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ORIGINAL ARTICLE

Formulation and stability of an extemporaneous 0.02% chlorhexidine digluconate ophthalmic solution



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KEYWORDS

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Background/Purpose: *Acanthamoeba* keratitis is difficult to treat because *Acanthamoeba* cysts are resistant to the majority of antimicrobial agents. Despite the efficacy of 0.02% chlorhexidine in treating *Acanthamoeba* keratitis, a lack of data in the literature regarding the formulation's stability limits its clinical use. The objective of this study was to develop an optimal extemporaneous 0.02% chlorhexidine digluconate ophthalmic formulation for patients in need. **Methods:** With available active pharmaceutical ingredients, 0.02% chlorhexidine digluconate sample solutions were prepared by diluting with BSS Plus Solution or acetate buffer. Influences of the buffer, type of container, and temperature under daily-open condition were assessed based on the changes of pH values and chlorhexidine concentrations of the test samples weekly. To determine the beyond-use date, the optimal samples were stored at 2–8°C or room temperature, and analyzed at time 0 and at Week 1, Week 2, Week 3, Week 4, Week 5, Week 8, Week 12, and Week 24.

Results: Despite chlorhexidine exhibiting better stability in acetate buffer than in BSS solution, its shelf-life was < 14 days when stored in a light-resistant low-density polyethylene container. The acetate-buffered 0.02% chlorhexidine digluconate solution stored in light-resistant high-density polyethylene eyedroppers did not exhibit significant changes in pH or strength at any time interval.

Conclusion: The acetate-buffered 0.02% chlorhexidine digluconate ophthalmic solution stored in light-resistant high-density polyethylene eyedroppers demonstrated excellent stability at 2

Conflicts of interest: The authors have no conflicts of interest relevant to this article.

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–25°C for 6 months after being sealed and for 1 month after opening. This finding will enable us to prepare 0.02% chlorhexidine digluconate ophthalmic solutions based on a doctor's prescription.

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Introduction

Acanthamoeba keratitis, a corneal infectious disease, is difficult to treat because *Acanthamoeba* cysts are resistant to the majority of existing antimicrobial agents. Compared with other pharmaceutical agents, biguanides have the lowest minimal amebicidal and cysticidal concentration, and 0.02% biguanide ophthalmic preparations are the drug of choice for *Acanthamoeba* keratitis.^{1,2} However, these products are not commercially available in Taiwan.

Chlorhexidine is a biguanide (Fig. 1) and the drug of choice for *Acanthamoeba* keratitis.³ It is a strong base, practically insoluble in water at 20°C, and available in different salt forms, including dihydrochloride, diacetate, and digluconate. Different chlorhexidine salts have varying water solubilities.^{4,5} Chlorhexidine digluconate is freely soluble in water, is manufactured as a 20% w/v stock aqueous solution, and should be protected from light during storage.⁶ Some licensed 20% chlorhexidine digluconate products obtained from domestic manufacturers and chlorhexidine digluconate concentrate imported from the reagent supplier Sigma-Aldrich (Steinheim, Germany) can be used as raw materials.

Chlorhexidine digluconate is extensively used as an antiseptic because of its broad-spectrum coverage of Gram (+) and Gram (–) bacteria.⁷ Chlorhexidine 0.005% has been used as a pharmaceutical preservative, particularly in ophthalmic solutions.^{5,6} However, high concentrations of chlorhexidine are irritating to mucous membranes, and a concentration not to exceed 0.05% is recommended when applied to wounds and burns to decrease the risk of anaphylactic reaction.⁸ Recently, several studies have demonstrated that 0.02% chlorhexidine possesses excellent amebicidal activity and minimal corneal epithelial toxicity.^{1–3} Despite reports of the efficacy of 0.02% chlorhexidine in the treatment of *Acanthamoeba* keratitis, a lack of data in the literature regarding the formulation's stability limits its clinical use. To the best of our knowledge, only Moorfields Pharmaceuticals (London) in the United Kingdom has commercialized 0.02% chlorhexidine ophthalmic products. Ordering the product from abroad is not only expensive, but also labor and time intensive, which may result in a delay in treatment. Therefore,

extemporaneous compounding of chlorhexidine digluconate 0.02% ophthalmic solutions is needed in Taiwan.

The aim of this study was to develop an optimal extemporaneous 0.02% chlorhexidine digluconate ophthalmic formulation with an appropriate pH value and osmolality for patients with *Acanthamoeba* keratitis, to ensure that the sterility, particulate matter, and assay of the final product meet the general characteristics of ophthalmic solutions in pharmacopoeia, and to determine the type of container, storage condition, and beyond-use date of the final product. This is the very first report on the formulation and stability of an optimal extemporaneous chlorhexidine digluconate 0.02% ophthalmic solution. Our hospital, with 60-year experiences in extemporaneous compounding and in-house quality control for medications, would like to share this formula with the world.^{9–12}

Methods

Materials

Chlorhexidine acetate standard was obtained from US Pharmacopeia (USP; Rockville, MD, USA). Chlorhexidine digluconate 20% was provided by Schutz Dishman Biotech Limited (Ahmedabad, India). Anhydrous sodium acetate (CH₃COONa), glacial acetic acid (CH₃COOH), sodium dihydrogen phosphate monohydrate (NaH₂PO₄·H₂O), triethylamine, and acetonitrile were purchased from Merck (Darmstadt, Germany). Light-resistant low-density polyethylene (LDPE) containers for ophthalmic solutions were provided by Sinphar (I-Lan, Taiwan), and light-resistant high-density polyethylene (HDPE) containers were provided by Chin-Tai Plastic Ind. Co., Ltd (Changhua, Taiwan). Balanced salt solution (BSS Plus Solution), which contains sodium chloride, potassium chloride, calcium chloride, magnesium chloride hexahydrate, sodium acetate, and sodium citrate, was obtained from Alcon Laboratories, Inc. (Fort Worth, TX, USA).

Experimental design and sample preparation

With available active pharmaceutical ingredients, we formulated a 0.02% chlorhexidine digluconate ophthalmic solution and investigated the effects of temperature and buffer on its stability in two types of containers, HDPE eyedroppers and LDPE eyedroppers. Other parameters, such as pH value, osmolality, particulate matter concentration, and sterility, were also assessed. The beyond-use date was determined through these tests.

Experimental implementation of various physical conditions encountered clinically is listed in Table 1 and can be divided into three stages. In the first stage, eight sets of

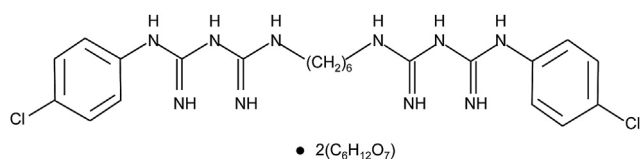


Figure 1 Chemical structure of chlorhexidine digluconate.

Table 1 Experimental design for developing an optimal 0.02% chlorhexidine digluconate ophthalmic preparation.

Experiment stage	Solvent	Type of container	Storage temperature	Opened thrice daily or sealed	Test time point
1	Acetate buffer	LDPE	RT	Opened thrice daily	D0, W1, W2, W3, W4
	Acetate buffer	LDPE	2–8°C	Opened thrice daily	D0, W1, W2, W3, W4
	Acetate buffer	LDPE	RT	Sealed	D0, W4
	Acetate buffer	LDPE	2–8°C	Sealed	D0, W4
	BSS	LDPE	RT	Opened thrice daily	D0, W1, W2, W3, W4
	BSS	LDPE	2–8°C	Opened thrice daily	D0, W1, W2, W3, W4
	BSS	LDPE	RT	Sealed	D0, W4
	BSS	LDPE	2–8°C	Sealed	D0, W4
2	Acetate buffer	Glass	RT	Opened thrice daily	D0, W1, W2
	Acetate buffer	HDPE	RT	Opened thrice daily	D0, W1, W2
3	Acetate buffer	HDPE	RT	Opened thrice daily	D0, W1, W2, W3, W4, W5
	Acetate buffer	HDPE	2–8°C	Opened thrice daily	D0, W1, W2, W3, W4, W5
	Acetate buffer	HDPE	RT	Sealed	D0, W4, W8, W12, W24
	Acetate buffer	HDPE	2–8°C	Sealed	D0, W4, W8, W12, W24

BSS = balanced salt solution; D0 = Day 0; HDPE = high-density polyethylene; LDPE = low-density polyethylene; RT = room temperature; W1 = Week 1; W2 = Week 2; W3 = Week 3; W4 = Week 4; W5 = Week 5; W8 = Week 8; W12 = Week 12; W24 = Week 24.

test samples were used to study the influence of buffer and temperature under daily-open or sealed conditions. Six test samples were prepared for every condition at each test time point. Three of them were used for pH value measurement and high-performance liquid chromatography (HPLC) analysis, and the others for sterility tests. Furthermore, the amount of particulate matter and osmolality were measured every 4 weeks, so another six test samples were prepared for Week 4 in every sample set, three for particulate matter measurement and the others for osmolality measurement. In the second stage, triplicate 0.02% acetate-buffered chlorhexidine digluconate test samples were prepared, stored in glass and HDPE containers, and analyzed on Day 0, Week 1, and Week 2. In the third stage, the stability of the final products was studied. Four sets of test samples were prepared and analyzed on Day 0 and Week 1, Week 2, Week 3, Week 4, Week 5, Week 8, Week 12, and Week 24. The study items at each test time point were the same as those in the first stage.

Test samples for chlorhexidine digluconate (0.2 mg/mL) solution was prepared by dilution with either acetate buffer (composed of 141.4mM CH₃COONa and 7.8mM CH₃COOH) or BSS Plus Solution in a laminar flow cabinet in an ISO class 5 clean room using an aseptic technique. After mixing, the solutions were filtered through a 0.22 µm membrane and stored in the eyedroppers. Each dropper was filled with 10 mL of the test sample. Influences of the buffer, type of container, and temperature under daily-open or sealed conditions were studied. The stability at room temperature was determined in a laboratory where the humidity was 52–65%, and the temperature was <25°C in the daytime and 25–28°C during the evening and nighttime. The schema in Fig. 2 presents the development of the formulation and the evaluation of its stability.

We simulated the contact time of the solution to the air in clinical settings by study design. In the daily-open groups, we opened the eyedroppers thrice daily (at 9:00 AM, 1:00 PM, and 5:00 PM), shook them for a while, and let the eyedroppers stand for 10 minutes prior to being

closed. Thus, the total time of exposure of the test samples to the environment, 30 minutes per day, was not significantly different from clinical scenarios where a 0.02% chlorhexidine ophthalmic solution is given every 2 hours.

Analysis by HPLC

Chlorhexidine digluconate was assayed by an HPLC method according to the USP. This method was validated for determining chlorhexidine digluconate in various pharmaceutical formulations.¹³ To achieve the optimal performance, the flow rate and mobile phase composition of the method were slightly modified.

The HPLC system was an Agilent 1100 LC (Agilent Technologies, Santa Clara, CA, USA) comprising a G1329A

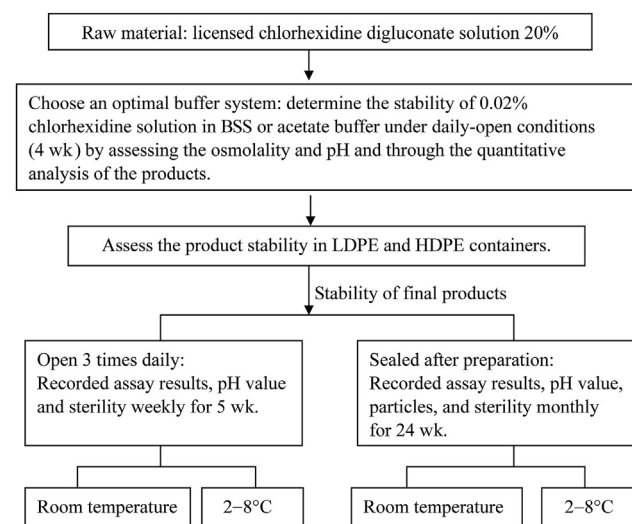


Figure 2 Formulation development and stability evaluation. BSS = balanced salt solution; HDPE = high-density polyethylene; LDPE = low-density polyethylene.

automatic injector, a G1322A degasser, a G1311A quaternary pump, a G1315B diode array detector, and ChemStation software. A reversal C18 column (LiChroCART 250-4 LiChroSphere C₁₈, 250 mm × 4 mm, particle size 5 µm) from Merck was set at a temperature of 40°C. The detector was operated at a wavelength of 239 nm, and the injection volume of the sample was 50 µL.

Mobile phase A was prepared by dissolving 27.6 g of monobasic sodium phosphate monohydrate and 5 mL of triethylamine in 750 mL of water (adjusted to a pH of 3.0 using phosphoric acid), diluting with water to 2 L, and mixing the resulting solution with acetonitrile (70:30). Mobile phase B was acetonitrile. The gradient setting was as follows: isocratic with 100% mobile phase A for 7 minutes, linear gradient to 50% mobile phase A from 7 minutes to 22 minutes, isocratic at 50% mobile phase A from 22 minutes to 27 minutes, linear gradient to 0% mobile phase B from 27 minutes to 27.1 minutes, and finally isocratic with 100% mobile phase A from 27.1 minutes to 35 minutes. The total running time was 35 minutes at a flow rate of 1.2 mL/minute. The retention time of chlorhexidine was 15 minutes.

The chlorhexidine acetate reference standard (molecular weight 625.55) 0.25 mg/mL stock solution was prepared in a 100 mL volumetric flask by dilution with mobile phase A. Working solutions of chlorhexidine acetate were prepared by diluting the stock solution with mobile phase A to six concentrations ranging from 0.01 mg/mL to 0.06 mg/mL.

Quality control samples were included in each analytical sequence in triplicate to ensure the linearity, intraday precision, interday precision, and accuracy of the analysis. A calibration curve was constructed by plotting the peak area of chlorhexidine as a function of the chlorhexidine acetate concentration (range 0.01–0.06 mg/mL). Prior to injection, 3 mL of the 0.02% chlorhexidine digluconate eye-drop samples were pipetted accurately into a 10 mL volumetric flask and diluted to volume with mobile phase A. Triplicate HPLC determinations were performed on all the samples of each test solution.

The initial concentrations (time 0) of chlorhexidine digluconate were referenced as 100%, and all subsequent concentrations were expressed as percentages of the initial concentration. The stability of the chlorhexidine gluconate in the ophthalmic solution was assessed by evaluating the percent change in the concentration from time 0. A decrease of more than 10% of the initial concentration was considered a significant loss of the drug.

Measurements of pH value, sterility, particulate matter, and osmolality

The pH value was measured by a Radiometer PHM210 pH meter (Radiometer, Lyon, France). The concentration of particulate matter was measured by APS 2000 (Particle Measuring Systems, Boulder, CO, USA). The osmolality was measured by an automatic autocal micro-osmometer (Roebeling, Berlin, Germany).

Microbial contamination of each sample was determined according to the USP sterility test using sterility-testing accessories, and microbiological media were purchased from Pall Corporation (New York, NY, USA).

Results

In this study, an optimal 0.02% extemporaneous chlorhexidine digluconate ophthalmic formulation, with a pH of 5.9 and an osmolality of 270 mOsmol/kg, was developed. When stored at 2–25°C, the formulation has a 6-month beyond-use date when sealed in a light-resistant HDPE dropper, and is stable for 1 month when opened for 10 minutes thrice daily.

Stability of chlorhexidine digluconate in BSS or acetate buffer stored in LDPE containers

The osmolality of the 0.02% chlorhexidine digluconate eye drops in BSS was 300 mOsmol/kg and remained unchanged during the testing period. However, the pH value increased from 7.7 to 8.3, and the concentration decreased drastically and was undetectable on Day 7 when stored at room temperature, as well on Day 14 when stored at 2–8°C (Fig. 3). The concentration of particulate matter measured in BSS was higher than that measured in the acetate buffer.

The pH value and osmolality of the 0.02% chlorhexidine solution in acetate buffer remained unchanged at near 5.9 and 270 mOsmol/kg, respectively, during the study period. On Day 14, concentrations of chlorhexidine decreased by > 10% from the initial value. Compared with the BSS solutions, 0.02% chlorhexidine in acetate buffer exhibited better stability, although its shelf-life was < 14 days when stored in an LDPE container (Fig. 3).

Change of container

LDPE containers are the most commonly used eyedroppers. However, these can adsorb chlorhexidine.⁶ A 2-week preliminary test to examine the concentration changes of the chlorhexidine gluconate solution in an acetate buffer stored in non-LDPE containers found that the 0.02% chlorhexidine digluconate concentration remained unchanged after 2 weeks. Therefore, an HDPE container was used for the following studies.

Stability of the final products

When opened for 10 minutes thrice daily, the chlorhexidine digluconate solution was stable throughout the 5-week study period, irrespective of whether it was refrigerated or stored at room temperature (Table 2). The chlorhexidine digluconate solution remained stable for 24 weeks when sealed in HDPE containers, regardless of whether it was refrigerated or stored at room temperature (Table 3).

The pH value of the tested eye-drop samples in acetate buffer was measured to be 5.9 initially and did not differ by more than one-tenth of a pH unit over the study period. The osmolality was similarly stable.

Ophthalmic solutions should be essentially free from particles large enough to be observed on visual inspection. The USP suggests that ophthalmic solutions should not exceed concentrations of 50 pieces/mL and 5 pieces/mL for particles ≥ 10 µm and ≥ 25 µm, respectively.¹⁴ Although the amount of particulate matter in the 0.02% chlorhexidine ophthalmic solutions increased slightly over time, it

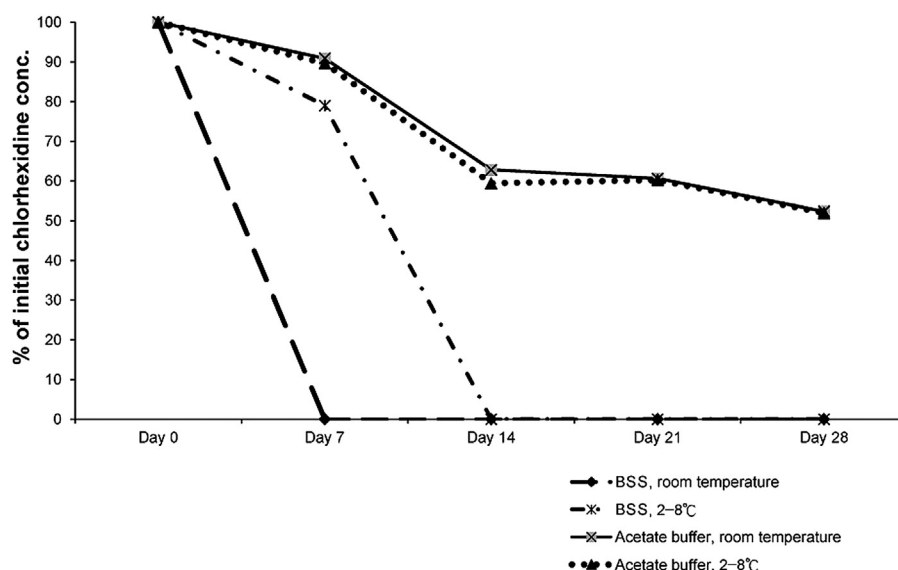


Figure 3 Stability of 0.02% chlorhexidine digluconate solution using BSS or acetate buffer as the solvent in LDPE containers when opened thrice daily. BSS = balanced salt solution; conc. = concentration; LDPE = low-density polyethylene.

remained within an acceptable range (Table 3, Fig. 4). No microbial contamination was evident under any of the experimental conditions.

Discussion

Formulation development

Preparation of ophthalmic solutions requires careful consideration of isotonicity and buffering agents. According to USP 34, an ideal ophthalmic solution should be isotonic to a 0.9% sodium chloride solution (normal saline). However, the eyes can tolerate osmolalities ranging from

190 mOsmol/kg (0.6% sodium chloride solution) to 650 mOsmol/kg (2.0% sodium chloride solution) without marked discomfort.^{15,16}

Normal tears have a pH of approximately 7.4 and possess some buffering capacity, making ophthalmic solutions with pH values between 3.5 and 8.5 acceptable.¹⁵ Aqueous solutions of chlorhexidine are most stable within a pH range of 5–8, and the optimal pH range for antimicrobial activity is 5.5–7.0.⁶ The type of buffer solution is an essential determinant of the safety and effectiveness of an eye drop. Chlorhexidine digluconate cannot be diluted with normal saline due to the low solubility of chlorhexidine hydrochloride (<1 mg/100 mL) and is incompatible with sodium lauryl sulfate, sodium carboxymethyl cellulose, etc.⁶

Table 2 Stability of a 0.02% chlorhexidine ophthalmic solution in a high-density polyethylene container when opened thrice daily.

Tests	Day 0	Week 1	Week 2	Week 3	Week 4	Week 5
Initial concentration (%)						
RT	100.0 ± 1.7	102.8 ± 2.0	97.3 ± 0.8	99.1 ± 0.2	101.1 ± 1.2	102.9 ± 0.4
2–8°C		102.3 ± 5.9	98.0 ± 0.5	100.1 ± 1.2	100.1 ± 0.6	102.4 ± 0.9
pH value						
RT	5.95 ± 0.01	5.92 ± 0.01	5.92 ± 0.02	5.97 ± 0.01	5.91 ± 0.01	5.92 ± 0.01
2–8°C		5.93 ± 0.02	5.93 ± 0.01	5.95 ± 0.02	5.93 ± 0	5.91 ± 0.02
Particulate matter (pieces/mL)						
RT ≥10 μm	0.1 ± 0.2	NP	NP	NP	1.7 ± 0.6	NP
RT ≥25 μm	ND	NP	NP	NP	0.1 ± 0.2	NP
2–8°C ≥10 μm	0.1 ± 0.2	NP	NP	NP	1.0 ± 1.7	NP
2–8°C ≥25 μm	ND	NP	NP	NP	ND	NP
Osmolality (mOsm/kg)						
RT	270.1 ± 0.6	NP	NP	NP	270.0 ± 0	NP
2–8°C		NP	NP	NP	272.7 ± 1.2	NP
Sterility						
RT	Sterile	Sterile	Sterile	Sterile	Sterile	Sterile
2–8°C		Sterile	Sterile	Sterile	Sterile	Sterile

ND = not detectable; NP = not performed; RT = room temperature.

Table 3 Stability of 0.02% chlorhexidine ophthalmic solutions in sealed HDPE containers under optimized conditions.

Examination items	Initial conditions	Open date of the sealed HDPE bottles				
	Day 0	Week 4	Week 8	Week 12	Week 24	
Initial concentration (%)						
RT	100.0 ± 1.7	99.7 ± 0.3	99.9 ± 0.3	97.3 ± 0.2	99.9 ± 0.4	
2–8°C		100.0 ± 0.1	99.8 ± 0.2	97.1 ± 0.4	98.8 ± 0.7	
pH value						
RT	5.95 ± 0.01	5.93 ± 0.04	5.89 ± 0.03	5.89 ± 0.01	5.93 ± 0.04	
2–8°C		5.89 ± 0.01	5.93 ± 0.04	5.87 ± 0.02	5.96 ± 0.04	
Particulate matter (pieces/mL)						
RT	≥10 μm	2.4 ± 0.5	1.3 ± 1.0	3.1 ± 0.5	4.2 ± 2.3	4.5 ± 5.1
	≥25 μm	ND	0.1 ± 0.2	ND	0.7 ± 0.9	0.4 ± 0.2
2–8°C	≥10 μm	2.4 ± 0.5	1.2 ± 0.5	2.4 ± 1.2	6.6 ± 2.8	6.1 ± 3.1
	≥25 μm	ND	ND	ND	0.1 ± 0.2	0.6 ± 0.4
Osmolality (mOsm/kg)						
RT	270.1 ± 0.6	273.0 ± 0	273.3 ± 0.3	272.8 ± 0.3	272.7 ± 1.2	
2–8°C		272.0 ± 0	273.3 ± 0.6	272.3 ± 1.2	272.0 ± 1.0	
Sterility						
RT	Sterile	Sterile	Sterile	Sterile	Sterile	
2–8°C		Sterile	Sterile	Sterile	Sterile	

HDPE = high-density polyethylene; ND = not detectable; RT = room temperature.

In this study, we had used sterile water to dilute the 20% chlorhexidine digluconate raw material to 0.02% chlorhexidine digluconate. The pH value of the 0.02% chlorhexidine digluconate solution in water was 5.9, but the osmolality was <10 mOsmol/kg. Because solutions of low osmolality may cause irritation and swelling of the cornea cells,¹⁷ addition of a buffering agent was necessary to adjust the tonicity of the chlorhexidine eye drops.

BSS Sterile Irrigating Solution is an isotonic solution for eye irrigation. Because some hospitals use BSS to compound the 0.02% chlorhexidine solution, it was included in our study. By contrast, Denton⁶ found that sodium acetate may

be used to adjust the tonicity of chlorhexidine solutions without causing precipitation; however, the pH value of the buffered solution may be as high as 8.0. Therefore, acetic acid was added to create an acetic acid–acetate buffer system. To prepare a solution with a pH value near 6, we adjusted the concentrations of acetic acid and sodium acetate according to the Henderson–Hasselbach equation.¹⁸ Chlorhexidine solution is not only stable, but also exhibits good antimicrobial activity at a pH value of about 6. After optimizing the pH value and osmolality, the acetate buffer solution comprising 141.4mM CH₃COONa and 7.8mM CH₃COOH (pH 5.9, 270 mOsmol/kg) was included in our study.

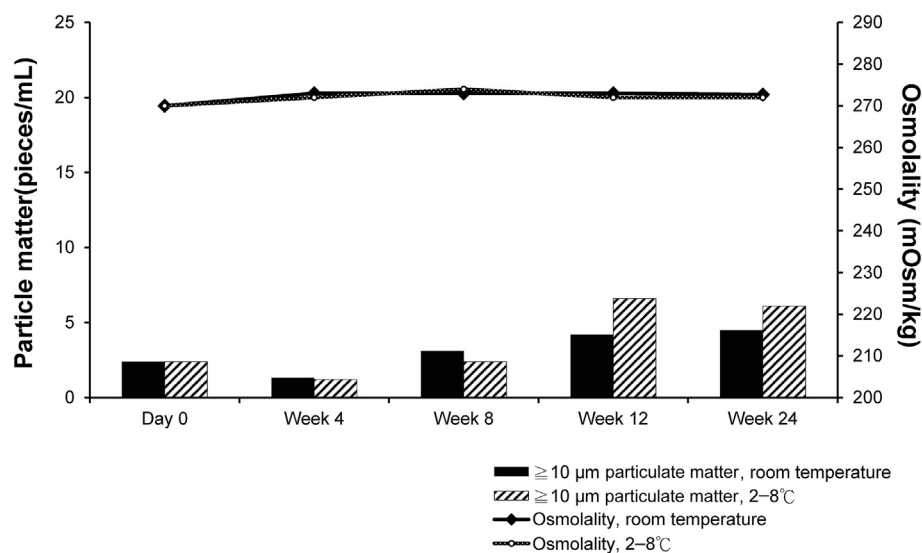


Figure 4 Osmolality and particulate matter concentration of 0.02% chlorhexidine digluconate ophthalmic solutions sealed in high-density polyethylene containers.

Compatibility of chlorhexidine digluconate with BSS and acetate buffer

The content of chlorhexidine digluconate in BSS-buffered solution stored in an LDPE eyedropper decreased drastically. The concentrations were close to 0 on Day 7 when stored at room temperature, and on Day 14 when stored at 2–8°C. However, the acetate-buffered chlorhexidine digluconate solution in LDPE containers retained 90% of the initial concentrations on Day 7, 60% on Day 14, and about 50% on Day 28. In addition, the amount of particulate matter measured in BSS was higher than that measured in the acetate buffer. Obviously, acetate buffer is a more appropriate solvent than BSS to compound the 0.02% chlorhexidine digluconate preparation. Moreover, chlorhexidine is incompatible with inorganic anions.⁶ BSS contains approximately 0.13 mEq/mL chloride, which might have made the BSS-buffered chlorhexidine digluconate solution unstable. Therefore, we chose only acetate buffer as a solvent for the 0.02% chlorhexidine digluconate preparation in the experiment of the next stage.

Influence of LDPE and HDPE containers on the stability of chlorhexidine digluconate

Although the content of chlorhexidine digluconate in acetate-buffered solution stored in a light-resistant LDPE eyedropper decreased over a certain period of time, the rate of decline slowed down after Day 14. The pH value did not change despite the drop in concentrations. There may be some factors other than buffer solutions that influenced the stability of acetate-buffered chlorhexidine solution. A statement "Low-density polyethylene may be unsuitable (for chlorhexidine) because of excessive adsorption..." was found in a textbook.⁶ Therefore, amber glass vials and light-resistant HDPE eyedroppers were chosen in the second stage. In the 2-week preliminary test, concentration of the 0.02% chlorhexidine digluconate remained unchanged. This result confirmed our speculation that LDPE containers can adsorb chlorhexidine. Therefore, the stability of the optimal preparation, 0.02% chlorhexidine digluconate eye drop in acetate buffer stored in light-resistant HDPE containers, was studied for 24 weeks in the third stage. The results show that the formulation has an excellent stability when stored in an HDPE eyedropper.

Conclusion

This is the very first report on the formulation and stability of an extemporaneous chlorhexidine digluconate 0.02% ophthalmic solution. An optimal extemporaneous chlorhexidine digluconate 0.02% ophthalmic solution was prepared by diluting a 20% chlorhexidine digluconate solution with acetate buffer (pH 5.9 and osmolality 270 mOsmol/kg) in a laminar flow hood in an ISO class 5 clean room, filtering the resulting solution through a 0.22 µm membrane, and dispensing into a sterile HDPE dropper aseptically. When stored at 2–25°C, the acetate-buffered 0.02% chlorhexidine digluconate ophthalmic solution stored in light-resistant HDPE eyedroppers demonstrated excellent

stability for 6 months after being sealed, and for 1 month after being opened. This stability will allow us to provide 0.02% chlorhexidine digluconate ophthalmic solutions to patients suffering from *Acanthamoeba* keratitis based on a doctor's prescription.

Acknowledgments

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