoxypropylene copolymers). In some embodiments, the viscous compositions contain from about 15% to about 21% of a viscosity enhancing agent (e.g., gelling components such as polyoxyethylene-polyoxypropylene copolymers). In some embodiments, a viscous ophthalmic composition described herein provides an apparent viscosity of from about 100,000 cP to about 1,000,000 cP. In some embodiments, a viscous ophthalmic composition described herein provides an apparent viscosity of from about 150,000 cP to about 500,000 cP. In some embodiments, a viscous ophthalmic composition described herein provides an apparent viscosity of from about 250,000 cP to about 500,000 cP. In some of such embodiments, a viscous ophthalmic composition is a liquid at room temperature and gels at about between room temperature and body temperature (including an individual with a serious fever, e.g., up to about 42 °C). In some embodiments, a viscous ophthalmic composition is administered as monotherapy for treatment of an ophthalmic disease or condition described herein.

[00247] In some embodiments, the viscosity of the gel formulations presented herein is measured by any means described. For example, in some embodiments, an LVDV-II+CP Cone Plate Viscometer and a Cone Spindle CPE-40 is used to calculate the viscosity of the gel formulation described herein. In other embodiments, a Brookfield (spindle and cup) viscometer is used to calculate the viscosity of the gel formulation described herein. In some embodiments, the viscosity ranges referred to herein are measured at room temperature. In other embodiments, the viscosity ranges referred to herein are measured at body temperature (e.g., at the average body temperature of a healthy human).

[00248] Gel/Ointment Dose-To-Dose Uniformity

[00249] Typical ophthalmic gels are packaged in eye drop bottles and administered as drops. For example, a single administration (i.e. a single dose) of an ophthalmic gel includes a single drop, two drops, three drops or more into the eyes of the patient. Furthermore, typical ophthalmic ointments are packaged in tubes or other squeezable containers with a dispensing nozzle through which strips of the ointment are delivered. For example, a single administration (i.e. a single dose) of an ophthalmic ointment includes a single strip, or multiple strips into the eyes of the patient. In some embodiments, one dose of the ophthalmic gel described herein is one drop of the gel composition from the eye drop bottle. In some embodiments, one dose of the ophthalmic ointment is one strip of the ointment composition dispensed through the nozzle of a dispersing tube.

[00250] In some cases, described herein include ophthalmic gel compositions which provide a dose-to-dose uniform concentrations. In some instances, the dose-to-dose uniform concentration does not present significant variations of drug content from one dose to another. In some instances, the dose-to-dose uniform concentration does provide consistent drug content from one dose to another.

[00251] In some cases, described herein include ophthalmic ointment compositions which provide a dose-to-dose uniform concentrations. In some instances, the dose-to-dose uniform concentration does not present significant variations of drug content from one dose to another. In some instances, the dose-to-dose uniform concentration does provide consistent drug content from one dose to another.

[00252] In some embodiments, the composition has a dose-to-dose ophthalmic agent concentration variation of less than 50%. In some embodiments, the composition has a dose-to-dose ophthalmic agent concentration variation of less than 40%. In some embodiments, the composition has a dose-to-dose ophthalmic agent concentration variation of less than 30%. In some embodiments, the composition has a dose-to-dose ophthalmic agent concentration variation of less than 20%. In some embodiments, the composition has a dose-to-dose ophthalmic agent concentration variation of less than 10%. In some embodiments, the composition has a dose-to-dose ophthalmic agent concentration variation of less than 5%.

[00253] In some embodiments, the dose-to-dose ophthalmic agent concentration variation is based on 10 consecutive doses. In some embodiments, the dose-to-dose ophthalmic agent concentration variation is based on 8 consecutive doses. In some embodiments, the dose-to-dose ophthalmic agent concentration variation is based on 5 consecutive doses. In some embodiments, the dose-to-dose ophthalmic agent concentration variation is based on 3 consecutive doses. In some embodiments, the dose-to-dose ophthalmic agent concentration variation is based on 2 consecutive doses.

[00254] A nonsettling formulation should not require shaking to disperse drug uniformly. A “no-shake” formulation is potentially advantageous over formulations that require shaking for the simple reason that patients’ shaking behavior is a major source of variability in the amount of drug dosed. It has been reported that patients often times do not or forget to shake their ophthalmic compositions that requires shaking before administering a dose, despite the instructions to shake that were clearly marked on the label. On the other hand, even for those patients who do shake the product, it is normally not possible to determine whether the shaking is adequate in intensity and/or duration to render the product uniform. In some embodiments, the ophthalmic gel compositions and ophthalmic ointment compositions described herein are “no-shake” formulations that maintained the dose-to-dose uniformity described herein.

[00255] To evaluate the dose-to-dose uniformity, drop bottles or tubes containing the ophthalmic aqueous compositions, the ophthalmic gel compositions, or ophthalmic ointment compositions are stored upright for a minimum of 12 hours prior to the start of the test. To simulate the recommended dosing of these products, predetermined number of drops or strips are dispensed from each commercial bottles or tubes at predetermined time intervals for an extended period of time or until no product was left in the bottle or tube. All drops and strips are dispensed into tared glass vials, capped, and stored at room temperature until analysis. Concentrations of a muscarinic antagonist such as atropine in the expressed drops were determined using a reverse-phase HPLC method.

[00256] **Methods of Treatment**

[00257] Disclosed herein are methods of arresting myopia development by administering to an eye of an individual in need thereof an effective amount of an ophthalmic composition as described above. Also disclosed herein are methods of preventing myopia development by administering to an eye of an individual in need thereof an effective amount of an ophthalmic composition as described above.

[00258] In some embodiments, the ophthalmic aqueous formulations described herein are packaged in eye drop bottles and administered as drops. For example, a single administration (i.e. a single dose) of an ophthalmic aqueous formulation includes a single drop, two drops, three drops or more into the eyes of the patient. In some embodiments, the ophthalmic gel formulations described herein are packaged in eye drop bottles and administered as drops. For example, a single administration (i.e. a single dose) of an ophthalmic gel includes a single drop, two drops, three drops or more into the eyes of the patient. In some embodiments, the ophthalmic ointment formulations described herein are packaged in tubes or other squeezable containers with a dispensing nozzle through which strips of the ointment are delivered. For example, a single administration (i.e. a single dose) of an ophthalmic ointment includes a single strip, or multiple strips into the eyes of the patient. In some embodiments, one dose of the

ophthalmic aqueous formulation described herein is one drop of the aqueous composition from the eye drop bottle. In some embodiments, one dose of the ophthalmic gel described herein is one drop of the gel composition from the eye drop bottle. In some embodiments, one dose of the ophthalmic ointment is one strip of the ointment composition dispensed through the nozzle of a dispensing tube.

[00259] In some embodiments of the disclosed method, the ophthalmic composition is stored below room temperature prior to first use. In some embodiments of the disclosed method, the ophthalmic composition is stored at between about 2 °C to about 10 °C prior to first use. In some embodiments of the disclosed method, the ophthalmic composition is stored at about 2 °C, about 3 °C, about 4 °C, about 5 °C, about 6 °C, about 7 °C, about 8 °C, about 9 °C, or about 10 °C prior to first use. In some embodiments of the disclosed method, the ophthalmic composition is stored at between about 4 °C to about 8 °C prior to first use.

[00260] In some embodiments of the disclosed method, the ophthalmic composition is stored at room temperature after first use. In some embodiments of the disclosed method, the ophthalmic composition is stored at between about 16 °C to about 26 °C after to first use. In some embodiments of the disclosed method, the ophthalmic composition is stored at about 16 °C, about 17 °C, about 18 °C, about 19 °C, about 20 °C, about 21 °C, about 22 °C, about 23 °C, about 24 °C, about 25 °C, or about 26 °C after to first use.

[00261] In some embodiments, the ophthalmic aqueous formulations are administered as follows: the lower lid of the eye to be administered was pulled down and a predetermined amount of the aqueous formulation (e.g. 1-3 drops) is applied to the inside of the eyelid. The ophthalmic tip of the dispensing mechanism does not touch any surface to avoid contamination and/or injury.

[00262] In some embodiments, the ophthalmic gel formulations are administered as follows: the lower lid of the eye to be administered was pulled down and a predetermined amount of gel (e.g. 1-3 drops) is applied to the inside of the eyelid. The ophthalmic tip of the dispensing mechanism does not touch any surface to avoid contamination and/or injury.

[00263] In some embodiments, the ophthalmic ointment formulations are administered as follows: the lower lid of the eye to be administered was pulled down and a small amount of ointment (approximately 0.25 inches) was applied to the inside of the eyelid. The ophthalmic tip of the dispensing mechanism does not touch any surface to avoid contamination and/or injury.

[00264] In some embodiments, the ophthalmic composition is administered at predetermined time intervals over an extended period of time. In some embodiments, the ophthalmic composition is administered once every day. In some embodiments, the ophthalmic composition is administered every other day. In some embodiments, the ophthalmic composition is administered over 1 week, 2 weeks, 1

month, 2 months, 3 months, 6 months, 1 year, 2 years, 3 years, 4 years, 5 years, 6 years, 7 years, 8 years, 9 years, 10 years, 11 years, or 12-15 years.

[00265] In some embodiments, the ophthalmic composition is administered in doses having a dose-to-dose ophthalmic agent concentration variation of less than 50%, less than 40%, less than 30%, less than 20%, less than 10%, or less than 5%.

[00266] The number of times a composition is administered to an individual in need thereof depends on the discretion of a medical professional, the disorder, the severity of the disorder, and the individual's response to the formulation. In some embodiments, a composition disclosed herein is administered once to an individual in need thereof with a mild acute condition. In some embodiments, a composition disclosed herein is administered more than once to an individual in need thereof with a moderate or severe acute condition. In the case wherein the patient's condition does not improve, upon the doctor's discretion the administration of an ophthalmic agent is administered chronically, that is, for an extended period of time, including throughout the duration of the patient's life in order to ameliorate or otherwise control or limit the symptoms of the patient's disease or condition.

[00267] In the case wherein the patient's condition does not improve, upon the doctor's discretion the administration of the ophthalmic agent is administered chronically, that is, for an extended period of time, including throughout the duration of the patient's life in order to ameliorate or otherwise control or limit the symptoms of the patient's disease or condition.

[00268] In the case wherein the patient's status does improve, upon the doctor's discretion the administration of the ophthalmic agent is given continuously; alternatively, the dose of drug being administered is temporarily reduced or temporarily suspended for a certain length of time (*i.e.*, a "drug holiday"). The length of the drug holiday varies between 2 days and 1 year, including by way of example only, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 10 days, 12 days, 15 days, 20 days, 28 days, 35 days, 50 days, 70 days, 100 days, 120 days, 150 days, 180 days, 200 days, 250 days, 280 days, 300 days, 320 days, 350 days, and 365 days. The dose reduction during a drug holiday is from 10%-100%, including by way of example only 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, and 100%.

[00269] Once improvement of the patient's ophthalmic conditions has occurred, a maintenance ophthalmic agent dose is administered if necessary. Subsequently, the dosage or the frequency of administration, or both, is optionally reduced, as a function of the symptoms, to a level at which the improved disease, disorder or condition is retained. In certain embodiments, patients require intermittent treatment on a long-term basis upon any recurrence of symptoms.

[00270] The amount of ophthalmic agent that will correspond to such an amount will vary depending upon factors such as the particular compound, disease condition and its severity, according to the particular circumstances surrounding the case, including, *e.g.*, the specific ophthalmic agent being administered, the route of administration, the condition being treated, the target area being treated, and the subject or host being treated. The desired dose is presented in a single dose or as divided doses administered simultaneously (or over a short period of time) or at appropriate intervals.

[00271] In some embodiments, the initial administration is a particular ophthalmic agent and the subsequent administration a different formulation or ophthalmic agent.

[00272] **Kits/Articles of Manufacture**

[00273] The disclosure also provides kits for preventing or arresting myopia development. Such kits generally will comprise one or more of the ophthalmic compositions disclosed herein, and instructions for using the kit. The disclosure also contemplates the use of one or more of the ophthalmic compositions, in the manufacture of medicaments for treating, abating, reducing, or ameliorating the symptoms of a disease, dysfunction, or disorder in a mammal, such as a human that has, is suspected of having, or at risk for developing myopia.

[00274] In some embodiments, kits include a carrier, package, or container that is compartmentalized to receive one or more containers such as vials, tubes, and the like, each of the container(s) including one of the separate elements to be used in a method described herein. Suitable containers include, for example, bottles, vials, syringes, and test tubes. In other embodiments, the containers are formed from a variety of materials such as glass or plastic.

[00275] The articles of manufacture provided herein contain packaging materials. Packaging materials for use in packaging pharmaceutical products are also presented herein. See, *e.g.*, U.S. Patent Nos. 5,323,907, 5,052,558 and 5,033,252. Examples of pharmaceutical packaging materials include, but are not limited to, drop bottles, tubes, pumps, bags, vials, containers, syringes, bottles, and any packaging material suitable for a selected formulation and intended mode of administration and treatment. A wide array of ophthalmic compositions provided herein are contemplated as are a variety of treatments for any disease, disorder, or condition that benefits by controlled release administration of an ophthalmic agent to the eye.

[00276] In some embodiments, a kit includes one or more additional containers, each with one or more of various materials (such as rinses, wipes, and/or devices) desirable from a commercial and user standpoint for use of a formulation described herein. Such materials also include labels listing contents and/or instructions for use and package inserts with instructions for use. A set of instructions is optionally included. In a further embodiment, a label is on or associated with the container. In yet a further

embodiment, a label is on a container when letters, numbers or other characters forming the label are attached, molded or etched into the container itself; a label is associated with a container when it is present within a receptacle or carrier that also holds the container, e.g., as a package insert. In other embodiments a label is used to indicate that the contents are to be used for a specific therapeutic application. In yet another embodiment, a label also indicates directions for use of the contents, such as in the methods described herein.

[00277] In certain embodiments, the ophthalmic compositions are presented in a dispenser device which contains one or more unit dosage forms containing a compound provided herein. In a further embodiment, the dispenser device is accompanied by instructions for administration. In yet a further embodiment, the dispenser is also accompanied with a notice associated with the container in form prescribed by a governmental agency regulating the manufacture, use, or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the drug for human or veterinary administration. In another embodiment, such notice, for example, is the labeling approved by the U.S. Food and Drug Administration for prescription drugs, or the approved product insert. In yet another embodiment, compositions containing a compound provided herein formulated in a compatible pharmaceutical carrier are also prepared, placed in an appropriate container, and labeled for treatment of an indicated condition.

EXAMPLES

Example 1 – Ophthalmic Formulations

[00278] Exemplary compositions for preparation of ophthalmic formulations are described in Tables 1-8.

Table 1 —Aqueous Solution Formulation (Atropine)

Ingredient	Quantity (mg/g)	Concentration (wt%)
Atropine	0.01-0.5	0.001-0.05 (wt%)
Buffer agent and/or pD adjusting agent (e.g. , borates and/or DCl)	-	q.s. for pD=4.2-7.9
Preservative (e.g. benzalkonium chloride, cetrimonium sodium perborate, etc.)	-	q.s. to prevent the growth of or to destroy microorganism introduced into the solution
Tonicity and/or Osmolarity adjustor (e.g. NaCl, mannitol, etc)	-	q.s. to 0.5-2.0 wt%
Deuterated Water	-	q.s. to 100 wt%

Table 2 —Aqueous Solution Formulation (Atropine Sulfate)

Ingredient	Quantity (mg/g)	Concentration (wt%)
Atropine sulfate	0.01-0.5	0.001-0.05 (wt%)
Buffer agent and/or pD adjusting agent (e.g. , borates and/or DCl)	-	q.s. for pD=4.2-7.9
Preservative (e.g. benzalkonium chloride, cetrimonium sodium perborate, etc.)	-	q.s. to prevent the growth of or to destroy microorganism introduced into the solution
Tonicity and/or Osmolarity adjustor (e.g. NaCl, mannitol, etc)	-	q.s. to 0.5-2.0 wt%
Deuterated Water	-	q.s. to 100 wt%

Table 3 —Aqueous Solution Formulation (Atropine Sulfate)

Ingredient	Quantity (mg/g)	Concentration (wt%)
Atropine sulfate	0.05-0.15	0.005-0.015 (wt%)
Buffer agent and/or pD adjusting agent (e.g. , borates and/or DCl)	-	q.s. for pD=4.2-7.9
Preservative (e.g. benzalkonium chloride, cetrimonium sodium perborate, etc.)	-	q.s. to prevent the growth of or to destroy microorganism introduced into the solution
Tonicity and/or Osmolarity adjustor (e.g. NaCl, mannitol, etc)	-	q.s. to 0.5-2.0 wt%
Deuterated Water	-	q.s. to 100 wt%

Table 4 —Mucus Penetrating Particle Formulation (Atropine)

Ingredient	Quantity (mg/g)	Concentration (wt%)
Atropine	0.01-0.5	0.001-0.05 (wt%)

Buffer agent and/or pD adjusting agent (e.g. , borates and/or DCl)	-	q.s. for pD=4.2-7.9
Preservative (e.g. benzalkonium chloride, cetrimonium sodium perborate, etc.)	-	q.s. to prevent the growth of or to destroy microorganism introduced into the solution
Mucus penetrating particles	-	q.s. to formulate atropine at 0.001-0.05 wt%
Deuterated Water	-	q.s. to 100 wt%

Table 5 —Mucus Penetrating Particle Formulation (Atropine Sulfate)

Ingredient	Quantity (mg/g)	Concentration (wt%)
Atropine sulfate	0.01-0.5	0.001-0.05 (wt%)
Buffer agent and/or pD adjusting agent (e.g. , borates and/or DCl)	-	q.s. for pD=4.2-7.9
Preservative (e.g. benzalkonium chloride, cetrimonium sodium perborate, etc.)	-	q.s. to prevent the growth of or to destroy microorganism introduced into the solution
Mucus penetrating particles	-	q.s. to formulate atropine at 0.001-0.05 wt%
Deuterated Water	-	q.s. to 100 wt%

Table 6 —Cellulose Gel Formulation (Atropine Sulfate)

Ingredient	Quantity (mg/g)	Concentration (wt%)
Atropine Sulfate	0.01-0.5	0.001-0.05 (wt%)
Viscosity enhancing agent (e.g. hydroxypropyl methylcellulose)	10-50	1-5 (wt%)
Buffer agent and/or pD adjusting agent (e.g. , sodium acetate and/or DCl)	-	q.s. for pD=4.2-7.9
Stabilizer (e.g. EDTA, cyclodextrin, etc.)	-	q.s. for low degradation of atropine sulfate (e.g. less than

		10%, 5% or 1% degradation)
Osmolarity modifier (e.g. NaCl)	-	q.s. 150-500 mOsm/L
Deuterated Water	-	q.s. to 100 wt%

Table 7 —Thermosetting Gel Formulation (Atropine Sulfate)

Ingredient	Quantity (mg/g)	Concentration (wt%)
Atropine sulfate	0.01-0.5	0.001-0.05 (wt%)
Viscosity enhancing agent (e.g. poloxamer 407)	100-250	10-25 (wt%)
Buffer agent and/or pD adjusting agent (e.g. , sodium acetate and/or DCl)	-	q.s. for pH=4.2-7.9
Stabilizer (e.g. EDTA, cyclodextrin, etc.)	-	q.s. for low degradation of atropine sulfate (e.g. less than 10%, 5% or 1% degradation)
Osmolarity modifier (e.g. NaCl)	-	q.s. 150-500 mOsm/L
Deuterated Water	-	q.s. to 100 wt%

Table 8 — Ointment Formulation (Atropine Sulfate)

Ingredient	Quantity (g) for 1000 mL solution	Concentration in 1000 mL aqueous solution
Atropine sulfate	0.01-0.5	0.001-0.05 (wt%)
Dispersing agent (e.g. polyethyleneglycol, and/or polyethoxylated castor oil and/or C12-C20 alcohol	10-200	1-20 (wt%)
Buffering agent pD adjusting agent (e.g. DCl)	-	q.s. for pD=4.2-7.9
Stabilizer (e.g. EDTA, cyclodextrin, etc.)	-	q.s. for low degradation of atropine sulfate (e.g. less than 10%, 5% or 1% degradation)
Osmolarity modifier (e.g. NaCl)	-	q.s. 150-500 mOsm/L

Ointment base (e.g. wool wax and/or petrolatum and/or liquid paraffin)		q.s. to 100 wt%

Example 2 - Preparation of an Aqueous Solution Formulation Containing 0.01% Atropine in D₂O

[00279] Stock 1% Solution

[00280] In a 100mL solution, 1 gram of atropine, and 0.77 g of NaCl (and other ingredients/components preferably in their dry state) are added along with a quantity sufficient to equal 100mL sterile deuterated water for injection. The solution is mixed in an appropriately sized beaker with a stir bar on a hot plate until all of the solid powders have dissolved and the solution has become clear with no visible particles. Next, the stir bar is removed, and the solution is poured into a filter bottle and vacuum filtered through a 0.22 micron pothyethersulfone membrane filter into a sterile bottle. The filter top is removed from the sterile stock bottle and the stock bottle is capped for storage with a sterile bottle cap.

[00281] Diluted 0.01% Solution

[00282] 0.3mL of the 1% solution was combined with a quantity sufficient to achieve 30mL total of sterile 0.9% Sodium Chloride For Injection USP. The solution was thoroughly mixed. The pH of the solution was recorded. A 0.22 micron filter was placed on the tip of the syringe and the solution was aliquotted into separate sterile containers.

Example 3 - Preparation of an Aqueous Solution Formulation Containing 0.01% Atropine Sulfate

[00283] Stock 1% Solution

[00284] In a 100mL solution, 1 gram of atropine sulfate, and 0.77 g of NaCl (and other ingredients/components preferably in their dry state) were added along with a quantity sufficient to equal 100mL sterile water for injection. The solution was mixed in an appropriately sized beaker with a stir bar on a hot plate until all of the solid powders had dissolved and the solution became clear with no visible particles. Next, the stir bar was removed, and the solution was poured into a filter bottle and vacuum filtered through a 0.22 micron pothyethersulfone membrane filter into a sterile bottle. The filter top was removed from the sterile stock bottle and the stock bottle was capped for storage with a sterile bottle cap.

[00285] Diluted 0.01% Solution

[00286] 0.3mL of the 1% solution was combined with a quantity sufficient to achieve 30mL total of sterile 0.9% Sodium Chloride For Injection USP. The solution was thoroughly mixed. The pH of the solution was recorded. A 0.22 micron filter was placed on the tip of the syringe and the solution was aliquotted into separate sterile containers.

Example 4 – Stability Analysis

[00287] Five 0.01% atropine sulfate solutions were prepared from the 1% atropine sulfate stock solution (preparation as described in Example 2). The pH of the five solutions was 5.87, 5.97, 5.90, 6.24, and 6.16 for solutions 1-5, respectively. Each solution was thoroughly mixed. A 0.22 micron filter was placed on the tip of the syringe and the solution was aliquotted into separate sterile containers according to Table 9.

Table 9. Container Filling Outline

Type of Container	Volume of 0.01% Atropine Sulfate Drug Product in Container	Total Containers Filled
Sterile Eyedroppers	5-mL	12
Sterile Glass Vials	5-mL	12

[00288] The samples were then stored at different conditions for stability analysis. The samples were analyzed at different time points up to 2 months. The storage conditions include: 40°C with 75% relative humidity (RH) (samples were transferred from 2-8°C condition after 3 days), 25°C with 60% RH, and 60°C. The time points were 1 week, 2 weeks, 1 month, and 2 months. At each of the time point, one plastic eyedropper (LDPE plastic) and one glass vial from each of the stored condition were removed and allowed to equilibrate to ambient conditions. Once equilibrated, both the plastic eyedropper and the glass vials were inverted 3 times. The solution in the eyedroppers was transferred to an HPLC vial in a drop wise fashion through the dropper. The solution in the glass vial was aliquotted into an HPLC vial using a glass Pasteur pipette. The samples were then tested for purity and potency using the UPLC method listed in Table 10.

Table 10. UPLC Method Parameters

Parameter	Condition
Column	EMD, Hiber HR PurospherSTAR C-18, 100 x 2.1 mm, 2 μ m
Mobile Phase/Diluent	87:13, 50 mM Potassium Phosphate: Acetonitrile, pH 3.5
Flow	Isocratic
Flow Rate	0.5 mL/min
Detection Wavelength	210 nm
Column Temperature	30 $\pm$ 3 °C
Autosampler Temperature	5 $\pm$ 3 °C
Run Time	6.0 minutes
Injection Volume	10 μ L*
Needle Wash Solution	90/10 Water: Acetonitrile

* Modified from original method to maintain sensitivity at 100 μ g/mL nominal.

[00289] Table 11 lists the stability data for the 0.01% atropine sulfate solutions.

Table 11. Stability Data for 0.01% Atropine Sulfate Solutions

Analyst	Container Type	Storage Condition	t=0			t=1 week		t=2 week ¹		t=1 month ²			t=2 month ³		
			Purity	Potency	pH	Purity	Potency	Purity	Potency	Purity	Potency	pH	Purity	Potency	pH
1	Eyedropper, LDPE (Plastic)	25 °C/60% RH	99.5	99.8	5.9	ND	ND	99.1	99.9	ND	ND	ND	95.4	97.4	6.3
		40 °C/75% RH				ND	ND	96.2	97.3	95.1	95.6	5.2	ND	ND	ND
		60 °C				80.8	83.3	86.2	88.6	88.3	91.5	4.2	ND	ND	ND
	Glass Vial	25 °C/60% RH	99.8	100.4	ND	ND	ND	92.2	93.1	80.7	80.5	7.8	73.0	74.5	7.3
		40 °C/75% RH				ND	ND	73.6	74.1	50.1	50.2	7.4	ND	ND	ND
		60 °C				43.1	43.9	28.3	28.4	ND	ND	ND	ND	ND	ND
2	Eyedropper, LDPE (Plastic)	25 °C/60% RH	99.7	99.9	6.0	ND	ND	99.1	99.6	ND	ND	ND	97.0	99.1	6.1
		40 °C/75% RH				ND	ND	96.6	97.2	95.5	95.8	5.6	ND	ND	ND
		60 °C				89.4	92.2	92.2	94.0	90.6	94.4	4.1	ND	ND	ND
	Glass Vial	25 °C/60% RH	99.8	100.2	ND	ND	ND	92.6	92.9	82.5	82.2	7.6	80.2	81.6	7.3
		40 °C/75% RH				ND	ND	74.7	75.1	59.1	59.0	7.2	ND	ND	ND
		60 °C				54.2	55.2	37.3	37.4	ND	ND	ND	ND	ND	ND
3	Eyedropper, LDPE (Plastic)	25 °C/60% RH	99.3	96.3	5.9	ND	ND	98.7	96.1	ND	ND	ND	95.8	94.8	6.3
		40 °C/75% RH				ND	ND	96.7	93.1	94.8	91.8	5.5	ND	ND	ND
		60 °C				88.8	89.0	88.0	86.8	88.6	87.7	4.1	ND	ND	ND
	Glass Vial	25 °C/60% RH	99.4	98.4	ND	ND	ND	94.1	91.2	85.0	81.9	7.5	79.3	78.3	7.3
		40 °C/75% RH				ND	ND	72.2	74.6	61.3	63.0	7.2	ND	ND	ND
		60 °C				48.6	51.1	34.1	34.9	ND	ND	ND	ND	ND	ND
4	Eyedropper, LDPE (Plastic)	25 °C/60% RH	99.8	99.6	6.2	ND	ND	99.1	98.8	ND	ND	ND	96.4	97.6	6.3
		40 °C/75% RH				ND	ND	96.3	97.0	94.5	94.2	5.6	ND	ND	ND
		60 °C				90.5	93.0	89.3	90.6	84.2	85.8	4.2	ND	ND	ND
	Glass Vial	25 °C/60% RH	99.8	98.8	ND	ND	ND	90.7	90.0	76.9	75.1	7.6	72.5	71.6	7.4
		40 °C/75% RH				ND	ND	71.0	68.7	57.0	56.7	7.2	ND	ND	ND
		60 °C				52.4	52.1	29.7	28.6	ND	ND	ND	ND	ND	ND
5	Eyedropper, LDPE (Plastic)	25 °C/60% RH	99.6	100.5	6.2	ND	ND	99.3	100.4	ND	ND	ND	97.8	100.5	6.2
		40 °C/75% RH				ND	ND	95.9	96.7	96.8	97.6	5.5	ND	ND	ND
		60 °C				91.2	94.6	91.4	93.6	90.3	92.8	4.2	ND	ND	ND
	Glass Vial	25 °C/60% RH	99.8	100.7	ND	ND	ND	90.5	91.3	79.3	79.7	7.8	72.8	74.6	7.3
		40 °C/75% RH				ND	ND	71.3	71.9	56.0	56.4	7.3	ND	ND	ND
		60 °C				46.3	47.4	29.5	29.6	ND	ND	ND	ND	ND	ND

¹ The 25 °C and the 60 °C samples were pulled at 15 days, the 40 °C samples were pulled at 11 days.

² The 25 °C and the 60 °C samples were pulled at 28 days, the 40 °C samples were pulled at 24 days.

³ The 25 °C and the 60 °C samples were pulled at 46 days.

[00290] A change in the pH of the 0.01% Atropine Sulfate solutions was observed over the course of the stability study. The plastic (LDPE) eyedroppers maintained pH around 6.2 when stored at 25 °C for 2 months. However at the same time point, the pH of the 0.01% atropine has increased to 7.2 when stored in glass vials. Additionally, when stored at elevated temperatures (e.g. 40°C and 60°C), the pH in the plastic (LDPE) eyedroppers dropped to approximately 4-5, while the pH maintained around 7.2 when stored in the glass vials.

[00291] There was also a significant difference in the rate of degradation for Atropine Sulfate (0.01%) when stored in plastic (LDPE) eyedroppers versus Type I glass vials. However, in both containers there was an increase of an early eluting related substance at relative retention time (RRT) =0.87-0.89. In some cases, this early eluting related substance is referred to as primary degradant. In some

instances, the primary degradant is referred to as RRT 0.87-0.89. This related substance is likely to be the first parameter to fail specification regardless of the container. The amount of this related substance was tracked at each time point and is listed in Table 12.

Table 12. Area (%) of the Main Degradation Species for 0.01% Atropine Sulfate (RRT 0.87-0.89)

Analyst	Temperature °C	t=0	t=1 week	t=2 week	t=1 month	t=2 months
1	25	0.08	NA	0.92	NA	3.98
	40	NA	NA	3.74	4.78	NA
	60	NA	17.78	13.49	11.51	NA
2	25	0.07	NA	0.88	NA	2.46
	40	NA	NA	3.26	4.37	NA
	60	NA	9.38	7.67	9.13	NA
3	25	0.07	NA	1.05	NA	2.88
	40	NA	NA	2.98	4.85	NA
	60	NA	9.59	11.57	10.55	NA
4	25	0.08	NA	0.92	NA	3.09
	40	NA	NA	3.43	5.32	NA
	60	NA	8.30	10.46	15.49	NA
5	25	0.08	NA	0.64	NA	1.66
	40	NA	NA	3.96	3.07	NA
	60	NA	7.61	8.35	9.7	NA
Average 25 °C		0.08	NA	0.88	NA	2.81
Average 40 °C		NA	NA	3.47	4.48	NA
Average 60 °C		NA	10.53	10.31	11.28	NA

[00292] Arrhenius based shelf life predictions were calculated using the related substance data from Table 12. These predictions are based on an assumption that the degradation is first order (linear). These predictions are illustrated in Figs. 1 and 2. Fig. 1 shows the shelf life prediction of 0.01% atropine sulfate solution with a primary degradant RRT 0.87-0.89, and a n.m.t. of 0.5% area, based on data obtained from samples stored at 25°C and 40°C. The pH range of the atropine sulfate solution is from 5.9-6.2. Fig. 2 shows the shelf life prediction of 0.01% atropine sulfate solution with a primary degradant RRT 0.87-0.89, and a n.m.t. of 0.5% area, based on data obtained from samples stored at 25°C and 60°C. The pH range of the atropine sulfate solution is from 5.9-6.2.

Example 5 – 1% Atropine Sulfate (Bausch + Lomb) Sample Analysis

[00293] The 1% atropine sulfate sample was obtained from Bausch + Lomb (Lot 198421). For comparison the pH of the 1% Atropine Sulfate drug product was determined in the neat solution as well as a sample that was diluted to the current nominal concentration (0.01% Atropine Sulfate) using the vehicle. Additionally a sample was diluted to the nominal concentration with method diluent. Both samples diluted to the nominal concentration were analyzed using the RP-UPLC method (Table 10). The results are listed in Table 13.

Table 13. pH and Purity of the Bausch + Lomb Atropine Sulfate Sample

Sample	pH	Purity (% area)
1% Atropine Sulfate	4.89	ND
0.01% Atropine Sulfate, <i>diluted with Vehicle</i>	6.16	99.6%
0.01% Atropine Sulfate, <i>diluted with Diluent</i>	ND	99.6%
Vehicle	7.94	ND

ND= not determined

Example 6 – Dose Uniformity (10-Dose)

[00294] To evaluate the dose-to-dose uniformity, drop bottles containing the ophthalmic aqueous composition are stored upright for a predetermined period of time (e.g. 12 hours) prior to the start of the test. To simulate the recommended dosing of the product, 10 drops of the aqueous composition are dispensed from each bottle at predetermined time intervals (e.g. consecutively, every 1 minute, every 10 minutes, every hour or every 24 hours). All drops are dispensed into tared glass vials, capped, and stored at room temperature until analysis. Concentrations of atropine in the expressed drops are determined using a reverse-phase HPLC method.

Example 7 – Dose Uniformity (5-Dose)

[00295] To evaluate the dose-to-dose uniformity, drop bottles containing the ophthalmic aqueous composition are stored upright for a predetermined period of time (e.g. 12 hours) prior to the start of the test. To simulate the recommended dosing of the product, 5 drops of the aqueous composition are dispensed from each bottle at predetermined time intervals (e.g. consecutively, every 1 minute, every 10 minutes, every hour or every 24 hours). All drops are dispensed into tared glass vials, capped, and stored at room temperature until analysis. Concentrations of atropine in the expressed drops are determined using a reverse-phase HPLC method.

Example 8 – Dose Uniformity (2-Dose)

[00296] To evaluate the dose-to-dose uniformity, drop bottles containing the ophthalmic aqueous composition are stored upright for a predetermined period of time (e.g. 12 hours) prior to the start of the test. To simulate the recommended dosing of the product, 2 drops of the aqueous composition are dispensed from each bottle at predetermined time intervals (e.g. consecutively, every 1 minute, every 10

minutes, every hour or every 24 hours). All drops are dispensed into tared glass vials, capped, and stored at room temperature until analysis. Concentrations of atropine in the expressed drops are determined using a reverse-phase HPLC method.

Example 9 – Formulation Stability Comparison

[00297] Atropine sulfate monohydrate (MP Bio; Lot Number 7825K) and tropic acid (Sigma Aldrich; Lot Number STBD6457V) were used for this experiment. Eight formulations illustrated in Table 14A were analyzed at $t=0$, 2 weeks, and 4 weeks. A RP-HPLC method was used to carry out the analysis.

Table 14A. Atropine sulfate formulations

Formulation	Atropine Sulfate Monohydrate	Benzalkonium Chloride (BAK)	Sodium Chloride	Acetic Acid	Citric Acid	pH/pD	Aqueous
1	0.010	0.01	0.90	0.01	-	4.2	SWFI
2	0.025	0.01	0.90	0.01	-	4.2	SWFI
3	0.010	0.01	0.90	0.01	-	4.8	SWFI
4	0.025	0.01	0.90	0.01	-	4.8	SWFI
5	0.010	0.01	0.90	-	0.04	5.8	SWFI
6	0.025	0.01	0.90	-	0.04	5.8	SWFI
7	0.010	0.01	0.90	0.01	-	5.2 (pD)	D ₂ O
8	0.010	0.01	0.90	-	0.04	6.2 (pD)	D ₂ O

[00298] The values are % w/v. The formulations were prepared at 100mL scale in volumetric glassware. The pD of Formulation 7 and Formulation 8 are 5.2 and 6.2, respectively. In some instances, the pD is calculated as $pD = 0.4 + pH^*$, in which pH^* is the measured or observed pH of the solution formulated in a solution containing deuterated water.

[00299] Table 14B illustrates analysis time points for the formulations listed in Table 14A.

Table 14B. Schedule for atropine sulfate formulation testing

Storage Condition (Horizontal)	Time Point		
	Initial (t=0)	2 Week	4 Week

25 °C/60%RH	X	X	X
40 °C/75%RH		X	X
60 °C		X	X

[00300] Table 15 illustrates the atropine sulfate purity data associated with each of the eight formulations. Purity is indicated as percentage of area under the curve.

Table 15. Atropine sulfate purity as Area-%

Solvent	Condition	t=0	t=2 weeks	t=4 weeks ¹
Formulation 1 pH 4.2	25/60	97.39	97.76	98.20
	40/75		97.25	97.04
	60 °C		94.98	93.87
Formulation 2 pH 4.2	25/60	98.85	99.03	99.08
	40/75		98.50	98.32
	60 °C		97.47	96.65
Formulation 3 pH 4.8	25/60	98.16	98.16	98.45
	40/75		97.98	97.35
	60 °C		95.94	94.65
Formulation 4 pH 4.8	25/60	98.81	98.75	98.46
	40/75		98.26	98.01
	60 °C		96.22	94.04
Formulation 5 pH 5.8	25/60	98.16	97.92	97.54
	40/75		95.88	93.51
	60 °C		80.94	66.83
Formulation 6 pH 5.8	25/60	99.08	98.91	98.46
	40/75		97.65	96.20
	60 °C		89.15	80.68
Formulation 7 pD 5.2	25/60	98.93	99.07	98.39
	40/75		98.51	97.55
	60 °C		96.70	94.01
Formulation 8 pD 6.2	25/60	98.93	98.95	98.51
	40/75		98.53	97.44
	60 °C		95.97	92.72

¹Some chromatographic interference were observed to occur late in the run (~27-32 minutes) for many of the t=4 week stability samples and in some instances is proposed to be system related.

[00301] After four weeks of storage at 60 °C, in some instances the atropine sulfate concentration have an impact on the stability for the formulations containing acetic acid at pH 4.2. For example, atropine sulfate concentration at 0.025 % w/v (Formulation 2) showed a 2.8% increase in % purity at pH

4.2 compared to the atropine sulfate concentration at 0.010 % w/v (Formulation 1). This trend was not observed for the acetic acid formulations at pH 4.8 (Formulations 3 and 4); rather a 0.6% decrease in % purity was observed for the higher doses.

[00302] The dose dependent stability trend that was observed at pH=4.2 was also seen in the formulations containing citric acid at pH 5.8 (Formulations 5 and 6). After four weeks of storage at 60 °C there is approximately 14% less degradation in the higher doses than observed in the lower dose.

[00303] At both the high and the low doses, more degradation is observed in the formulations that start at a higher pH. This degradation is predominantly the growth of tropic acid. In some instances, buffer species plays a role in the observed degradation between the different pH values.

[00304] The percentage of tropic acid observed for each of the formulations at t=4 weeks and at 60°C are as follow:

[00305] Formulation 1- Tropic acid observed is 0.54%.

[00306] Formulation 2-Tropic acid observed is 0.93%.

[00307] Formulation 3-Tropic acid observed is 1.58%.

[00308] Formulation 4-Tropic acid observed is 3.03%.

[00309] Formulation 5-Tropic acid observed is 29.13%.

[00310] Formulation 6-Tropic acid observed is 16.84%.

[00311] Formulation 7-Tropic acid observed is 1.07%.

[00312] Formulation 8-Tropic acid observed is 4.03%.

[00313] In some embodiments, switching the water source to deuterated water (D₂O) has an impact on stabilizing the growth of the tropic acid peak for the formulation containing acetic acid at pH 5.2 (Formulation 7), see Fig. 4. In addition, in the formulation containing citric acid at pH 6.2 (Formulation 8), the deuterated water also stabilizes atropine sulfate, see Fig. 5.

[00314] Table 16 illustrates tropic acid as area under the curve for each of the eight formulations. Tropic acid is a degradant of atropine sulfate. In some instances, LOQ was previously found to be 0.05% for the RP-HPLC method.

Table 16. Tropic acid as area-%

Solvent	Condition	t=0	t=2 weeks	t=4 weeks
Formulation 1 pH 4.2	25/60	<LOQ	0.08	<LOQ
	40/75		0.10	0.10
	60 °C		0.37	0.51
Formulation 2 pH 4.2	25/60	<LOQ	0.05	<LOQ
	40/75		0.11	0.12
	60 °C		0.46	0.93

Formulation 3 pH 4.8	25/60	<LOQ	0.12	0.05
	40/75		0.19	0.27
	60 °C		0.90	1.58
Formulation 4 pH 4.8	25/60	<LOQ	0.10	0.13
	40/75		0.31	0.53
	60 °C		1.84	3.03
Formulation 5 pH 5.8	25/60	<LOQ	0.40	0.71
	40/75		2.22	4.35
	60 °C		16.62	29.13
Formulation 6 pH 5.8	25/60	<LOQ	0.24	0.42
	40/75		1.30	2.44
	60 °C		9.32	16.84
Formulation 7 pD 5.2	25/60	<LOQ	0.07	0.08
	40/75		0.14	0.24
	60 °C		0.71	1.07
Formulation 8 pD 6.2	25/60	<LOQ	0.11	0.14
	40/75		0.33	0.65
	60 °C		2.32	4.03

[00315] Table 17 illustrates percentage of potency of atropine in the eight formulations.

Table 17. % Potency

Solvent	Condition	t=0	t=2 weeks	t=4 weeks
Formulation 1 pH 4.2	25/60	109.4	110.3	112.8
	40/75		111.0	112.4
	60 °C		112.8	114.8
Formulation 2 pH 4.2	25/60	102.9	107.1	109.7
	40/75		108.4	109.6
	60 °C		109.4	111.0
Formulation 3 pH 4.8	25/60	106.3	108.0	109.6
	40/75		108.1	110.0
	60 °C		108.0	109.9
Formulation 4 pH 4.8	25/60	102.5	107.9	109.2
	40/75		107.4	108.9
	60 °C		107.9	108.8
Formulation 5 pH 5.8	25/60	105.0	105.9	107.1
	40/75		103.8	103.5
	60 °C		90.2	77.7
Formulation 6	25/60	107.2	107.1	109.1

pH 5.8	40/75		106.8	107.1
	60 °C		99.0	93.7
Formulation 7 pD 5.2	25/60	107.3	111.3	112.9
	40/75		111.6	113.5
	60 °C		111.8	113.5
Formulation 8 pD 6.2	25/60	99.0	103.0	105.0
	40/75		104.9	104.7
	60 °C		101.6	103.0

[00316] After 4 weeks of storage, the observed potency values were elevated from the t=0 and 2 week time points, with the exception of Formulations 5 and 6 at 60 °C where the potencies dropped due to degradation. In some instances, these potency values are within the error of the HPLC method, but appear to be trending upward. Mass balance was calculated for the 60 °C data and results were consistent across the formulations and levels of degradation, although skewed lower due to the higher than anticipated potency values at 4 weeks, see Figure 3.

[00317] Table 18 illustrates pH or pD stability of the eight formulations.

Table 18. pH/pD Stability

Solvent	Condition	t=0	t=2 weeks	t=4 weeks
Formulation 1 (pH)	25/60	4.21	3.93	4.02
	40/75		3.86	3.96
	60 °C		3.71	3.86
Formulation 2 (pH)	25/60	4.26	4.11	4.25
	40/75		4.04	4.17
	60 °C		3.93	4.10
Formulation 3 (pH)	25/60	4.85	4.44	4.61
	40/75		4.41	4.54
	60 °C		4.32	4.40
Formulation 4 (pH)	25/60	4.98	4.93	5.05
	40/75		4.89	4.98
	60 °C		4.77	4.77
Formulation 5 (pH)	25/60	5.87	5.93	6.03
	40/75		5.96	5.96
	60 °C		5.82	5.78
Formulation 6 (pH)	25/60	5.80	5.69	5.77
	40/75		5.65	5.67
	60 °C		5.54	5.50
Formulation 7	25/60	5.31	5.10	5.24

(pD)	40/75		5.08	5.15
	60 °C		5.00	4.93
Formulation 8 (pD)	25/60	6.25	5.72	5.88
	40/75		5.74	5.78
	60 °C		5.58	5.50

[00318] The italicized values are pD values for a deuterated sample. In some embodiments, the pD of the deuterated samples are $pD = pH_{\text{reading}} + 0.4$ (Glasoe, *et al.* "Use of glass electrodes to measure acidities in deuterium oxide" J. Physical Chem. 64(1): 188-190 (1960)).

[00319] At the two lower temperatures, the pH values at t=4 week are slightly elevated from the t=2 week time point. These data were generated using a new glass pH probe. In some instances, the observed differences are due to the probe differences or additional variables such as for example, the age of the standard buffers or temperature gradients within the laboratory environment. The downward pH trend for each formulation with increasing temperatures at t=4 week is consistent with previous data and is consistent with the increase in the amount of tropic acid present in the stability sample.

Example 10-Determination of Shelf Life and Activation Energy

[00320] Activation energy was calculated for the eight formulations disclosed in Example 9 and comparison with a reference standard was made with Formulations 4-7.

[00321] Table 19 illustrates the activation energy (Ea) calculation. The Ea minimum is 17.8 Kcal/mol, the Ea maximum is 21.3 Kcal/mol, and the Ea mean is 19.5 Kcal/mol. Mean is +/- 3* stdev. Figs. 6 and 7 illustrate the poor correlation between RS and tropic acid with Formulation 4 and Formulation 7, respectively. Figs. 8 and 9 illustrate improved correlation between RS and tropic acid with Formulation 5 and Formulation 6, respectively. At a lower pH (e.g. pH 4.8 or lower), there was a poor correlation observed (Formulation 4 and Formulation 7). This was due to a slowed hydrolysis and increased alternative degradation pathways. At a higher pH (e.g., pH 5.8 or higher), an improved or better correlation was observed (Formulation 5 and Formulation 6). This was due to the hydrolysis of atropine as the primary degradant. It is noted that the activation energy is for the specific acid catalyzed degradation to tropic acid- the predominant degradation product and degradation mechanism operating at pH 5.8 or higher.

Table 19. Activation energy for total related substance (RS) and tropic acid.

	Total RS	Tropic Acid
1	Poor	Poor

	Corr	Corr	
2	12.2	Poor	
		Corr	
3	Poor	18.3	
		Corr	
4	16.8	18.1	
5	19.8	19.7	
6	19.2	20.0	
7	13.2	15.5	
8	Poor	18.9	
		Corr	
Mean	16.2	18.4	Kcal/mole
Stdev	3.4	1.6	
RSD	21%	9%	

[00322] Table 20 illustrates the rate of RS or tropic acid formation per week at 40°C.

Table 20.

	Formulation	Rate 40°C (total RS %/wk)	Rate 40°C (Tropic acid %/wk)
Formulation 5	0.01% Atr Citrate pH5.8	1.16	1.09
Formulation 6	0.025% Atr Citrate pH5.8	0.72	0.61
Formulation 8	0.01% Atr Citrate pD 6.2 D ₂ O		0.163

[00323] Table 21 illustrates the activation energy and predicted shelf life at 30°C calculated based on Table 20. It is assumed for the calculation that tropic acid and total RS is 5% (self-life).

Table 21A.

	Rate @30°C (Total RS %/wk)			Estimated Shelf life @30°C (mo)		
Formulation	Ea min	Ea mean	Ea max	Ea min	Ea mean	Ea max
5	0.45	0.41	0.38	2.78	3.04	3.33
6	0.28	0.26	0.23	4.47	4.90	5.37
8	--	--	--	--	--	--

Table 21B.

	Rate @30°C (Tropic acid %/wk)			Estimated Shelf life @30°C (mo)		
Formulation	Ea min	Ea mean	Ea max	Ea min	Ea mean	Ea max
5	0.42	0.39	0.35	2.95	3.24	3.54
6	0.24	0.22	0.20	5.28	5.78	6.33
8	0.06	0.06	0.05	19.75	21.64	23.70

[00324] At pD 6.2, the deuterated formulation (Formulation 8) has a predicted shelf life of close to 2 years at 30°C.

[00325] Table 22 illustrate the predicted shelf life at temperatures of 40°C, 30°C, 25°C, and 2-8°C for Formulations 4-8 for total RS and tropic acid, respectively.

Table 22

Stability Prediction		RS		Tropic Acid		
Formulation	Temperature (°C)	weeks	months	Temperature (°C)	weeks	months
4	40	16.5	4.1	40	7.7	1.9
	30	40.2	10.1	30	20.0	5.0
	25	64.2	16.0	25	33.0	8.3
	2-8	493.4	123.4	2-8	296.8	74.2
5	40	2.8	0.7	40	0.9	0.2
	30	7.9	2.0	30	2.7	0.7
	25	13.7	3.4	25	4.6	1.2
	2-8	151.1	37.8	2-8	50.5	12.6
6	40	5.8	1.4	40	1.7	0.4
	30	15.9	4.0	30	4.8	1.2
	25	27.3	6.8	25	8.4	2.1
	2-8	281.6	70.4	2-8	95.9	24.0
7	40	11.5	2.9	40	16.9	4.2
	30	23.2	5.8	30	38.4	9.6
	25	33.4	8.4	25	59.1	14.8
	2-8	165.7	41.4	2-8	388.2	97.1
8	40	-	-	40	6.2	1.6
	30	-	-	30	17.0	4.3
	25	-	-	25	28.9	7.2
	2-8	-	-	2-8	287.1	71.8

Example 11-Additional Formulation Stability Comparison

[00326] Atropine sulfate monohydrate (MP Bio; Lot Number 7825K) and tropic acid (Sigma Aldrich; Lot Number STBD6457V) were used for this experiment. Thirteen formulations illustrated in

Table 23A were analyzed. Formulations 1-8 had been analyzed at t=0, 2 weeks, 4 weeks, and 8 weeks. Formulations 9-13 had been analyzed at t=0, 2 weeks, and 4 weeks. The pH values reported herein are the measured pH values obtained using the Thermo Scientific, Orion Dual Star pH/ISE benchtop pH meter and the Orion Double Junction Micro pH probe S/N S01-18520 calibrated with H₂O based standards.

Table 23A. Atropine sulfate Formulations

Formulation	Atropine Sulfate Monohydrate	Benzalkonium Chloride (BAK)	Sodium Chloride	Acetic Acid	Citric Acid	pH/pD	Aqueous
1	0.010	0.01	0.90	0.01	-	4.2	SWFI
2	0.025	0.01	0.90	0.01	-	4.2	SWFI
3	0.010	0.01	0.90	0.01	-	4.8	SWFI
4	0.025	0.01	0.90	0.01	-	4.8	SWFI
5	0.010	0.01	0.90	-	0.04	5.8	SWFI
6	0.025	0.01	0.90	-	0.04	5.8	SWFI
7	0.010	0.01	0.90	0.01	-	5.2 (pD)	D ₂ O
8	0.010	0.01	0.90	-	0.04	6.2 (pD)	D ₂ O
9	0.010	-	0.90	-	0.04	6.8 (pD)	D ₂ O
10	0.010	-	0.90	-	0.04	6.4	H ₂ O (control)
11	0.010	-	0.90	-	0.08	6.4	H ₂ O (control)
12	0.010	-	0.90	-	0.04	7.2 (pD)	D ₂ O
13	0.010	-	0.90	-	0.04	6.8	H ₂ O (control)

[00327] The values are % w/v. The formulations were prepared at 100 mL scale in volumetric glassware and filled into LDPE eye droppers. In some instances, the pD is calculated as $pD = 0.4 + pH^*$, in which pH^* is the measured or observed pH of the solution formulated in a solution containing deuterated water.

[00328] Table 23B illustrates analysis time points for the formulations listed in Table 23A.

Table 23B. Schedule for atropine sulfate formulation testing

Storage Condition (Horizontal)	Time Point		
	Initial (t=0)	2 Week	4 Week
25 °C/60%RH	X	X	X
40 °C/75%RH		X	X
60 °C		X	X

[00329] Table 24A and Table 24B illustrate atropine sulfate purity data associated with the atropine sulfate formulations. Purity is indicated as percentage of area under the curve. The ↑ & ↓ indicate the high or low concentration of atropine sulfate monohydrate (0.01% and 0.025%). The *A* & *C* indicate the buffer species used, acetic acid and citric acid respectively.

Table 24A. Atropine Sulfate Purity as Area-% for H₂O Formulations

Solvent	Condition	t=0	t=2 weeks	t=4 weeks
Formulation 3 ↓ <i>A</i> H ₂ O pH 4.8	25/60	98.16	98.16	98.45
	40/75		97.98	97.35
	60 °C		95.94	94.65
Formulation 5 ↓ <i>C</i> H ₂ O pH 5.8	25/60	98.16	97.92	97.54
	40/75		95.88	93.51
	60 °C		80.94	66.83
Formulation 10 ↓ <i>C</i> H ₂ O pH 6.4	25/60	98.66	96.67	95.81
	40/75		91.07	85.27
	60 °C		59.77	42.87
Formulation 11 ↓ <i>C</i> (2x) H ₂ O pH 6.4	25/60	99.47	97.87	96.69
	40/75		90.97	84.26
	60 °C		54.96	34.40
Formulation 13 ↓ <i>C</i> H ₂ O pH 6.8	25/60	97.21	95.42	93.24
	40/75		83.05	73.00
	60 °C		43.99	27.50

Table 24B. Atropine Sulfate Purity as Area-% for D₂O Formulations

Solvent	Condition	t=0	t=2 weeks	t=4 weeks
Formulation 7 ↓ <i>A</i> D ₂ O pD 5.2	25/60	98.93	99.07	98.39
	40/75		98.51	97.55
	60 °C		96.70	94.01
Formulation 8 ↓ <i>C</i> D ₂ O pD 6.2	25/60	98.93	98.95	98.51
	40/75		98.53	97.44
	60 °C		95.97	92.72
Formulation 9 ↓ <i>C</i> D ₂ O pD 6.8	25/60	99.29	98.42	98.07
	40/75		95.20	93.22
	60 °C		75.17	65.97
Formulation 12	25/60	98.53	97.17	95.99

↓C D ₂ O pD 7.2	40/75		90.75	84.64
	60 °C		56.78	46.05

[00330] Table 25A and Table 25B illustrate tropic acid formation associated with the atropine sulfate formulations. Tropic acid is a degradant of atropine sulfate, and is indicated as percentage of area under the curve. LOQ was found to be 0.05% for the RP-HPLC method. The ↑ & ↓ indicate the high or low concentration of atropine sulfate monohydrate (0.01% and 0.025%). The *A* & *C* indicate the buffer species used, acetic acid and citric acid, respectively.

Table 25A. Tropic Acid as Area-% for H₂O Formulations

Solvent	Condition	t=0	t=2 weeks	t=4 weeks
Formulation 3 ↓A H ₂ O pH 4.8	25/60	<LOQ	0.12	0.05
	40/75		0.19	0.27
	60 °C		0.90	1.58
Formulation 5 ↓C H ₂ O pH 5.8	25/60	<LOQ	0.40	0.71
	40/75		2.22	4.35
	60 °C		16.62	29.13
Formulation 10 ↓C H ₂ O pH 6.4	25/60	0.74	1.90	3.21
	40/75		7.61	13.49
	60 °C		37.44	54.06
Formulation 11 ↓C(2x) H ₂ O pH 6.4	25/60	0.09	1.31	2.64
	40/75		7.61	14.68
	60 °C		42.43	62.23
Formulation 13 ↓C H ₂ O pH 6.8	25/60	2.21	3.66	6.11
	40/75		15.47	25.80
	60 °C		53.24	69.34

Table 25B. Tropic Acid as Area-% for D₂O Formulations

Solvent	Condition	t=0	t=2 weeks	t=4 weeks
Formulation 7 ↓A D ₂ O pD 5.2	25/60	<LOQ	0.07	0.08
	40/75		0.14	0.24
	60 °C		0.71	1.07
Formulation 8 ↓C D ₂ O pD 6.2	25/60	<LOQ	0.11	0.14
	40/75		0.33	0.65
	60 °C		2.32	4.03
Formulation 9 ↓C D ₂ O pD 6.8	25/60	0.06	0.55	1.06
	40/75		3.16	6.29
	60 °C		21.09	29.25
Formulation 12 ↓C D ₂ O pD 7.2	25/60	0.42	1.35	2.62
	40/75		7.27	13.53
	60 °C		38.58	48.15

[00331] Table 26A and Table 26B illustrate the percentage of potency of atropine in the formulations. The ↑ & ↓ indicate the high or low concentration of atropine sulfate monohydrate (0.01% and 0.025%). The *A* & *C* indicate the buffer species used, acetic acid and citric acid respectively.

Table 26A. Percentage of potency for H₂O Formulations

Solvent	Condition	t=0	t=2 weeks	t=4 weeks
Formulation 3 ↓ <i>A</i> H ₂ O pH 4.8	25/60	106.3	108.0	109.6
	40/75		108.1	110.0
	60 °C		108.0	109.9
Formulation 5 ↓ <i>C</i> H ₂ O pH 5.8	25/60	105.0	105.9	107.1
	40/75		103.8	103.5
	60 °C		90.2	77.7
Formulation 10 ↓ <i>C</i> H ₂ O pH 6.4	25/60	101.7	100.0	98.0
	40/75		89.4	87.0
	60 °C		63.7	45.7
Formulation 11 ↓ <i>C</i> (2x) H ₂ O pH 6.4	25/60	97.5	96.1	94.3
	40/75		89.4	82.0
	60 °C		55.7	35.20
Formulation 13 ↓ <i>C</i> H ₂ O pH 6.8	25/60	99.4	96.9	94.1
	40/75		85.0	74.0
	60 °C		46.4	29.8

Table 26B. Percentage of potency for D₂O Formulations

Solvent	Condition	t=0	t=2 weeks	t=4 weeks
Formulation 7 ↓ <i>A</i> D ₂ O pD 5.2	25/60	107.3	111.3	112.9
	40/75		111.6	113.5
	60 °C		111.8	113.5
Formulation 8 ↓ <i>C</i> D ₂ O pD 6.2	25/60	99.0	103.0	105.0
	40/75		104.9	104.7
	60 °C		101.6	103.0
Formulation 9 ↓ <i>C</i> D ₂ O pD 6.8	25/60	101.4	99.9	100.1
	40/75		97.4	93.2
	60 °C		78.7	68.9
Formulation 12 ↓ <i>C</i> D ₂ O pD 7.2	25/60	104.9	103.5	101.6
	40/75		96.9	89.1
	60 °C		62.5	50.9

[00332] Table 27A and Table 27B illustrate the stability of pH or pD for the atropine sulfate formulations. The ↑ & ↓ indicate the high or low concentration of atropine sulfate monohydrate (0.01% and 0.025%). The *A* & *C* indicate the buffer species used, acetic acid and citric acid respectively.

Table 27A. Stability of pH for H₂O Formulations

Solvent	Condition	t=0	t=2 weeks	t=4 weeks
Formulation 3	25/60	4.85	4.44	4.61

↓A H ₂ O pH 4.8	40/75		4.41	4.54
	60 °C		4.32	4.40
Formulation 5 ↓C H ₂ O pH 5.8	25/60	5.87	5.93	6.03
	40/75		5.96	5.96
	60 °C		5.82	5.78
Formulation 10 ↓C H ₂ O pH 6.4	25/60	6.43	6.41	6.46
	40/75		6.62	6.67
	60 °C		6.01	5.92
Formulation 11 ↓C(2x) H ₂ O pH 6.4	25/60	6.44	6.47	6.72
	40/75		6.66	6.61
	60 °C		6.27	6.23
Formulation 13 ↓C H ₂ O pH 6.8	25/60	6.77	6.91	6.91
	40/75		6.65	6.62
	60 °C		6.30	6.19

Table 27B. Stability of pD for D₂O Formulations

Solvent	Condition	t=0	t=2 weeks	t=4 weeks
Formulation 7 ↓A D ₂ O pD 5.2	25/60	5.31	5.10	5.24
	40/75		5.08	5.15
	60 °C		5.00	4.93
Formulation 8 ↓C D ₂ O pD 6.2	25/60	6.25	5.72	5.88
	40/75		5.74	5.78
	60 °C		5.58	5.50
Formulation 9 ↓C D ₂ O pD 6.8	25/60	6.76	6.80	6.81
	40/75		6.78	6.86
	60 °C		6.45	6.24
Formulation 12 ↓C D ₂ O pD 7.2	25/60	7.25	7.18	7.26
	40/75		7.14	7.15
	60 °C		6.52	6.36

Example 12. Determination of shelf life and activation energy for atropine sulfate formulations of**Example 11**

[00333] Activation energy was calculated for the atropine sulfate formulations disclosed in Example 11. Specifically, activation energies were calculated from the total % of related substances (RS) at 40°C and 60°C (2 point calculations) and from tropic acid formation at 40°C and 60°C (2 point calculations). These values were then averaged. Table 28 illustrates the activation energy calculation. Table 29 illustrates estimated shelf-lives from the 40°C rate of formation of %RS and tropic acid, respectively. Figure 10 illustrates estimated shelf lives for D₂O and H₂O formulations.

Table 28. Activation Energy

Atropine Formulations	Total RS	Tropic Acid
7	14	19

3	16	17
8	20	21
5	14	Poor Corr
6	15	16
Mean	16.3	18.7
Stdev	2.68	1.90
RSD	16%	10%
Poor Corr:	One or more curve had $R^2 < 0.95$	

Table 29. Estimated Shelf Life

	Estimated Shelf life/mo			
	Total related substances % (limit=8%)		Tropic acid % (limit=5%)	
	8°C	25°C	8°C	25°C
0.01% w/v Atr 0.01% w/v Acetate 0.9% w/v NaCl 0.01% w/v BAK pD 5.2 D₂O (Formulation 7)	189	26	1427	147
0.01% w/v Atr 0.01% w/v Acetate 0.9% w/v NaCl 0.01% w/v BAK pH4.8 H₂O (Formulation 3)	211	29	1095	113
0.01% w/v Atr 0.04% w/v Citrate 0.9% w/v NaCl 0.01% w/v BAK pD 6.2 D₂O (Formulation 8)	158	22	369.8	38

0.01% w/v Atr 0.04% w/v Citrate 0.9% w/v NaCl 0.01% w/v BAK pH5.8 H₂O (Formulation 5)	37	5.2	54	5.5
0.01% Atr 0.9% w/v NaCl pH5.9 H₂O extemporaneous preparation	13.6	2.6		

[00334] Tables 30 illustrate the predicted shelf life at temperatures of 40°C, 30°C, 25°C, and 2-8°C for Formulations 2-8 for total RS and tropic acid, respectively.

Table 30

Stability Prediction		RS		Tropic Acid		
Formulation	Temperature (°C)	weeks	months	Temperature (°C)	weeks	months
2	40	64.5	16.1	40	-	-
	30	153.2	38.3	30	-	-
	25	241.2	60.3	25	-	-
	2-8	1747.9	437.0	2-8	-	-
3	40	31.1	7.8	40	99.5	24.9
	30	73.9	18.5	30	268.3	67.1
	25	116.3	29.1	25	451.8	113.0
	2-8	842.9	210.7	2-8	4382.0	1095.5
4	40	30.7	7.7	40	42.1	10.5
	30	73.0	18.2	30	113.7	28.4
	25	114.9	28.7	25	191.5	47.9
	2-8	832.6	208.1	2-8	1857.0	464.2
5	40	5.5	1.4	40	4.9	1.2
	30	13.1	3.3	30	13.2	3.3
	25	20.6	5.2	25	22.2	5.5

	2-8	149.3	37.3	2-8	215.0	53.8
6	40	10.7	2.7	40	8.8	2.2
	30	25.5	6.4	30	23.7	5.9
	25	40.1	10.0	25	39.8	10.0
	2-8	290.5	72.6	2-8	386.5	96.6
7	40	27.9	7.0	40	129.6	32.4
	30	66.4	16.6	30	349.6	87.4
	25	104.5	26.1	25	588.7	147.2
	2-8	757.3	189.3	2-8	5709.4	1427.4
8	40	23.3	5.8	40	33.6	8.4
	30	55.3	13.8	30	90.6	22.6
	25	87.2	21.8	25	152.5	38.1
	2-8	631.6	157.9	2-8	1479.2	369.8

Example 13 - Effect of pH on Ophthalmic Acceptance in Guinea Pigs

[00335] A cohort of guinea pigs is administered 50 µL of ophthalmic formulations having different pH values described herein. For example, ophthalmic formulations comprising H₂O or deuterated water (e.g., D₂O) are administered to the animals. Animal behavior is recorded at predetermined time intervals to evaluate the acceptance of the ophthalmic formulations

Example 14 – In vivo Rabbit Eye Irritation Test

[00336] The exemplary compositions disclosed herein are subjected to rabbit eye irritation test to evaluate their safety profile. The test composition are tested for eye irritation test in New Zealand Rabbits (see for example Abraham M H, et al., *Draize rabbit eye test compatibility with eye irritation thresholds in humans: a quantitative structure-activity relationship analysis*. Toxicol Sci. 2003 December; 76(2):384-91. Epub 2003 Sep. 26; see also Gettings S D et al., *A comparison of low volume, Draize and in vitro eye irritation test data. III. Surfactant-based formulations*. Food Chem Toxicol. 1998 March; 36(3):209-31). The study involves single ocular administration into the right eye and the same volume of its placebo in the left eye of each of the three rabbits. Rabbits are examined immediately and after instillation of the compositions for 4, 24, 48 and 72 hours post instillation to note the signs/symptoms of eye irritation, if any. The test compositions show no sign of irritancy in cornea, iris and conjunctivae of the rabbit eyes.

Example 15 – In vivo Testing of Ophthalmic Aqueous Formulation in Guinea Pigs

[00337] Focus deprivation myopia (FDM) is achieved using a latex shield to cover one eye. For defocus-induced myopia, a latex-made facemask was held in place by a rubber-band around the head of animals, leaving both eyes, the nose, mouth and ears freely exposed. A -4.00 D lens is glued onto a plastic lens frame. The lens frame is then attached to the facemask around one eye by a fabric hook-and-loop fastener after the optical center of the lens was aligned with the pupil center. The lens is detached and cleaned on both sides with a water-wetted gauze at least once daily followed by re-attachment to the facemask. All the animals are maintained on a cycle of 12-h illumination (500 Lux) and 12-h darkness during the experimental period

[00338] A cohort of guinea pigs at age of 3 weeks are randomly assigned to FDM (a facemask worn monocularly) or defocus-induced myopia (a -4.00 D lens worn monocularly) and control groups. The FDM groups were treated with the ophthalmic aqueous formulation, the ophthalmic carrier (without the ophthalmic agent), or FDM-only. The defocus-induced myopia groups were treated with the ophthalmic aqueous formulation, the ophthalmic carrier (without the ophthalmic agent), or defocus-only. The control groups were treated with the ophthalmic aqueous formulation, the ophthalmic carrier (without the ophthalmic agent), or no treatment. Ocular biometric parameters are measured in both eyes of individual animals before and at 11 days of treatment

[00339] Biometric parameters (e.g. refraction, corneal curvature, and axial components of the eye) are measured by an optometrist, orthoptist, or ophthalmologist with help from an animal care assistant during the light cycle (daytime) after removal of the facemask or lens. The optometrist, orthoptist, or ophthalmologist is masked in regard to the treatment conditions for each animal.

[00340] Refraction is measured by retinoscopy after the pupil is completely dilated by topical administration of 1% cyclopentolate hydrochloride. The results of retinoscopy are recorded as the mean value of the horizontal and vertical meridians.

[00341] Corneal curvature is measured with a keratometer modified by attachment of an +8 D lens onto the anterior surface of the keratometer. A group of stainless steel balls with diameters from 5.5 to 11.0 mm are measured by the modified keratometer. Three readings are recorded for each measurement to provide a mean result. The radius of corneal curvature is then deduced from the readings on the balls with known radii.

[00342] A-scan ultrasonograph is used to measure axial components of the eye (lens thickness and vitreous length and axial length). The conducting velocity was 1,723.3 m/s for measurement of the lens thickness and 1,540 m/s for measurement of the vitreous length as described previously. Each of the axial components is calculated as the mean of 10 repeated measurements.

Example 16 – Safety and Efficacy Studies of Ophthalmic Aqueous Formulation

[00343] A clinical trial is performed to investigate the efficacy and safety of ophthalmic aqueous formulations described herein in patients with myopia. In some instances, the study is open-label, single blind, or double blind study. Patient selection criteria include myopic refraction of at least 1.0D in both eyes, and additional factors such as astigmatism, a documented myopic progression, age, sex, and/or health conditions.

[00344] The patients are randomized to receive 0.05%, 0.01%, or 0.001 atropine aqueous formulation formulated in either H₂O or deuterated water (e.g., D₂O) once nightly in both eyes. Allocation ratio in some instances is defined based the patient population.

[00345] The patients are evaluated on day 0 (baseline), day 14, day 30, and then at 2, 3, 4, 5, 6, 8, 10, 12, 18, 20, 24, and 36 months. At each visit, best-corrected distance logMar visual acuity (BCVA) is assessed by an optometrist, orthoptist, or ophthalmologist using the Early Treatment Diabetic Retinopathy study chart. Near visual acuity is assessed using best-corrected distance spectacle correction with a reduced logMar reading chart placed at 40cm under well-lit conditions. The near point of accommodation (NPA) is measured using a RAF rule using best-corrected distance spectacle correction. Patients are instructed to move the target inwards till the N5 print becomes slightly blurred and then outwards till it just becomes clear. Accommodation amplitude is calculated as the inverse of NPA. Mesopic pupil size is measured with Procyon 3000 pupillometer. Photopic pupil size is measured using the Neuroptics pupillometer.

[00346] Cycloplegic autorefraction is determined 30 minutes after 3 drops of cyclopentolate 1% are administered at 5 minutes apart using a Canon RK-F1 autorefractor. A Zeiss IOL Master, a non-contact partial coherence interferometry, is used to measure the ocular axial length.

[00347] The primary outcome is myopia progression over the time period of the study. Safety is assessed by adverse events including allergic reactions, irritation, or development of blurring of vision in one or both eyes.

Example 17 – Preparation of an Ointment Formulation Containing Atropine Sulfate

[00348] Atropine sulfate is mixed with the dispersing agent (e.g. polyethyleneglycol) under heating and sonication and this mixture is further thoroughly mixed with a molten ointment base (e.g. a mixture of wool wax, white petrolatum, and liquid paraffin). The mixture is placed in a pressure vessel, and sterilized at 125 °C for 30-45 minutes and cooled to room temperature. In another embodiment, autoclaving is conducted under nitrogen. The resulting ophthalmic ointment is aseptically filled into pre-sterilized containers (e.g. tubes).

Example 18 – Atropine-Mucus Penetrating Particle Composition

[00349] A 0.01% atropine-mucus penetrating particle composition was prepared utilizing a milling procedure. An aqueous dispersion containing atropine particles and an MPP-enabling mucus penetrating agent was milled with grinding medium until particle size was reduced to approximately 200nm with a polydispersity index less than 0.15 as measured by dynamic light scattering. Additional agents such as preservatives are also added during the milling procedure. Subsequently, the atropine-MPP composition are be stored at temperatures of between about 15°C and about 25°C.

Example 19 – Atropine Sulfate-Mucus Penetrating Particle Composition

[00350] A 0.01% atropine sulfate-mucus penetrating particle composition was prepared utilizing a milling procedure. An aqueous dispersion containing atropine particles and an MPP-enabling mucus penetrating agent was milled with grinding medium until particle size was reduced to approximately 200nm with a polydispersity index less than 0.15 as measured by dynamic light scattering. Additional agents such as preservatives are also be added during the milling procedure. Subsequently, the atropine-MPP composition are be stored at temperatures of between about 15°C and about 25°C.

[00351] According to another aspect of the disclosure, described herein is an ophthalmic composition that comprises from about 0.001 wt% to about 0.05 wt% of a muscarinic antagonist and water, at a pH of from about 3.8 to about 7.5.

[00352] In some instances, the muscarinic antagonist comprises atropine, atropine sulfate, noratropine, atropine-N-oxide, tropine, tropic acid, hyoscine, scopolomine, tropicamide, cyclopentolate, pirenzapine, homatropine, or a combination thereof. In some cases, the muscarinic antagonist is atropine. In some cases, the muscarinic antagonist is atropine sulfate.

[00353] In some instances, the ophthalmic composition comprises one of: at least about 80%, at least about 85%, at least about 90%, at least about 93%, at least about 95%, at least about 97%, at least about 98%, or at least about 99% of the muscarinic antagonist based on initial concentration after extended period of time under storage condition.

[00354] In some instances, the ophthalmic composition has a pH of one of: less than about 7.3, less than about 7.2, less than about 7.1, less than about 7, less than about 6.8, less than about 6.5, less than about 6.4, less than about 6.3, less than about 6.2, less than about 6.1, less than about 6, less than about 5.9, less than about 5.8, less than about 5.2, less than about 4.8, or less than about 4.2 after extended period of time under storage condition.

[00355] In some instances, the ophthalmic composition further has a potency of one of: at least 80%, at least 85%, at least 90%, at least 93%, at least 95%, at least 97%, at least 98%, or at least 99% after extended period of time under storage condition.

[00356] In some instances, the extended period of time is one of: about 1 week, about 2 weeks, about 3 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 8 months, about 10 months, about 12 months, about 18 months, about 24 months, about 36 months, about 4 years, or about 5 years.

[00357] In some instances, the storage condition has a storage temperature of one of: about 25°C, about 40°C, or about 60°C. In some cases, the storage condition has a storage temperature of from about 2°C to about 10°C or from about 16°C to about 26°C. In some cases, the storage condition has a relative humidity of about 60% or about 75%.

[00358] In some instances, the ophthalmic composition is in the form of an aqueous solution. In some cases, the muscarinic antagonist is present in the composition at a concentration of one of: from about 0.001 wt% to about 0.04 wt%, from about 0.001 wt% to about 0.03 wt%, from about 0.001 wt% to about 0.025 wt%, from about 0.001 wt% to about 0.02 wt%, from about 0.001 wt% to about 0.01 wt%, from about 0.001 wt% to about 0.008 wt%, or from about 0.001 wt% to about 0.005 wt%.

[00359] In some instances, the ophthalmic composition further comprises an osmolarity adjusting agent. In some cases, the osmolarity adjusting agent is sodium chloride.

[00360] In some instances, the ophthalmic composition further comprises a preservative. In some cases, the preservative is selected from benzalkonium chloride, cetrimonium, sodium perborate, stabilized oxychloro complex, SofZia, polyquaternium-1, chlorobutanol, edetate disodium, polyhexamethylene biguanide, or combinations thereof.

[00361] In some instances, the ophthalmic composition further comprises a buffer agent. In some cases, the buffer agent is selected from borates, borate-polyol complexes, phosphate buffering agents, citrate buffering agents, acetate buffering agents, carbonate buffering agents, organic buffering agents, amino acid buffering agents, or combinations thereof.

[00362] In some instances, the ophthalmic composition further comprises a tonicity adjusting agent. In some cases, the tonicity adjusting agent is selected from sodium chloride, sodium nitrate, sodium sulfate, sodium bisulfate, potassium chloride, calcium chloride, magnesium chloride, zinc chloride, potassium acetate, sodium acetate, sodium bicarbonate, sodium carbonate, sodium thiosulfate, magnesium sulfate, disodium hydrogen phosphate, sodium dihydrogen phosphate, potassium dihydrogen phosphate, dextrose, mannitol, sorbitol, dextrose, sucrose, urea, propylene glycol, glycerin, or a combination thereof.

[00363] In some instances, the ophthalmic composition is stored in a plastic container. In some cases, the material of the plastic container comprises low-density polyethylene (LDPE).

[00364] In some instances, the ophthalmic composition has a dose-to-dose muscarinic antagonist concentration variation of one of: less than 50%, less than 40%, less than 30%, less than 20%, less than 10%, or less than 5%. In some cases, the dose-to-dose muscarinic antagonist concentration variation is based on one of: 10 consecutive doses, 8 consecutive doses, 5 consecutive doses, 3 consecutive doses, or 2 consecutive doses.

[00365] In some instances, the ophthalmic composition has a pH of one of: from about 3.8 to about 7.5, from about 4.2 to about 7.5, from about 4.8 to about 7.3, from about 5.2 to about 7.2, from about 5.8 to about 7.1, from about 6.0 to about 7.0, or from about 6.2 to about 6.8.

[00366] In some instances, the ophthalmic composition further comprises a pH adjusting agent. In some cases, the pH adjusting agent comprises HCl, NaOH, CH₃COOH, or C₆H₈O₇.

[00367] In some instances, the ophthalmic composition comprises one of: less than 5% of D₂O, less than 4% of D₂O, less than 3% of D₂O, less than 2% of D₂O, less than 1% of D₂O, less than 0.5% of D₂O, less than 0.1% of D₂O, or 0% D₂O. In some cases, the ophthalmic composition is essentially free of D₂O.

[00368] In some instances, the ophthalmic composition further comprises a pharmaceutically acceptable carrier.

[00369] In some instances, the ophthalmic composition is formulated as an ophthalmic solution for the treatment of an ophthalmic disorder. In some cases, the ophthalmic disorder or condition is pre-myopia, myopia, or progression of myopia.

[00370] In some instances, the ophthalmic composition is not formulated as an injectable formulation.

[00371] While preferred embodiments of the present disclosure have been shown and described herein, such embodiments are provided by way of example only. Various alternatives to the embodiments described herein are optionally employed in practicing the disclosure. It is intended that the following claims define the scope of the disclosure and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

WHAT IS CLAIMED IS:

1. An ophthalmic composition, comprising from about 0.001 wt% to about 0.05 wt% of a muscarinic antagonist and deuterated water, at a pD of from about 4.2 to about 7.9.
2. The ophthalmic composition of claim 1, wherein the muscarinic antagonist comprises atropine, atropine sulfate, noratropine, atropine-N-oxide, tropine, tropic acid, hyoscine, scopolomine, tropicamide, cyclopentolate, pirenzapine, homatropine, or a combination thereof.
3. The ophthalmic composition of claim 2, wherein the muscarinic antagonist is atropine, or atropine sulfate.
4. The ophthalmic composition of claim 1, wherein the ophthalmic composition has a pD of one of: less than about 7.3, less than about 7.2, less than about 7.1, less than about 7, less than about 6.8, less than about 6.5, less than about 6.4, less than about 6.3, less than about 6.2, less than about 6.1, less than about 6, less than about 5.9, less than about 5.8, less than about 5.2, or less than about 4.8 after extended period of time under storage condition.
5. The ophthalmic composition of claim 1, wherein the ophthalmic composition comprises one of: at least about 80%, at least about 85%, at least about 90%, at least about 93%, at least about 95%, at least about 97%, at least about 98%, or at least about 99% of the muscarinic antagonist based on initial concentration after extended period of time under storage condition.
6. The ophthalmic composition of claim 1, wherein the ophthalmic composition further has a potency of one of: at least 80%, at least 85%, at least 90%, at least 93%, at least 95%, at least 97%, at least 98%, or at least 99% after extended period of time under storage condition.
7. The ophthalmic composition of claim 1, wherein the extended period of time is one of: about 1 week, about 2 weeks, about 3 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 8 months, about 10 months, about 12 months, about 18 months, about 24 months, about 36 months, about 4 years, or about 5 years.

8. The ophthalmic composition of claim 1, wherein the storage condition has a storage temperature of from about 2°C to about 10°C or from about 16°C to about 26°C.
9. The ophthalmic composition of claim 1, wherein the muscarinic antagonist is present in the composition at a concentration of one of: from about 0.001 wt% to about 0.04 wt%, from about 0.001 wt% to about 0.03 wt%, from about 0.001 wt% to about 0.025 wt%, from about 0.001 wt% to about 0.02 wt%, from about 0.001 wt% to about 0.01 wt%, from about 0.001 wt% to about 0.008 wt%, or from about 0.001 wt% to about 0.005 wt%.
10. The ophthalmic composition of claim 1, wherein the ophthalmic composition further comprises an osmolarity adjusting agent.
11. The ophthalmic composition of claim 10, wherein the osmolarity adjusting agent is sodium chloride.
12. The ophthalmic composition of claim 1, wherein the ophthalmic composition further comprises a preservative.
13. The ophthalmic composition of claim 12, wherein the preservative is selected from benzalkonium chloride, cetrimonium, sodium perborate, stabilized oxychloro complex, SofZia, polyquaternium-1, chlorobutanol, edetate disodium, polyhexamethylene biguanide, or combinations thereof.
14. The ophthalmic composition of claim 1, wherein the ophthalmic composition further comprises a buffer agent.
15. The ophthalmic composition of claim 14, wherein the buffer agent is selected from borates, borate-polyol complexes, phosphate buffering agents, citrate buffering agents, acetate buffering agents, carbonate buffering agents, organic buffering agents, amino acid buffering agents, or combinations thereof.
16. The ophthalmic composition of claim 1, wherein the ophthalmic composition is essentially free of procaine and benactyzine, or pharmaceutically acceptable salts thereof.

17. The ophthalmic composition of claim 1, wherein the ophthalmic composition has a dose-to-dose muscarinic antagonist concentration variation of one of: less than 50%, less than 40%, less than 30%, less than 20%, less than 10%, or less than 5%.
18. The ophthalmic composition of claim 17, wherein the dose-to-dose muscarinic antagonist concentration variation is based on one of: 10 consecutive doses, 8 consecutive doses, 5 consecutive doses, 3 consecutive doses, or 2 consecutive doses.
19. The ophthalmic composition of claim 1, wherein the ophthalmic composition further comprises a pD adjusting agent.
20. The ophthalmic composition of claim 19, wherein the pD adjusting agent comprises DCl, NaOD, CD₃COOD, or C₆D₈O₇.
21. The ophthalmic composition of claim 1, wherein the ophthalmic composition comprises one of: less than 5% of H₂O, less than 4% of H₂O, less than 3% of H₂O, less than 2% of H₂O, less than 1% of H₂O, less than 0.5% of H₂O, less than 0.1% of H₂O, or 0% of H₂O.
22. The ophthalmic composition of claim 1, wherein the ophthalmic composition is not formulated as an injectable formulation.
23. The ophthalmic composition of claim 1, wherein the ophthalmic composition is formulated as an ophthalmic solution for the treatment of pre-myopia, myopia, or progression of myopia.
24. An ophthalmic solution, comprising from about 0.001 wt% to about 0.05 wt% of a muscarinic antagonist and deuterated water, at a pD of from about 4.2 to about 7.9.
25. The ophthalmic solution of claim 24, wherein the ophthalmic solution has a pD of one of: less than about 7.3, less than about 7.2, less than about 7.1, less than about 7, less than about 6.8, less than about 6.5, less than about 6.4, less than about 6.3, less than about 6.2, less than about 6.1, less than about 6, less than about 5.9, less than about 5.8, less than about 5.2, or less than about 4.8 after extended period of time under storage condition.

26. The ophthalmic solution of claim 24, wherein the muscarinic antagonist comprises atropine, atropine sulfate, noratropine, atropine-N-oxide, tropine, tropic acid, hyoscine, scopolomine, tropicamide, cyclopentolate, pirenzapine, homatropine, or a combination thereof.
27. The ophthalmic solution of claim 24, wherein the ophthalmic solution comprises one of: less than 5% of H₂O, less than 4% of H₂O, less than 3% of H₂O, less than 2% of H₂O, less than 1% of H₂O, less than 0.5% of H₂O, less than 0.1% of H₂O, or 0% of H₂O.
28. A method of arresting or preventing myopia development, comprising administering to an eye of an individual in need thereof an effective amount of an ophthalmic composition comprising from about 0.001 wt% to about 0.05 wt% of a muscarinic antagonist and deuterated water, at a pD of from about 4.2 to about 7.9.
29. The method of claim 28, wherein the ophthalmic composition is stored at between about 2 °C to about 10 °C prior to first use.
30. The method of claim 28, wherein the ophthalmic composition is stored at between about 16 °C to about 26 °C after first use.

OPHTHALMIC COMPOSITION

ABSTRACT

[00372] Provided herein is an ophthalmic composition. In some embodiments, the ophthalmic composition includes a low concentration of an ophthalmic agent for treatment of an ophthalmic disorder or condition; and an ophthalmically acceptable carrier, wherein the ophthalmic agent is distributed with substantial uniformity throughout the ophthalmically acceptable carrier. Further disclosed herein include an ophthalmic composition including a low concentration of an ophthalmic agent and deuterated water. Also disclosed herein are methods of arresting or preventing myopia development by administering to an eye of an individual in need thereof an effective amount of an ophthalmic composition as described herein.

Fig. 1A

Weeks							
	Temp (°C)	0	1	1.571429	2.142857	3.428571	6.571429
T1	25	0.08			0.88		2.81
T2	40	0.08		3.47		4.48	

Fig. 1B

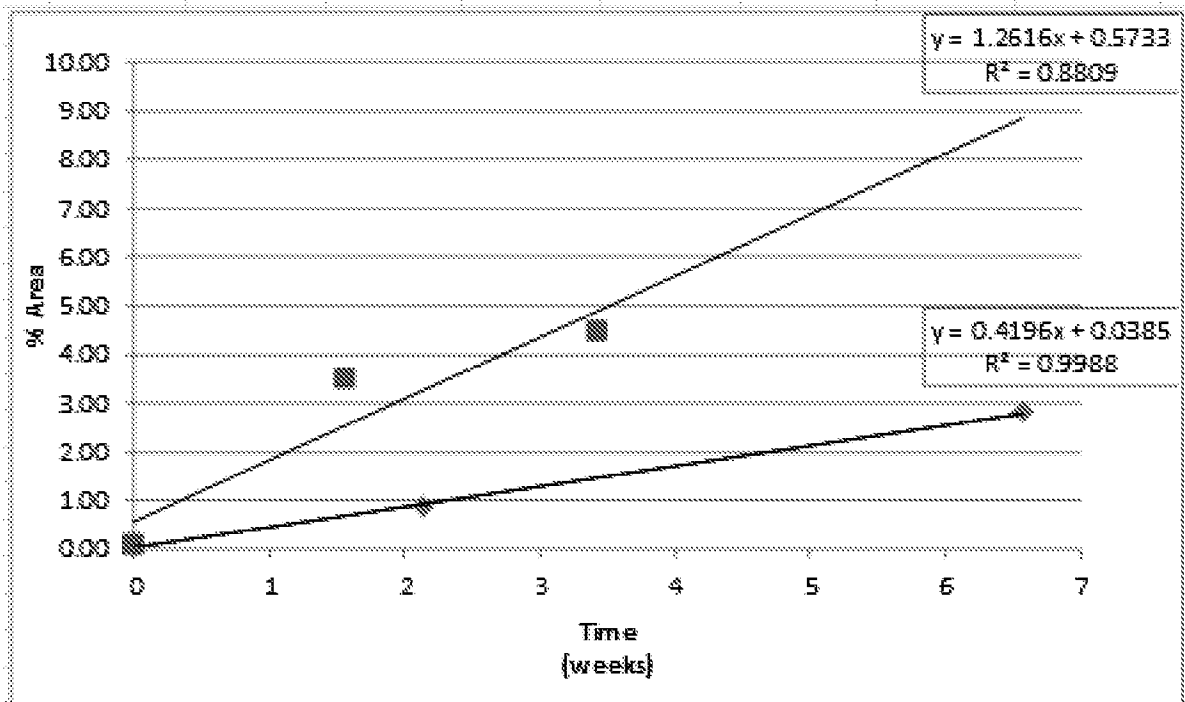


Fig. 1C

Formulation:		Average of Analysts		
Stability Prediction:		RRT 0.87		
	spec limit:	0.50	% (not more than)	
			shelf life	
			weeks	months
rate	1.24844	at 40C	0.4	0.1
rate	0.60617	at 30C	0.8	0.2
rate	0.41477	at 25C	1.2	0.3
rate	0.07932	at 2-8C	6.3	1.6
rate	0.00694	at -20C	N/A	N/A

Fig. 2A

Weeks						
	Temp (°C)	0	1	2.142857	4	6.571429
T1	25	0.08		0.9		2.8
T2	60	0.08	10.5		11.3	

Fig. 2B

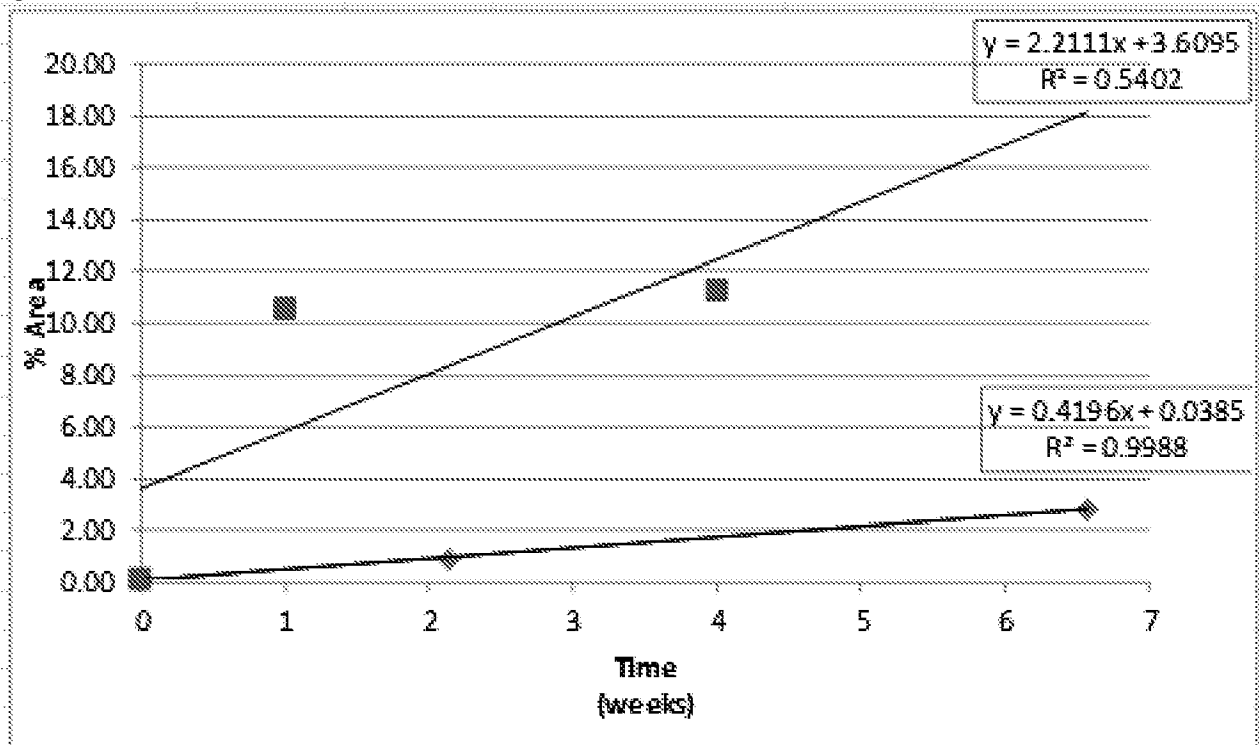


Fig. 2C

Formulation:		Average of Analysts		
Stability Prediction:		RRT 0.87		
	spec limit:	0.50	% (not more than)	
			shelf life	
			weeks	months
rate	0.88876 at 40C		0.6	0.1
rate	0.54051 at 30C		0.9	0.2
rate	0.41627 at 25C		1.2	0.3
rate	0.13331 at 2-8C		3.8	0.9
rate	0.02493 at -20C		N/A	N/A

Fig. 3

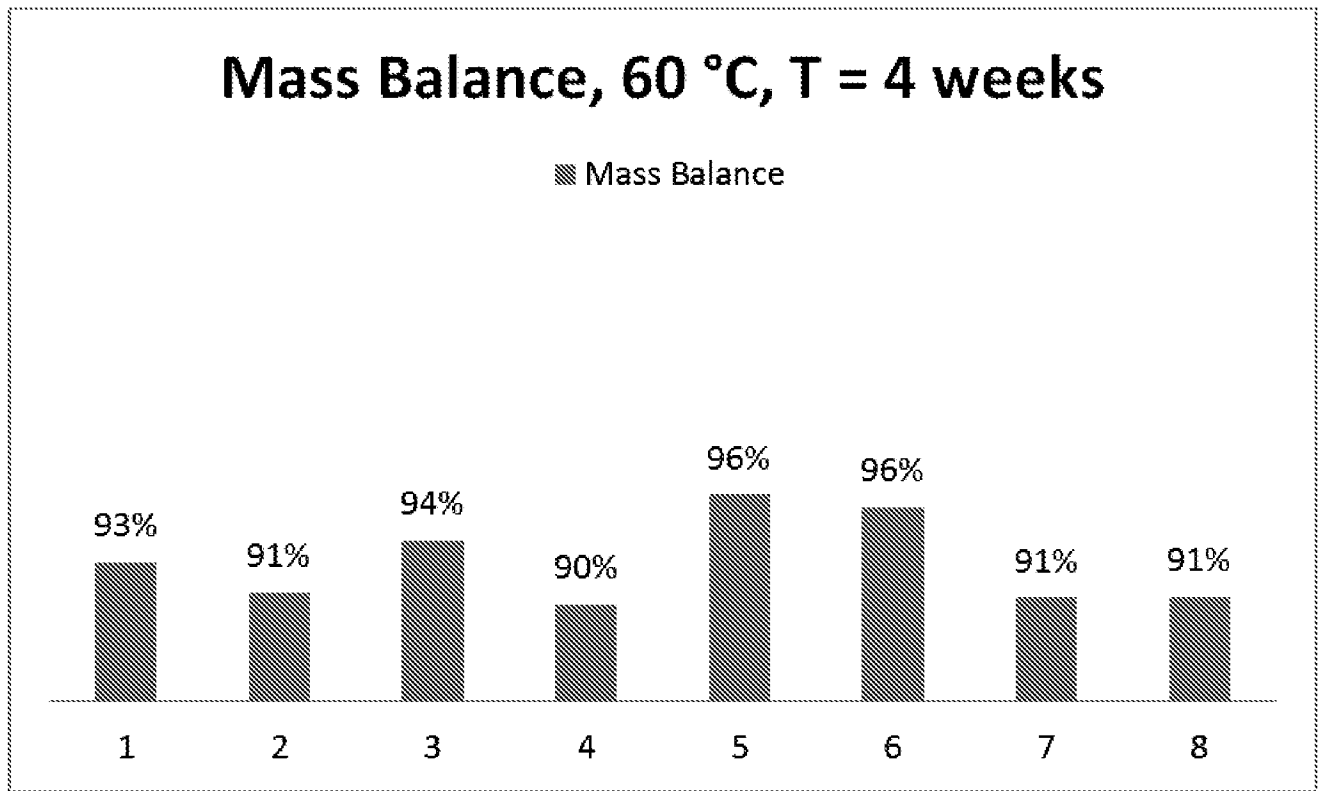


Fig. 4

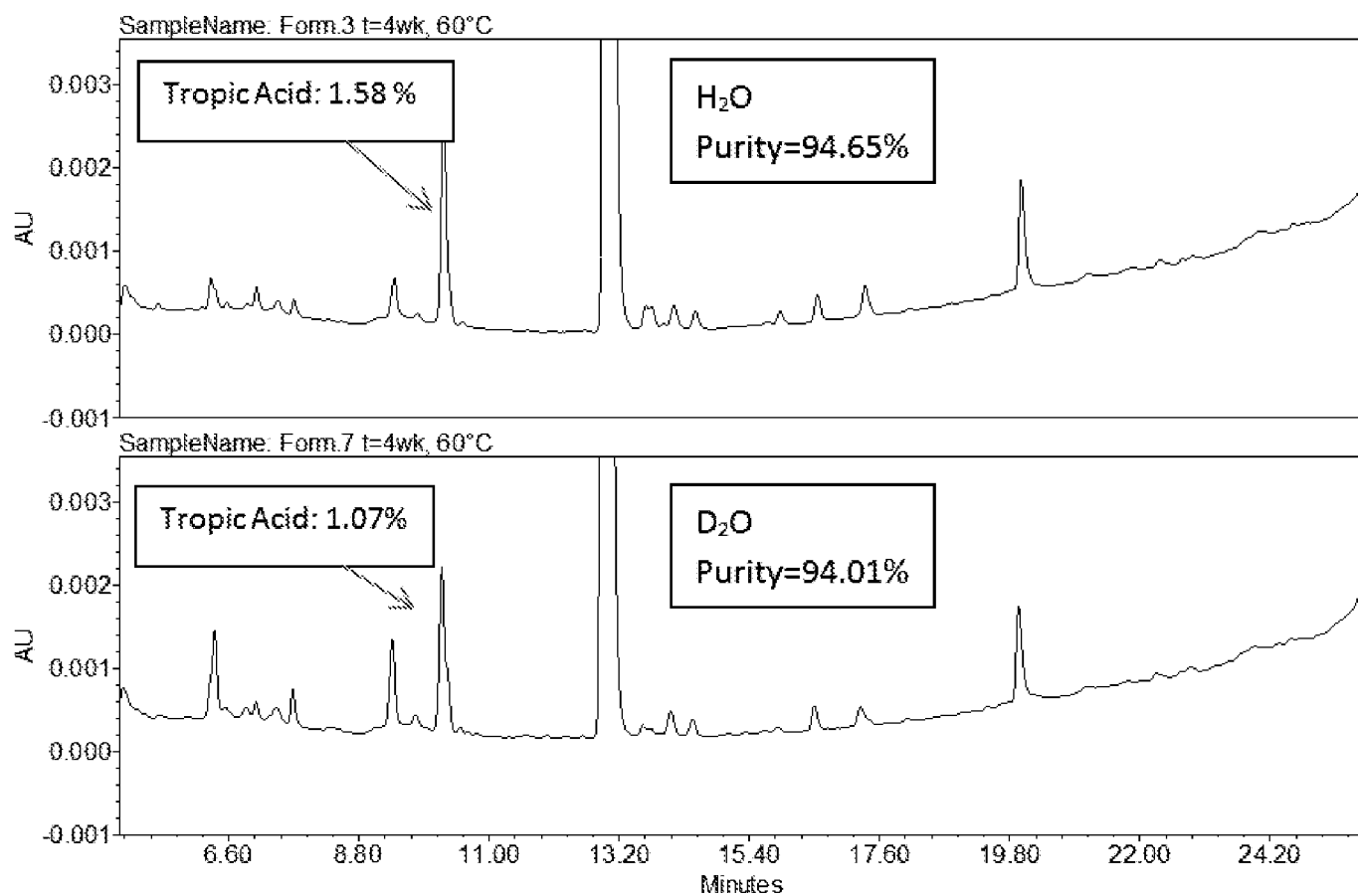


Fig. 5

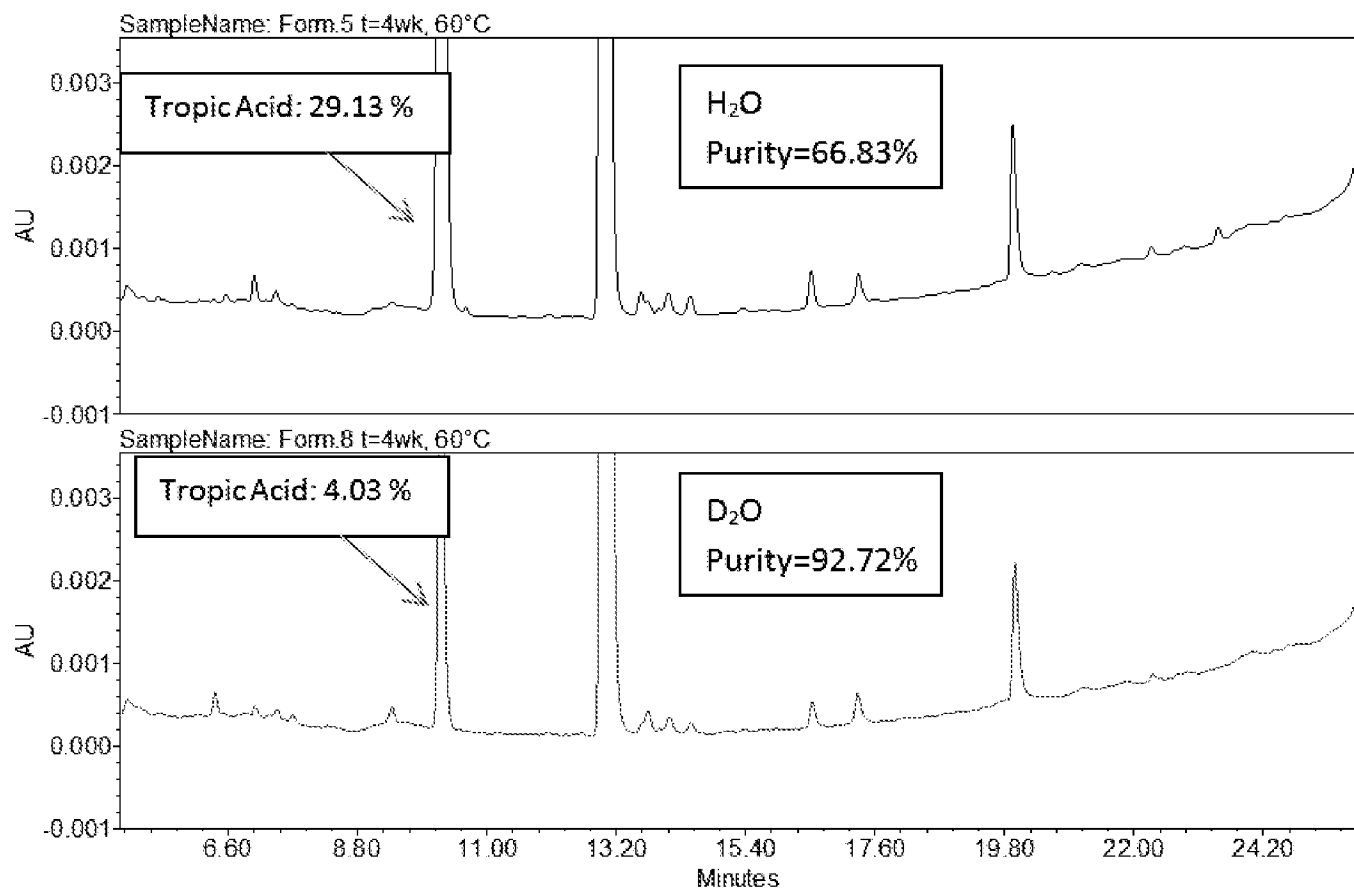


Fig. 6

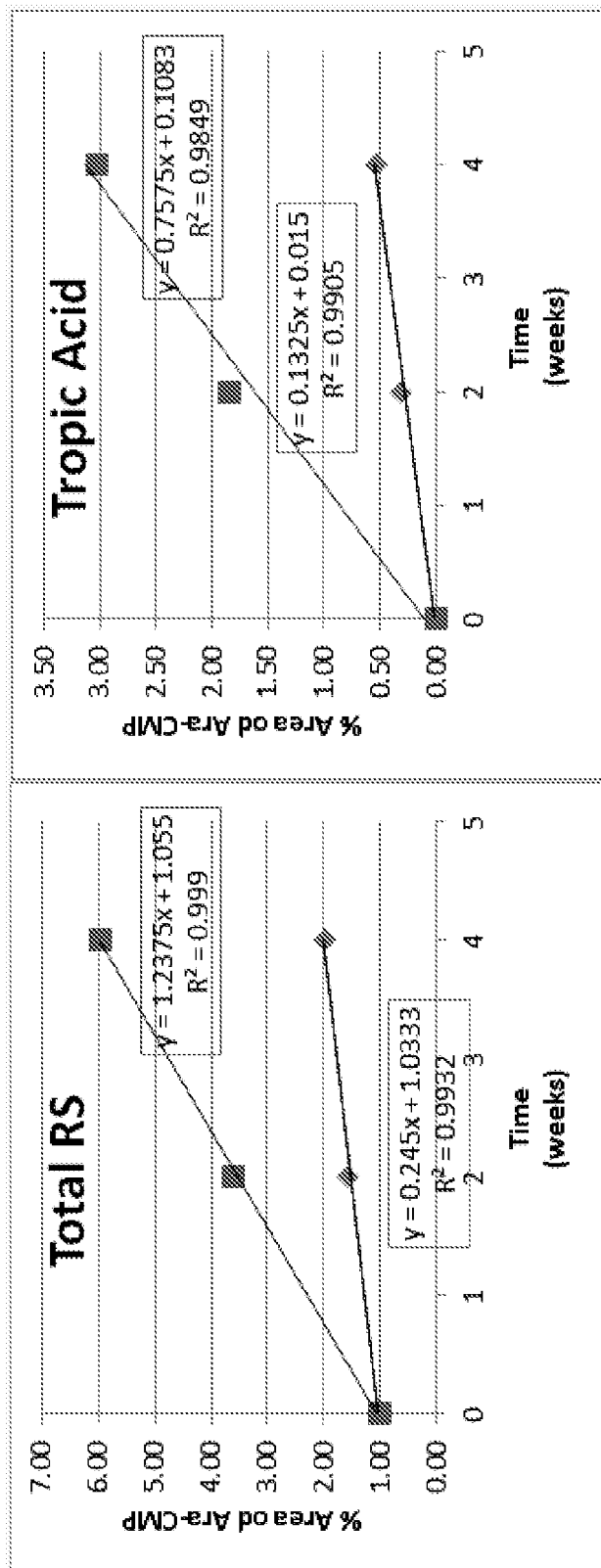


Fig. 7

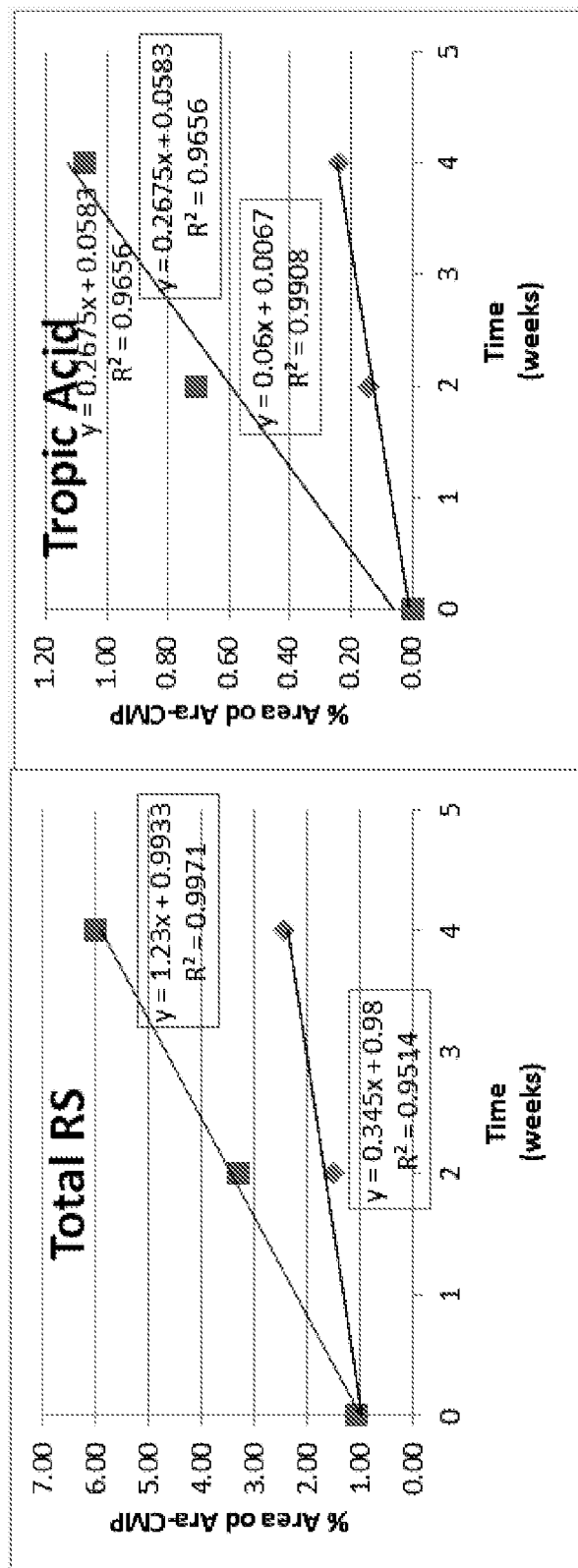


Fig. 8

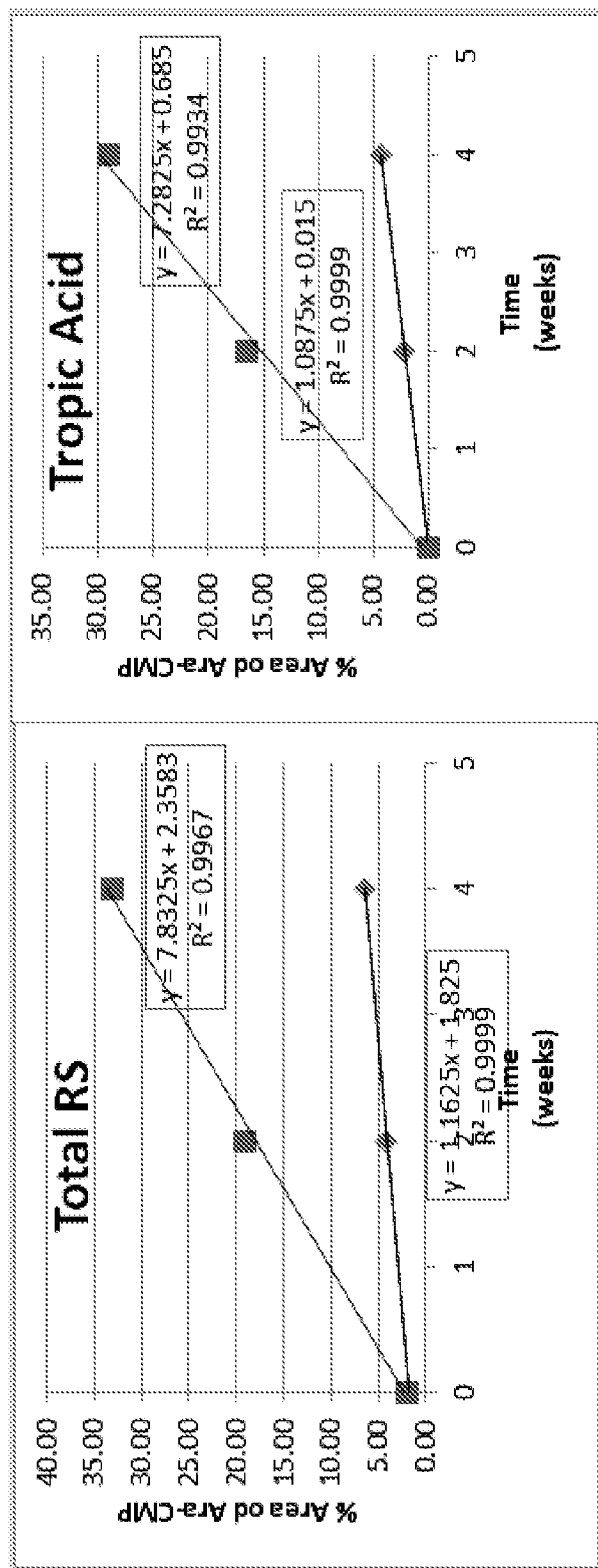


Fig. 9

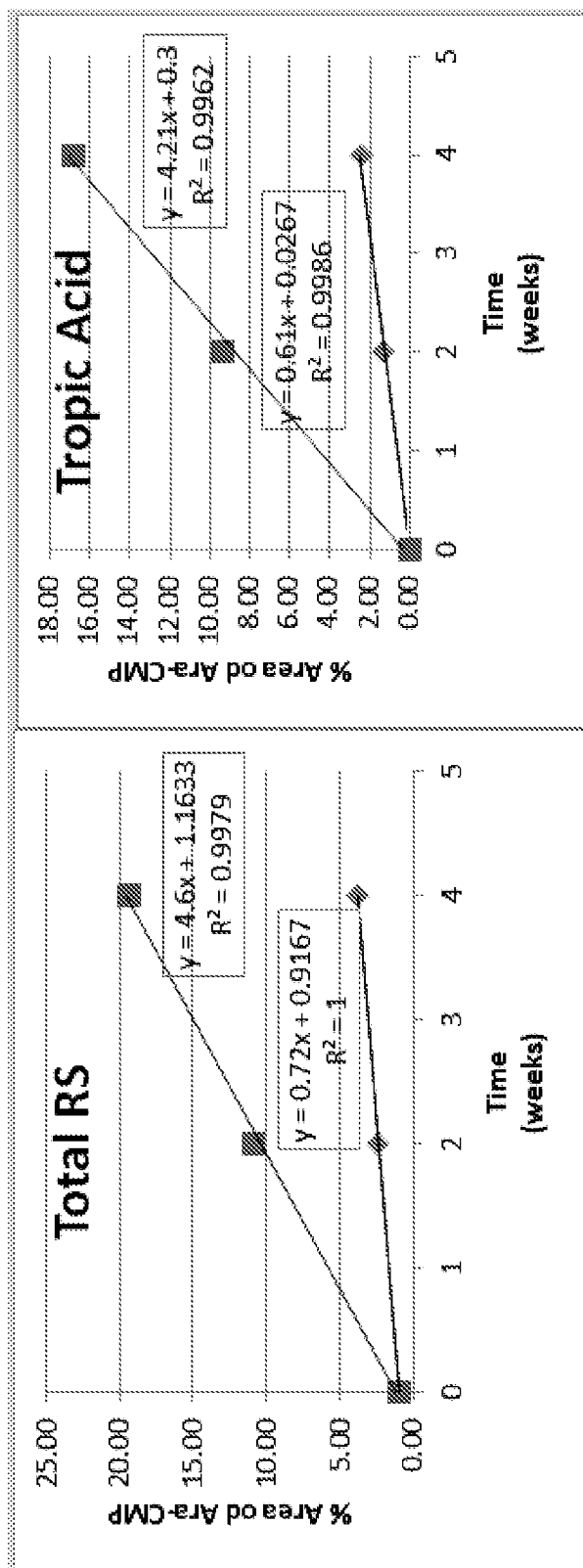
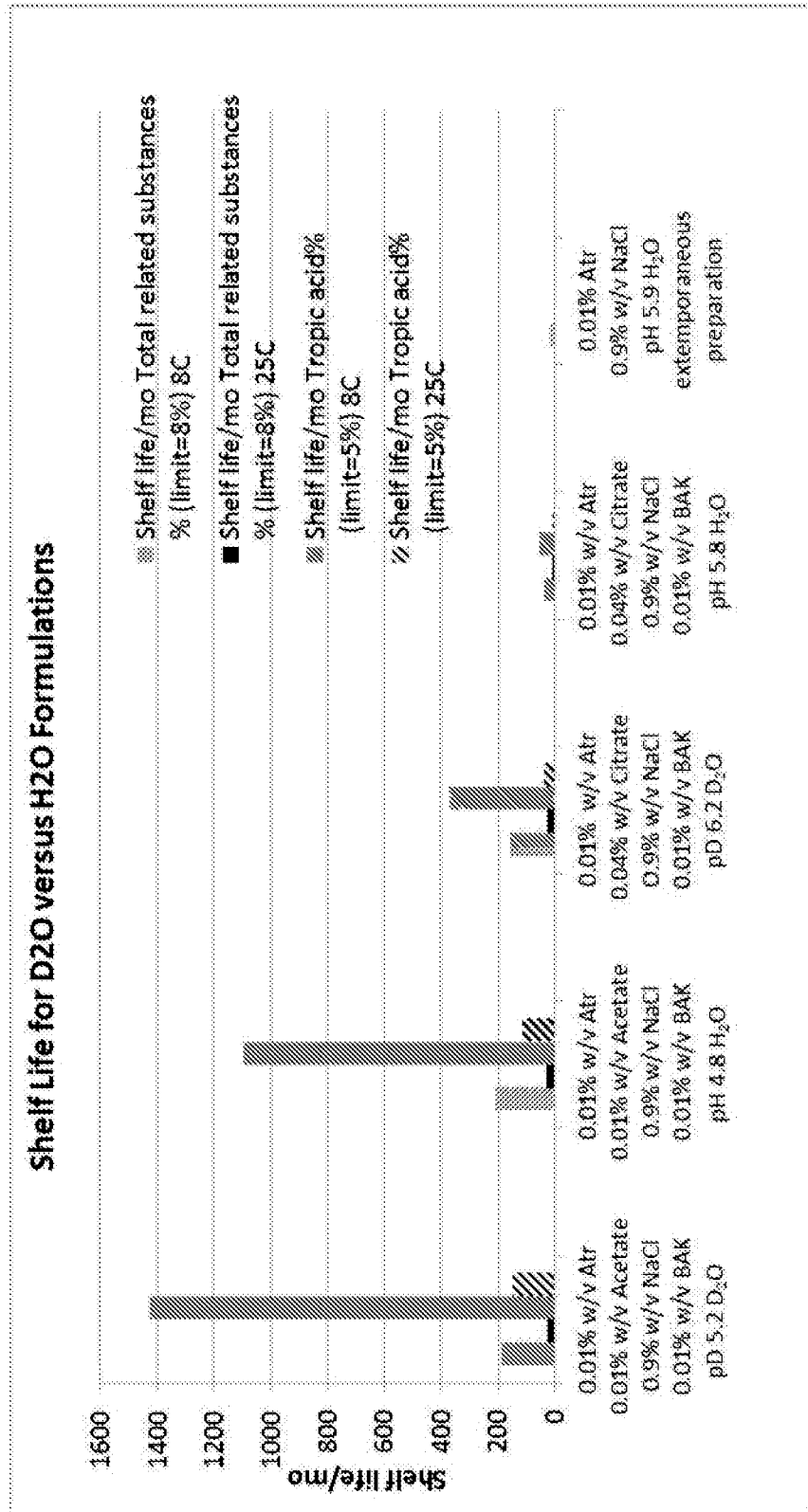


Fig. 10



Electronic Patent Application Fee Transmittal				
Application Number:				
Filing Date:				
Title of Invention:		OPHTHALMIC COMPOSITION		
First Named Inventor/Applicant Name:		Gregory I. Ostrow		
Filer:		Michael J. Hostetler/Linda Anders		
Attorney Docket Number:		46682-701.201		
Filed as Small Entity				
Filing Fees for Track I Prioritized Examination - Nonprovisional Application under 35 USC 111(a)				
Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
Utility filing Fee (Electronic filing)	4011	1	70	70
Utility Search Fee	2111	1	300	300
Utility Examination Fee	2311	1	360	360
Request for Prioritized Examination	2817	1	2000	2000
Pages:				
Claims:				
Claims in excess of 20	2202	10	40	400
Miscellaneous-Filing:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Publ. Fee- Early, Voluntary, or Normal	1504	1	0	0
PROCESSING FEE, EXCEPT PROV. APPLS.	2830	1	70	70
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
Total in USD (\$)				3200

Electronic Acknowledgement Receipt

EFS ID:	22489119
Application Number:	14726139
International Application Number:	
Confirmation Number:	7389
Title of Invention:	OPHTHALMIC COMPOSITION
First Named Inventor/Applicant Name:	Gregory I. Ostrow
Customer Number:	21971
Filer:	Michael J. Hostetler/Linda Anders
Filer Authorized By:	Michael J. Hostetler
Attorney Docket Number:	46682-701.201
Receipt Date:	29-MAY-2015
Filing Date:	
Time Stamp:	18:15:09
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	\$ 3200
RAM confirmation Number	5081
Deposit Account	232415
Authorized User	ANDERS, LINDA

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	TrackOne Request	46682-701-201-TrackOneRequest.pdf	74566	no	1
			9225f6318ce10ed823b921c878809a43831ed2a5		
Warnings:					
Information:					
2	Transmittal of New Application	46682-701-201-Transmittal.pdf	276323	no	2
			3c3477005da382953ff2c960ab096a3cf88bbdca		
Warnings:					
Information:					
3	Application Data Sheet	46882-701-201-ADS.pdf	1561636	no	7
			ad74839ccf05c424574e70293c95898d1b93c1cf		
Warnings:					
Information:					
4		46682-701-201-Application.pdf	819891	yes	108
			ebaa6f27d9d6aa6c42bee93b8bce8708642de0c		
	Multipart Description/PDF files in .zip description				
	Document Description		Start	End	
	Specification		1	103	
	Claims		104	107	
	Abstract		108	108	
Warnings:					
Information:					
5	Drawings-only black and white line drawings	46682-701.pdf	428948	no	10
			74ea25eeec5f5b89a5b6a7cfa1798bede17aa0c9		
Warnings:					
Information:					
6	Fee Worksheet (SB06)	fee-info.pdf	41750	no	2
			4bf3e871ed9dea0d9034cdce062376992ff5aef3		

Warnings:	
Information:	
Total Files Size (in bytes):	3203114
This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503. <u>New Applications Under 35 U.S.C. 111</u> If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application. <u>National Stage of an International Application under 35 U.S.C. 371</u> If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course. <u>New International Application Filed with the USPTO as a Receiving Office</u> If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.	

**CERTIFICATION AND REQUEST FOR PRIORITIZED EXAMINATION
UNDER 37 CFR 1.102(e) (Page 1 of 1)**

First Named Inventor:	Gregory I. Ostrow	Nonprovisional Application Number (if known):	
Title of Invention:	OPHTHALMIC COMPOSITION		

APPLICANT HEREBY CERTIFIES THE FOLLOWING AND REQUESTS PRIORITIZED EXAMINATION FOR THE ABOVE-IDENTIFIED APPLICATION.

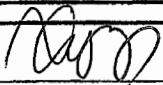
1. The processing fee set forth in 37 CFR 1.17(i)(1) and the prioritized examination fee set forth in 37 CFR 1.17(c) have been filed with the request. The publication fee requirement is met because that fee, set forth in 37 CFR 1.18(d), is currently \$0. The basic filing fee, search fee, and examination fee are filed with the request or have been already been paid. I understand that any required excess claims fees or application size fee must be paid for the application.
2. I understand that the application may not contain, or be amended to contain, more than four independent claims, more than thirty total claims, or any multiple dependent claims, and that any request for an extension of time will cause an outstanding Track I request to be dismissed.
3. The applicable box is checked below:

I. ☒ Original Application (Track One) - Prioritized Examination under § 1.102(e)(1)

- i. (a) The application is an original nonprovisional utility application filed under 35 U.S.C. 111(a). This certification and request is being filed with the utility application via EFS-Web.
---OR---
(b) The application is an original nonprovisional plant application filed under 35 U.S.C. 111(a). This certification and request is being filed with the plant application in paper.
- ii. An executed inventor's oath or declaration under 37 CFR 1.63 or 37 CFR 1.64 for each inventor, or the application data sheet meeting the conditions specified in 37 CFR 1.53(f)(3)(i) is filed with the application.

II. ☐ Request for Continued Examination - Prioritized Examination under § 1.102(e)(2)

- i. A request for continued examination has been filed with, or prior to, this form.
- ii. If the application is a utility application, this certification and request is being filed via EFS-Web.
- iii. The application is an original nonprovisional utility application filed under 35 U.S.C. 111(a), or is a national stage entry under 35 U.S.C. 371.
- iv. This certification and request is being filed prior to the mailing of a first Office action responsive to the request for continued examination.
- v. No prior request for continued examination has been granted prioritized examination status under 37 CFR 1.102(e)(2).

Signature 	Date 05/29/2015
Name (Print/Typed) Xiaofan Yang	Practitioner Registration Number 61,459
Note: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. Submit multiple forms if more than one signature is required.*	
☒ *Total of <u>1</u> forms are submitted.	

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor: Gregory I. Ostrow et al.

Serial Number: 14/726,139

Filing Date: May 28, 2015

Title: OPHTHALMIC COMPOSITION

Group Art Unit: Not assigned

Examiner: Not assigned

CONFIRMATION NO: 7389

FILED ELECTRONICALLY ON: May 29, 2015

Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

INFORMATION DISCLOSURE STATEMENT
UNDER 37 CFR §1.97

Madam/Sir:

An Information Disclosure Statement along with attached PTO/SB/08 is hereby submitted. A copy of each listed publication is submitted, if required, pursuant to 37 CFR §§1.97-1.98, as indicated below.

The Examiner is requested to review the information provided and to make the information of record in the above-identified application. The Examiner is further requested to initial and return the attached PTO/SB/08 in accordance with MPEP §609.

The right to establish the patentability of the claimed invention over any of the information provided herewith, and/or to prove that this information may not be prior art, and/or to prove that this information may not be enabling for the teachings purportedly offered, is hereby reserved.

This statement is not intended to represent that a search has been made or that the information cited in the statement is, or is considered to be, prior art or material to patentability as defined in §1.56.

A. ☒ 37 CFR §1.97(b). This Information Disclosure Statement should be considered by the Office because:

☒ (1) It is being filed within 3 months of the filing date of a national application and is other than a continued prosecution application under §1.53(d);

-- OR --

☐ (2) It is being filed within 3 months of entry of the national stage as set forth in §1.491 in an international application;

-- OR --

- ☐ (3) It is being filed before the mailing of a first Office action on the merits;

-- OR --

- ☐ (4) It is being filed before the mailing of a first Office action after the filing of a request for continued examination under §1.114.

- B. ☐ *37 CFR §1.97(c)*. Although this Information Disclosure Statement is being filed after the period specified in *37 CFR §1.97(b)*, above, it is filed before the mailing date of the earlier of (1) a final office action under §1.113, (2) a notice of allowance under §1.311, or (3) an action that otherwise closes prosecution on the merits, this Information Disclosure Statement should be considered because it is accompanied by one of:

- ☐ a statement as specified in §1.97(e) provided concurrently herewith;

-- OR --

- ☐ a fee of \$180.00 as set forth in §1.17(p) authorized below, enclosed, or included with the payment of other papers filed together with this statement.

- C. ☐ *37 CFR §1.97(d)*. Although this Information Disclosure Statement is being filed after the mailing date of the earlier of (1) a final office action under §1.113 or (2) a notice of allowance under §1.311, it is being filed before payment of the issue fee and should be considered because it is accompanied by:

- i. a statement as specified in §1.97(e);

-- AND --

- ii. a fee of \$180.00 as set forth in §1.17(p) is authorized below, enclosed, or included with the payment of other papers filed together with this Statement.

- D. ☐ *37 CFR §1.97(e)*. Statement.

- ☐ A statement is provided herewith to satisfy the requirement under 37 CFR §§1.97(c);

-- AND/OR --

- ☐ A statement is provided herewith to satisfy the requirement under 37 CFR §§1.97(d);

-- AND/OR --

- ☐ A copy of a dated communication from a foreign patent office clearly showing that the information disclosure statement is being submitted within 3 months of the filing date on the communication is provided in lieu of a statement under 37 C.F.R. § 1.97(e)(1) as provided for under MPEP 609.04(b) V.

- E. ☐ *Statement Under 37 C.F.R. §1.704(d)*. Each item of information contained in the information disclosure statement was first cited in a communication from a patent office in a counterpart foreign or international application or from the Office, or is a communication that was issued by a patent office in a counterpart foreign or international application or by the Office, that was received by an individual designated in § 1.56(c) not more than thirty (30) days prior to the filing of this information disclosure statement. This statement is made pursuant to the requirements of 37 C.F.R. §1.704(d) to avoid reduction of the period of adjustment of the patent term for Applicant(s) delay.

- F. ☒ *37 CFR §1.98(a)(2)*. The content of the Information Disclosure Statement is as follows:

- ☐ Copies of each of the references listed on the attached Form PTO/SB/08 are enclosed herewith.

-- OR --

- ☒ Copies of U.S. Patent Documents (issued patents and patent publications) listed on the attached Form PTO/SB/08 are NOT enclosed.

-- AND/OR --

- ☒ Copies of Foreign Patent Documents and/or Non Patent Literature Documents listed on the attached Form PTO/SB/08 are enclosed in accordance with 37 CFR §1.98 (a)(2).

-- AND/OR --

- ☐ Copies of pending unpublished U.S. patent applications are enclosed in accordance with 37 CFR §1.98(a)(2)(iii).

G. ☒ 37 CFR §1.98(a)(3). The Information Disclosure Statement includes non-English patents and/or references.

- ☐ Pursuant to 37 CFR §1.98(a)(3)(i), a concise explanation of the relevance of each patent, publication or other information provided that is not in English is provided herewith.

- ☐ Pursuant to MPEP 609(B), an English language copy of a foreign search report is submitted herewith to satisfy the requirement for a concise explanation where non-English language information is cited in the search report.

-- OR --

- ☐ A concise explanation of the relevance of each patent, publication or other information provided that is not in English is as follows: _____

- ☒ Pursuant to 37 CFR §1.98(a)(3)(ii), a copy of a translation, or a portion thereof, of the non-English language reference(s) is provided herewith.

H. ☐ 37 CFR §1.98(d). Copies of patents, publications and pending U.S. patent applications, or other information specified in 37 C.F.R. § 1.98(a) are not provided herewith because:

- ☐ Pursuant to 37 CFR §1.98(d)(1) the information was previously submitted in an Information Disclosure Statement, or cited by examiner, for another application under which this application claims priority for an earlier effective filing date under 35 U.S.C. 120.

Application in which the information was submitted: _____

Information Disclosure Statement(s) filed on: _____

AND

- ☐ The information disclosure statement submitted in the earlier application complied with paragraphs (a) through (c) of 37 CFR §1.98.

- I. ☒ *Fee Authorization.* The Commissioner is hereby authorized to charge the above-referenced fees of \$0.00 and charge any additional fees or credit any overpayment associated with this communication to Deposit Account No. 23-2415 (Docket No. 46682-701.201).

Respectfully submitted,

WILSON SONSINI GOODRICH & ROSATI

Dated: May 29, 2015

By: /Xiaofan Yang/
Xiaofan Yang, Reg. No. 61,459

650 Page Mill Road
Palo Alto, CA 94304-1050
(650) 493-9300
Customer No. 021971

SCORE Placeholder Sheet for IFW Content

Application Number: 14726139

Document Date: 05/29/2015

The presence of this form in the IFW record indicates that the following document type was received in electronic format on the date identified above. This content is stored in the SCORE database.

- Drawings – Other than Black and White Line Drawings

Since this was an electronic submission, there is no physical artifact folder, no artifact folder is recorded in PALM, and no paper documents or physical media exist. The TIFF images in the IFW record were created from the original documents that are stored in SCORE.

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