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(54) **Pharmaceutical compositions with deuterium oxide**

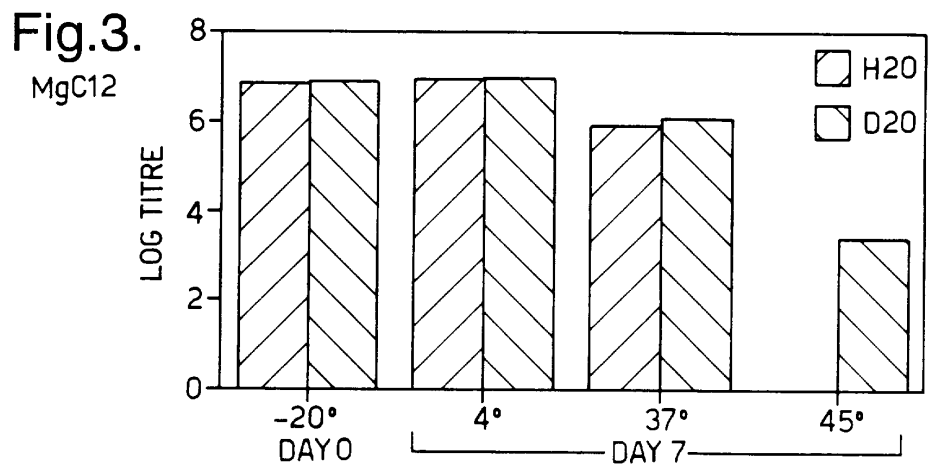
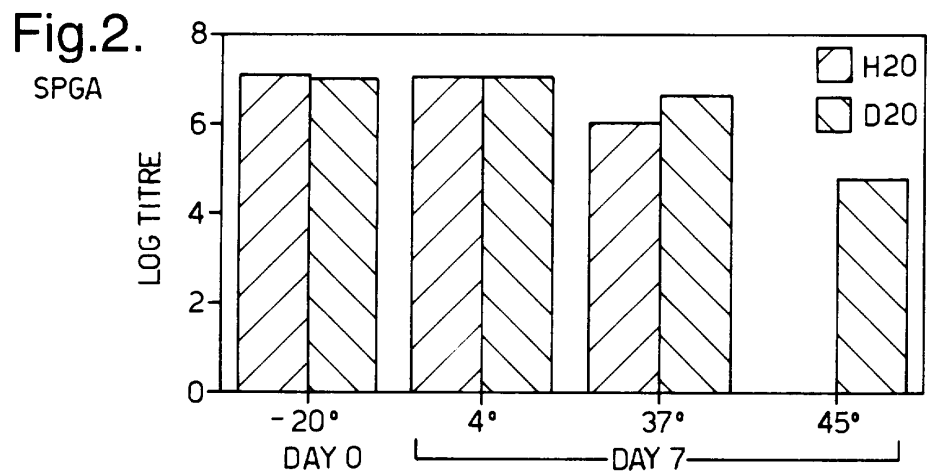
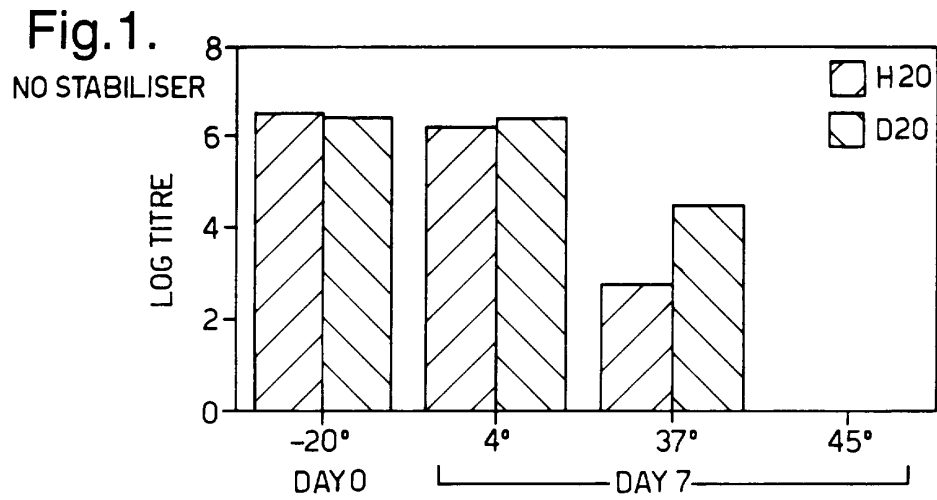
(57) A pharmaceutical composition comprises a solution or suspension of a biological agent in a diluent comprising D<sub>2</sub>O, which solution or suspension contains sufficient amounts of a sugar, phosphate, glutamic acid or a physiologically acceptable salt thereof and, optionally, a non-toxic protein, a peptone or a mixture of two or more amino acids besides glutamic acid to enhance the stability of the agent. Typically, the composition is a suspension of a live attenuated poliovirus or an inactivated poliovirus of each of poliovirus types 1, 2 and 3. Preferably the solution or suspension contains stabilising amounts of sucrose, KH<sub>2</sub>PO<sub>4</sub>, K<sub>2</sub>HPO<sub>4</sub>, L-glutamic acid or sodium glutamate and human serum albumin (SPGA).

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At least one drawing originally filed was informal and the print reproduced here is taken from a later filed formal copy.

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GRAPHS OF MEAN LOG<sub>10</sub> TITRE AT DAY 0 AND DAY 7



PHARMACEUTICAL COMPOSITIONS

The present invention relates to stabilised pharmaceutical compositions and to their preparation.

Biological agents such as proteins, nucleic acids and  
5 live attenuated or inactivated viruses can be formulated in a  
number of ways for pharmaceutical use. They may be formulated  
as aqueous solutions or dispersions. Such solutions or  
dispersions should, however, have a shelf-life of several  
months or, better, a year or more to take account of the time  
10 that may elapse between manufacture and use.

It is impossible to store solutions or dispersions of  
many biological agents at room temperature for prolonged  
periods of time without deterioration. This problem is overcome  
in some instances by freeze-drying to form a lyophilisate. The  
15 lyophilisate then has to be reconstituted prior to use which  
can itself be a difficulty. Alternatively, solutions or  
dispersions may be stored at a reduced temperature such as in a  
refrigerator in order to reduce degradation.

We have now found that solutions or dispersions of  
20 biological agents can be better stabilised by using deuterium  
oxide as the principal diluent in combination with a number of  
other components. Especially, live virus vaccines can be  
better stabilised.

Accordingly, the present invention provides a  
25 pharmaceutical composition which comprises a solution or  
suspension of a biological agent in a diluent comprising  $D_2O$ ,  
which solution or suspension contains sufficient amounts of a  
sugar, phosphate, glutamic acid or a physiologically acceptable  
salt thereof and, optionally, a non-toxic protein, a peptone or

a mixture of two or more amino acids besides glutamic acid to enhance the stability of the agent.

The invention can be applied to biological macromolecules such as proteins and nucleic acids. It may be applied to  
5 recombinant proteins. The proteins may be cytokines, enzymes or antibodies such as interferons, interleukins, antigens and enzymes. The protein may be erythropoetin, blood clotting factor VIII, insulin, human growth hormone or another growth factor. The nucleic acid may be DNA or RNA.

10 The invention can also be applied to viruses, in particular to live attenuated or inactivated viruses. Such viruses may be used in vaccines. They are not therefore virulent. The invention is particularly applicable to live virus vaccines.

15 The virus may be a RNA virus or a DNA virus. The virus may be influenza virus or poliovirus. The invention can thus be applied to live attenuated or inactivated poliovirus types 1, 2 or 3. A preferred composition of the invention is a suspension of a live attenuated poliovirus or of an inactivated poliovirus  
20 of each of poliovirus types 1, 2 and 3.

Any vaccine strain of poliovirus may be employed. The strain may therefore be a Sabin strain type 1, 2 or 3 poliovirus. Sabin strains are live attenuated polioviruses. Further, attenuated polioviruses can be constructed by genetic  
25 mutation, for example by genetic mutation of the 5' non-coding region of the poliovirus genome. Alternatively, the invention can be applied to Salk type 1, 2 and 3 polioviruses. Salk vaccines are poliovirus vaccines containing inactivated poliovirus.

The sugar in the pharmaceutical composition of the invention is typically a mono-, di-saccharide or tri-saccharide such as glucose, fructose, sucrose, lactose, maltose or cellobiose Preferably it is sucrose.

5       The phosphate is typically an orthophosphate. It may be potassium dihydrogen orthophosphate,  $\text{KH}_2\text{PO}_4$ , and/or dipotassium hydrogen orthophosphate,  $\text{K}_2\text{HPO}_4$ . The phosphate can act as a buffer, for example to ensure that a solution or suspension according to the invention has a pH of from 6.2 to 7.2 such as  
10 from 6.5 to 7.0.

Preferably the glutamic acid is L-glutamic acid. The preferred salt is the monosodium salt.

The optional non-toxic protein, peptone or mixture of two or more amino acids other than glutamic acid is generally  
15 albumin. The albumin can be bovine serum albumin or preferably human serum albumin.

The biological agent is dissolved or dispersed in a diluent comprising  $\text{D}_2\text{O}$ . The diluent thus consists essentially of  $\text{D}_2\text{O}$ .  $\text{D}_2\text{O}$  is the principal diluent. Minor amounts of other  
20 physiologically acceptable diluents miscible with  $\text{D}_2\text{O}$  may be present, typically  $\text{H}_2\text{O}$ . For example, another diluent such as  $\text{H}_2\text{O}$  may be present in an amount of up to 30%, for example up to 20%, by weight of the composition; for example from 1, or from 5, to 10, or to 15%, by weight.

25       The amount of the biological agent varies depending upon the nature of the agent and the desired final concentration of the agent. The agent may be present in the pharmaceutical composition of the invention in an amount of from 1 to 20% by weight, for example from 5 to 10% by weight.

In the case of viruses, the amount present may be represented by a titre. A suitable titre may therefore be from  $10^{6.5}$  to  $10^{7.5}$  per ml, for example  $10^{6.8}$  to  $10^{7.2}$  per ml and more especially about  $10^{6.9}$  per ml. Such titres are particularly  
5 appropriate in the case of a poliovirus.

An advantage of the present invention is that less of an active agent may be provided in a composition than would otherwise be the case. An excess or "overage" of an agent may be initially provided in a composition to take account of  
10 degradation of the active agent in the period of time between manufacture of the composition and use. As the present compositions exhibit enhanced stability, this overage can be reduced.

The amount of  $D_2O$  in the composition may vary depending  
15 upon, for example, the amount of biological agent that is present. Typically the amount of  $D_2O$  is from 70 to 95% by weight of the composition, for example from 75 to 90% or from 75 to 85%. The  $D_2O$  itself has an effect in increasing the stability of the compositions of the invention.

20 Sufficient amounts of (a) a sugar, (b) phosphate, (c) glutamic acid or a physiologically acceptable salt thereof and, optionally, (d) a non-toxic protein, a peptone or a mixture of two or more amino acids besides glutamic acid are required so that the stability of the agent in the composition is enhanced.  
25 Suitable stabilising amounts of each component are:

- (a) 0.5 to 3%, preferably 0.8 to 1.5%, by weight such as about 1% by weight;
- (b) 0.5 to 1.5%, preferably 0.7 to 1%, by weight such as about 0.8% by weight;

- (c) 0.2 to 1%, preferably 0.3 to 0.5%, by weight such as about 0.4% by weight; and
- (d) 0.5 to 3%, preferably 1 to 2%, by weight such as about 1.5% by weight.

5       When (c) is glutamic acid, preferably a composition of the invention contains a weight ratio of (a):(b):(c):optionally (d) of about 1:0.76:0.37:0.46. This ratio especially applies when (a) is sucrose, (b) is (b')KH<sub>2</sub>PO<sub>4</sub> and (b'')K<sub>2</sub>HPO<sub>4</sub>, (c) is L-glutamic acid and (d) is human serum albumin. In this event,  
10 the preferred weight ratio of (a):(b'):(b''):(c):optionally (d) is about 1:0.40:0.36:0.37:0.46.

      When (c) is sodium glutamate, preferably a composition of the invention contains a weight ratio of (a):(b):(c):optionally (d) of about 1:0.76:0.49:0.46. This ratio  
15 especially applies when (a) is sucrose, (b) is (b')KH<sub>2</sub>PO<sub>4</sub> and (b'')K<sub>2</sub>HPO<sub>4</sub>, (c) is sodium glutamate and (d) is human serum albumin. In this event, the preferred weight ratio of (a):(b'):(b''):(c): optionally (d) is about 1:0.40:0.36:0.49:0.46.

20       It is believed that a synergistic effect may be operating to increase the stability of the compositions of the invention. Preferably, therefore, (a) to (c) and optionally (d) and, indeed, the D<sub>2</sub>O and (a) to (c) and optionally (d) are present in respective amounts which provide a synergistic stabilising  
25 effect.

      A particularly useful composition according to the invention comprises a biological agent dissolved or dispersed in a D<sub>2</sub>O SPG or SPGA solution. D<sub>2</sub>O SPG and SPGA solutions are:

SPG solution

a solution in D<sub>2</sub>O of: 1.09%, for example 1.085%, w/v sucrose,  
0.43%, for example 0.432%, w/v KH<sub>2</sub>PO<sub>4</sub>,  
0.39%, for example 0.392%, w/v K<sub>2</sub>HPO<sub>4</sub>,  
5 and  
0.40%, for example 0.403%, w/v  
L-glutamic acid, or 0.53%, for example  
0.539%, w/v sodium glutamate;

SPGA solution

10 a solution in D<sub>2</sub>O of: 1.09%, for example 1.085%, w/v sucrose,  
0.43%, for example 0.432%, w/v KH<sub>2</sub>PO<sub>4</sub>,  
0.39%, for example 0.392%, w/v K<sub>2</sub>HPO<sub>4</sub>,  
0.40%, for example 0.403%, w/v  
L-glutamic acid or 0.53%, for example  
15 0.529%, w/v sodium glutamate, and  
0.50% w/v human serum albumin.

A SPG solution may alternatively be defined as 0.218 M  
sucrose, 0.0038 M KH<sub>2</sub>PO<sub>4</sub>, 0.0072 M K<sub>2</sub>HPO<sub>4</sub> and 0.0049 M  
monosodium glutamate in D<sub>2</sub>O. Similarly, a SPGA solution may be  
20 defined as 0.218 M sucrose, 0.0038 M KH<sub>2</sub>PO<sub>4</sub>, 0.0072 M K<sub>2</sub>HPO<sub>4</sub>,  
0.0049 M monosodium glutamate and 1% by weight albumin in D<sub>2</sub>O.

A composition of the invention may be a single-strength  
D<sub>2</sub>O SPG or SPGA solution as defined above. Alternatively, it  
may be above or below a single-strength solution. It may be  
25 from a half to five times single-strength, for example from  
one to four times single-strength. A D<sub>2</sub>O SPG or SPGA solution  
of variable strength may thus be present.

A composition of the invention may contain additional  
components. For example, a poliovirus vaccine may contain one



or more antibiotics. Typically, the vaccine may contain trace amounts of polymixin and/or neomycin. The poliovirus vaccine will necessarily contain live attenuated strains of all three types of poliovirus or inactivated strains of all three types  
5 of poliovirus.

A composition of the invention is prepared according to the invention by a process which comprises dissolving or dispersing the biological agent in a D<sub>2</sub>O solution of the said amounts of the sugar, phosphate, glutamic acid or salt thereof  
10 and, optionally, non-toxic protein, peptone or mixture of two or more amino acids other than glutamic acid.

This may be achieved in any appropriate fashion. In the case where the biological agent is a poliovirus, the poliovirus is first grown in tissue culture such as in human diploid cells  
15 or monkey kidney cells. The tissue culture fluid is harvested and sterilised through a filter. Concentrates obtained in this way of poliovirus strains of each of types 1, 2 and 3 can then be blended together. Then the resulting blend is dissolved or dispersed in the D<sub>2</sub>O solution according to the invention. The  
20 D<sub>2</sub>O solution can first be sterilised, for example by passing through a sterilising filter.

The solution or dispersion obtained by the process of the invention is generally sterilised by passing through a sterilising filter. The sterilised solution or dispersion may  
25 then be dispensed into containers, glass or plastic, which are then sealed. The containers are typically unit-dose or multi-dose containers such as ampoules or vials.

These containers can be stored under appropriate conditions. Depending upon the nature of the biological agent,

they may be stored at 4 to 8°C such as in a refrigerator or at room temperature such as on the shelf. The solutions or dispersions within the containers do, however, exhibit enhanced stability and so may be stored for longer than previously under the same conditions as were used previously. Alternatively, they may not need to be stored in a refrigerator when previous solutions were so stored.

The following Examples illustrate the invention. In the accompanying drawings, Figures 1, 2 and 3 are graphs of mean  $\log_{10}$  titre of poliovirus in  $H_2O$  and in  $D_2O$  at day 0 and at day 7 when no stabiliser is added (Figure 1), when SPGA is added as a stabiliser (Figure 2) and when  $MgCl_2$  is added as a stabiliser (Figure 3).

15

Example 1: Preparation of diluent solutions

The following diluent solutions were prepared.  $D_2O$  was from Sigma, product no. D-4501, stated to be 99.9%  $D$ .  $MgCl_2$  was from Fisons, product no. M/0600/53. Albumin was human albumin 20% B.P. (British Pharmacopoeia). No antibiotics were added to the solutions. The solutions were sterilised by passing through a sterilising filter. s.s. = single strength and 4x = four times single strength.

(a) 4x SPG

25 $KH_2PO_4$	0.173 g
$K_2HPO_4$	0.157 g
Sucrose	0.434 g
L-Glutamic acid	0.161 g
Water for Injections (WFI) or $D_2O$	to 10 ml

(b) s.s. SPGA

KH <sub>2</sub> PO <sub>4</sub>	0.432 g
K <sub>2</sub> HPO <sub>4</sub>	0.392 g
Sucrose	1.085 g
5 L-Glutamic acid	0.403 g
Albumin (added after pH adjustment and sterilisation)	7.5 ml
WFI or D <sub>2</sub> O.....to 100 ml	
4M KOH was added to adjust the pH to 6.6.	

10

(c) 1M MgCl<sub>2</sub>

20.3g of MgCl<sub>2</sub>.6H<sub>2</sub>O was dissolved in 100ml of WFI or D<sub>2</sub>O

(d) 3M MgCl<sub>2</sub>

15 6.1g of MgCl<sub>2</sub>.6H<sub>2</sub>O was dissolved in 10ml of WFI

Example 2: Preparation of Formulations

Formulations of poliovirus type 3 Sabin strain were prepared. A target titre of 10<sup>6.1</sup> per 0.15ml dose was required which corresponded to a titre of 10<sup>6.92</sup> per ml. A batch of the poliovirus was grown in human diploid cells in culture to a titre of approximately 10<sup>8.0</sup>. The culture was sterilised by passing through a sterilising filter. The following formulations were prepared:

25

Formulation	Formula	ml/100ml
1. No Stabiliser	Type III virus	8.3
	WFI	91.7

2. No Stabiliser + D <sub>2</sub> O	Type III virus	8.3
	WFI	7.7
	D <sub>2</sub> O	84.0
3. SPGA	Type III virus	8.3
	4xSPG(made in WFI)	2.8
	Albumin	0.8
	s.s. SPGA(made in WFI)	88.1
4. SPGA + D <sub>2</sub> O	Type III virus	8.3
	4xSPG(made in D <sub>2</sub> O)	2.8
	Albumin	0.8
	s.s. SPGA(made in D <sub>2</sub> O)	88.1
5 5. MgCl <sub>2</sub>	Type III virus	8.3
	3M MgCl <sub>2</sub> (made in WFI)	4.2
	1M MgCl <sub>2</sub> (made in WFI)	87.5
6. MgCl <sub>2</sub> + D <sub>2</sub> O	Type III virus	8.3
	3M MgCl <sub>2</sub> (made in WFI)	4.2
	1M MgCl <sub>2</sub> (made in WFI)	3.5
	1M MgCl <sub>2</sub> (made in D <sub>2</sub> O)	84.01

Formulations 3 and 4 were in fact single strength SPGA. Thorough mixing of the blends was ensured with minimum 10 agitation. Solutions containing D<sub>2</sub>O were opened to the air for as short a time as possible to prevent exchange with hydrogen ions from atmospheric H<sub>2</sub>O. For each formulation, the solutions were mixed in the order indicated in the Table. Using an Eppendorf repeater pipette fitted with a 50ml syringe filler, 15 the formulations were aliquoted into 1ml samples in bijou bottles, 80 samples per formulation. Each of the formulations

was sampled at day 0 and stored at 4°C, 37°C and 45°C for sampling on future days as below:

5	Temperature	Day	No. of replicates
		0	8
	4°C	1	8
		2	8
		7	8
	37°C	1	8
		2	8
		7	8
	45°C	1	8
		2	8
		7	8

10

The samples were then kept at -20°C until ready for analysis. Virus titres were determined by an Infectivity Titre test. The Infectivity Titre test complies with WHO TRS800. The results are as follows:

15 Day 0

	Sample Description	Total Virus Content (TCID <sub>50</sub> /ml)	Mean Titre (95% Fiducial Limits)
20	1. No Stabiliser	10 <sup>7.02</sup> , 10 <sup>6.57</sup> , 10 <sup>6.44</sup> *	10 <sup>6.50</sup> (10 <sup>6.32</sup> -10 <sup>6.68</sup> )

5	2. No Stabiliser + D <sub>2</sub> O	10 <sup>6.58</sup> , 10 <sup>6.33</sup> , 10 <sup>6.39</sup> *	10 <sup>6.42</sup> (10 <sup>6.28</sup> -10 <sup>6.56</sup> )
	3. SPGA	10 <sup>7.19</sup> , 10 <sup>6.94</sup> , 10 <sup>7.04</sup>	10 <sup>7.07</sup> (10 <sup>6.94</sup> -10 <sup>7.19</sup> )
	4. SPGA + D <sub>2</sub> O	10 <sup>7.02</sup> , 10 <sup>6.96</sup> , 10 <sup>7.02</sup>	10 <sup>7.00</sup> (10 <sup>6.86</sup> -10 <sup>7.15</sup> )
	5. MgCl <sub>2</sub>	10 <sup>6.86</sup> , 10 <sup>6.81</sup> , 10 <sup>6.93</sup>	10 <sup>6.85</sup> (10 <sup>6.71</sup> -10 <sup>6.99</sup> )
	6. MgCl <sub>2</sub> + D <sub>2</sub> O	10 <sup>7.01</sup> , 10 <sup>6.81</sup> , 10 <sup>6.86</sup>	10 <sup>6.89</sup> (10 <sup>6.75</sup> -10 <sup>7.03</sup> )
	Type III Standard	10 <sup>8.12</sup> , 10 <sup>8.21</sup> , 10 <sup>8.05</sup>	10 <sup>8.13</sup> (10 <sup>7.99</sup> -10 <sup>8.27</sup> )

\*Outside limits

Day 7 at 4°C

5	Sample Description	Total Virus Content (TCID <sub>50</sub> /ml)	Mean Titre (95% Fiducial Limits)
	1. No Stabiliser	10 <sup>6.19</sup> 10 <sup>6.19</sup>	10 <sup>6.19</sup> (10 <sup>6.00</sup> -10 <sup>6.38</sup> )
	2. No Stabiliser + D <sub>2</sub> O	10 <sup>6.45</sup> 10 <sup>6.28</sup>	10 <sup>6.37</sup> (10 <sup>6.15</sup> -10 <sup>6.58</sup> )
	3. SPGA	10 <sup>7.01</sup> 10 <sup>7.06</sup>	10 <sup>7.04</sup> (10 <sup>6.86</sup> -10 <sup>7.21</sup> )
10	4. SPGA + D <sub>2</sub> O	10 <sup>6.96</sup> 10 <sup>7.14</sup>	10 <sup>7.04</sup> (10 <sup>6.89</sup> -10 <sup>7.19</sup> )
	5. MgCl <sub>2</sub>	10 <sup>6.97</sup> 10 <sup>6.96</sup>	10 <sup>6.96</sup> (10 <sup>6.80</sup> -10 <sup>7.14</sup> )
	6. MgCl <sub>2</sub> + D <sub>2</sub> O	10 <sup>7.01</sup> 10 <sup>6.95</sup>	10 <sup>6.98</sup> (10 <sup>6.80</sup> -10 <sup>7.16</sup> )
	Type III Standard	10 <sup>8.08</sup> 10 <sup>8.13</sup>	10 <sup>8.11</sup> (10 <sup>7.95</sup> -10 <sup>8.28</sup> )

15

Day 7 at 37°C

20	Sample Description	Total Virus Content (TCID <sub>50</sub> /ml)	Mean Titre (95% Fiducial Limits)
	1. No Stabiliser	10 <sup>2.67</sup> 10 <sup>2.87</sup>	10 <sup>2.77</sup> (10 <sup>2.59</sup> -10 <sup>2.95</sup> )
	2. No Stabiliser + D <sub>2</sub> O	10 <sup>4.33</sup> 10 <sup>4.64</sup>	10 <sup>4.49</sup> (10 <sup>4.33</sup> -10 <sup>4.64</sup> )
	3. SPGA	10 <sup>6.00</sup> 10 <sup>6.06</sup>	10 <sup>6.03</sup> (10 <sup>5.86</sup> -10 <sup>6.20</sup> )
	4. SPGA + D <sub>2</sub> O	10 <sup>6.65</sup> 10 <sup>6.58</sup>	10 <sup>6.61</sup> (10 <sup>6.44</sup> -10 <sup>6.78</sup> )
25	5. MgCl <sub>2</sub>	10 <sup>5.88</sup> 10 <sup>5.92</sup>	10 <sup>5.91</sup> (10 <sup>5.75</sup> -10 <sup>6.06</sup> )
	6. MgCl <sub>2</sub> +D <sub>2</sub> O	10 <sup>6.13</sup> 10 <sup>6.07</sup>	10 <sup>6.09</sup> (10 <sup>5.92</sup> -10 <sup>6.26</sup> )
	Type III Standard	10 <sup>8.08</sup> 10 <sup>8.13</sup>	10 <sup>8.11</sup> (10 <sup>7.95</sup> -10 <sup>8.28</sup> )

Day 7 at 45°C

5	Sample Description	Total Virus Content (TCID <sub>50</sub> /ml)	Mean Titre (95% Fiducial Limits)
	1. No Stabiliser	No viable poliovirus	
	2. No Stabiliser + D <sub>2</sub> O	No viable poliovirus	
10	3. SPGA	No viable poliovirus	
	4. SPGA + D <sub>2</sub> O	10 <sup>4.81</sup> 10 <sup>4.77</sup>	10 <sup>4.79</sup> (10 <sup>4.52</sup> -10 <sup>4.97</sup> )
	5. MgCl <sub>2</sub>	No viable poliovirus	
	6. MgCl <sub>2</sub> + D <sub>2</sub> O	10 <sup>3.51</sup> 10 <sup>3.32</sup>	10 <sup>3.42</sup> (10 <sup>3.20</sup> -10 <sup>3.64</sup> )
	Type III Standard	10 <sup>8.08</sup> 10 <sup>8.13</sup>	10 <sup>8.11</sup> (10 <sup>7.95</sup> -10 <sup>8.28</sup> )

15

The results can be summarised as follows:

	Mean Titre	No Stabiliser		SPGA		MgCl <sub>2</sub>	
		-D <sub>2</sub> O	+D <sub>2</sub> O	-D <sub>2</sub> O	+D <sub>2</sub> O	-D <sub>2</sub> O	+D <sub>2</sub> O
20	Day 0	10 <sup>6.50</sup>	10 <sup>6.42</sup>	10 <sup>7.07</sup>	10 <sup>7.00</sup>	10 <sup>6.85</sup>	10 <sup>6.89</sup>
	Day 7 4°C	10 <sup>6.19</sup>	10 <sup>6.37</sup>	10 <sup>7.04</sup>	10 <sup>7.04</sup>	10 <sup>6.96</sup>	10 <sup>6.98</sup>
	Day 7 37°C	10 <sup>2.77</sup>	10 <sup>4.49</sup>	10 <sup>6.08</sup>	10 <sup>6.61</sup>	10 <sup>5.91</sup>	10 <sup>6.09</sup>
25	Day 7 45°C	None detected	None detected	None detected	10 <sup>4.79</sup>	None detected	10 <sup>3.42</sup>

These results are represented graphically in Figures 1, 2  
30 and 3 of the accompanying drawings. The initial titre of the  
Day 0 No Stabiliser formulation was low. This may have been due  
to the time that the virus stock stood at room temperature  
prior to blending. The target titre of 10<sup>6.92</sup>/ml was achieved at  
4°C after 7 days for the SPGA and MgCl<sub>2</sub> blends but not for the  
35 blend with No Stabiliser. At the higher temperatures of 37°C



and 45°C, the addition of D<sub>2</sub>O to the samples improved the stability of the virus. The loss in titre is expressed below as a logarithmic percentage of the initial antigen content:

5	Log <sub>10</sub> Titre	No Stabiliser		SPGA		MgCl <sub>2</sub>	
		-D <sub>2</sub> O	+D <sub>2</sub> O	-D <sub>2</sub> O	+D <sub>2</sub> O	-D <sub>2</sub> O	+D <sub>2</sub> O
	Day 0	6.50	6.42	7.07	7.00	6.85	6.89
	Day 7 4°C Loss%	6.19 51%	6.37 11%	7.04 7%	(7.04) (10%)	(6.96) (29%)	(6.98) (23%)
10	Day 7 37°C Loss%	2.77 100%	4.49 99%	6.03 91%	6.61 59%	5.91 89%	6.09 84%
15	Day 7 45°C Loss%	Not Det. 100%	Not Det. 100%	Not Det. 100%	4.79 99%	Not Det. 100%	3.42 100%

All percentages have been rounded up to the nearest whole number, hence the apparent 100% loss even where some antigen is detected. The figures in brackets were higher than the initial  
20 (Day 0) titre. This apparent increase was due to the variability of the assay. The results emphasise the superiority of the formulations containing both SPGA and D<sub>2</sub>O.

CLAIMS

1. A pharmaceutical composition which comprises a solution or suspension of a biological agent in a diluent comprising D<sub>2</sub>O, which solution or suspension contains  
5 sufficient amounts of a sugar, phosphate, glutamic acid or a physiologically acceptable salt thereof and, optionally, a non-toxic protein, a peptone or a mixture of two or more amino acids besides glutamic acid to enhance the stability of the agent.
- 10 2. A pharmaceutical composition according to claim 1, wherein the biological agent is a virus.
3. A pharmaceutical composition according to claim 1, which is a suspension of a live attenuated poliovirus or an inactivated poliovirus of each of poliovirus types 1, 2 and 3.
- 15 4. A pharmaceutical composition according to any one of the preceding claims, wherein the solution or suspension contains from 0.5 to 3% by weight of a sugar, from 0.5 to 1.5% by weight of phosphate, from 0.2 to 1% by weight of glutamic acid or sodium glutamate and, optionally, from 0.5 to 3% by  
20 weight of the non-toxic protein, peptone or said mixture of amino acids.
5. A pharmaceutical composition according to any one of the preceding claims, wherein the solution or suspension contains (a) sucrose as the sugar, (b') KH<sub>2</sub>PO<sub>4</sub> and (b'') K<sub>2</sub>HPO<sub>4</sub>  
25 as the phosphate, (c) L-glutamic acid or sodium glutamate and optionally (d) human serum albumin as the non-toxic protein, peptone or said mixture of amino acids in a weight ratio of (a):(b'):(b''):(c):optionally (d) of about 1:0.40:0.36:0.37 when (c) is glutamic acid or 0.49 when (c) is sodium glutamate:0.46.

6. A pharmaceutical composition according to any one of the preceding claims, wherein the solution or suspension comprises the biological agent and a D<sub>2</sub>O SPG or SPGA solution as follows:

5 SPG

a solution in D<sub>2</sub>O of: 1.09% w/v sucrose,  
0.43% w/v KH<sub>2</sub>PO<sub>4</sub>,  
0.39% w/v K<sub>2</sub>HPO<sub>4</sub>, and  
0.40% w/v L-glutamic acid or 0.53% w/v  
10 sodium glutamate;

SPGA

a solution in D<sub>2</sub>O of: 1.09% w/v sucrose,  
0.43% w/v KH<sub>2</sub>PO<sub>4</sub>,  
15 0.39% w/v K<sub>2</sub>HPO<sub>4</sub>,  
0.40% w/v L-glutamic acid or 0.53% w/v  
sodium glutamate, and  
0.50% w/v human serum albumin.

7. A process for the preparation of a pharmaceutical  
20 composition as defined in claim 1, which process comprises dissolving or dispersing the biological agent in a D<sub>2</sub>O solution of the said amounts of the sugar, phosphate, glutamic acid or salt thereof and, optionally, non-toxic protein, peptone or said mixture of amino acids.

25 8. A process according to claim 7, wherein the resulting solution or suspension is sterilised by passing through a sterilising filter and dispensed into unit- or multi-dose containers which are then sealed.

9. A pharmaceutical composition which comprises a

solution or suspension of a biological agent in a diluent comprising D<sub>2</sub>O, which solution or suspension is buffered to a pH at which the biological agent is stable by means of a phosphate buffer and contains sufficient amounts of a sugar, 5 glutamic acid or a physiologically acceptable salt thereof and, optionally, a non-toxic protein, a peptone or a mixture of two or more amino acids besides glutamic acid to enhance the stability of the agent.

10. Use of a D<sub>2</sub>O SPG or SPGA solution to stabilise a 10 solution or suspension of a biological agent.

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**Patents Act 1977**  
**Examiner's report to the Comptroller under Section 17**  
**(The Search report)**

Application number  
GB 9418609.5

**Relevant Technical Fields**

- (i) UK Cl (Ed.N)      A5B (BAB, BLB, BNB)  
(ii) Int Cl (Ed.6)      A61K 47/02, 47/16, 47/26, 47/36,  
47/18

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J F JENKINS

Date of completion of Search  
18 DECEMBER 1995

**Databases** (see below)

- (i) UK Patent Office collections of GB, EP, WO and US patent specifications.

Documents considered relevant following a search in respect of Claims :-  
1 TO 10

- (ii) ONLINE: WPI, CAS - ONLINE

**Categories of documents**

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| <p><b>X:</b> Document indicating lack of novelty or of inventive step.</p> <p><b>Y:</b> Document indicating lack of inventive step if combined with one or more other documents of the same category.</p> <p><b>A:</b> Document indicating technological background and/or state of the art.</p> | <p><b>P:</b> Document published on or after the declared priority date but before the filing date of the present application.</p> <p><b>E:</b> Patent document published on or after, but with priority date earlier than, the filing date of the present application.</p> <p><b>&amp;:</b> Member of the same patent family; corresponding document.</p> |
|--|---|

Category	Identity of document and relevant passages	Relevant to claim(s)
X	EP 0568726 A2 (RESEARCH FOUND. OSAKA UNIV.) see Reference Examples 5 and 6	1, 2, 5 and 9
A	EP 0332826 A1 (TEVA PHARMACEUTICAL) see Claim 1	
X, E	WO 94/21298 A1 (INSTITUT PASTEUR) see Example 4	1-3
X	WPI Acc. No. 94 - 307582/38 & JP - 6234659 (ZH-HANDAI BISEIBUTSUBYOK) 23 August 1994 see Abstract	1, 2, 5 and 9

**Databases:** The UK Patent Office database comprises classified collections of GB, EP, WO and US patent specifications as outlined periodically in the Official Journal (Patents). The on-line databases considered for search are also listed periodically in the Official Journal (Patents).