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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :		(11) International Publication Number: WO 93/16714
A61K 37/02 // (A61K 37/02 A61K 33:42, 33:14, 33:00 A61K 31:70)	A1	(43) International Publication Date: 2 September 1993 (02.09.93)

(21) International Application Number: PCT/US93/01408

(22) International Filing Date: 17 February 1993 (17.02.93)

(30) Priority data:
07/837,759 19 February 1992 (19.02.92) US

(71) Applicant: ALLERGAN, INC. [US/US]; 2525 Dupont Drive, P.O. Box 19534, Irvine, CA 92713-9534 (US).

(72) Inventor: NEWTON, Walter, A. ; 810 Taylorsville, Lenoir, NC 28645 (US).

(74) Agents: LAMBERT, Howard, R. et al.; Allergan, Inc., 2525 Dupont Drive, Post Office Box 19534, Irvine, CA 92713-9534 (US).

(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: TISSUE IRRIGATING SOLUTION

(57) Abstract

In a tissue irrigating solution of the type containing the combination of glutathione, bicarbonate, and Ringer solution (GBR), the bicarbonate and glutathione are freeze-dried, packaged and separately stored in lyophilized powder form until just preceding the operation, at which time the lyophilized powder containing the bicarbonate and glutathione is dissolved directly into a conventional I.V. solution.

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TISSUE IRRIGATING SOLUTION

Cross Reference to Related Applications

This application is a continuation-in-part of my copending application Ser. No. 577,306, filed Sept. 4, 1990 (now U.S. Patent No. _____, issued _____) which, in turn, was a divisional of Ser. No. 243,085, filed Sept. 9, 1988 and issued as U.S. Patent No. 4,975,419 on Dec. 4, 1990.

Field of the Invention

The present invention is directed to tissue irrigating solutions and, more particularly, to improved techniques for formulating and packaging the components of a tissue irrigating solution containing glutathione, bicarbonate, and Ringer solution.

Background of the Invention

During surgical procedures, it is important to minimize disturbance of the environment of tissue and cells as much as possible. A traumatic change in the environment surrounding internal cells may, for example, lead to the destruction of such cells or the destruction of the function of such cells. The destruction of cell function may even lead to destruction of other cells which are dependent upon a proper functioning of the destroyed cells. Therefore, during surgical procedures such as, for example, intraocular surgery, it is very important that the exposed tissue be continuously irrigated with solutions which approximate natural body fluids. Such solutions are called "tissue irrigating solutions". One of the earliest tissue irrigating solutions for ophthalmic procedures was an isotonic saline. However, it was quickly recognized that the isotonic saline was not adequate as an ophthalmic irrigating solution because it resulted in endothelial cell swelling, cell damage, and consequent corneal clouding.

Alternatively, various electrolyte solutions have been proposed as tissue irrigating solutions, particularly in ophthalmic procedures, because such

solutions more closely resemble the aqueous humor of the eye. The earliest electrolyte solution was known as Ringer's solution, which was a combination of sodium, calcium and potassium ions along with sodium lactate. Another solution intended for tissue irrigation is known as a balanced salt solution (referred to as "BSS") which contains the essential sodium, potassium, calcium, and magnesium salt ions along with an acetate-citrate buffer system. It was successful and was used almost exclusively until several years ago. Within the last 10-15 years, there has developed a tissue irrigating solution which is a combination of the Ringer solution along with glutathione and sodium bicarbonate. This is sometimes referred to GBR, and in recent years has become a recognized tissue irrigating solution, especially for ophthalmic procedures. When dextrose, sodium hydrogen phosphate (Na_2HPO_4), and sometimes adenosine are added to GBR, there results a fortified or enhanced balanced salt solution (sometimes referred to as "BSS Plus"), which has heretofore proven to be very effective for intraocular surgery.

The problem with GBR solutions and particularly the fortified or enhanced balanced salt solution is that they do not exhibit long term stability. Because they must be mixed essentially at the operative site, it is difficult to control and maintain sterility. There are various reasons why GBR type solutions are not stable. First, bicarbonate and phosphate tend to precipitate in the presence of the magnesium and calcium ions. Therefore, once mixed, the sodium bicarbonate quickly loses its ability to act as a pumping agent for causing the endothelium to perform its fluid transport function of maintaining an outward fluid transport to the stromal layer, which results in damage to the cornea. Stated otherwise, the purpose of the bicarbonate is to act as a pump and, when mixed with the magnesium or calcium ions, it quickly loses its pumping action. A second reason why

the GBR solutions are not stable is that bicarbonate decomposes at a pH of less than about 8 and becomes carbon dioxide which again causes the bicarbonate to fail to act as a chemical pump during the surgical procedure. Finally, the glutathione is unstable at a pH greater than about 5. Therefore, the glutathione cannot exist in a basic solution and the bicarbonate cannot exist for extended periods in an acid solution.

A solution to this problem has been offered in United States Patents Nos. 4,443,432 and 4,550,022, both issued to Garabedian et al. According to these two patents, initially two solutions are prepared, a first, basic solution providing the bicarbonate and sodium phosphate, and a second, acidic solution which provides the calcium and magnesium ions, as well as the dextrose and glutathione. The solutions are packaged and stored separately and mixed within 24 hours of use.

While the resulting irrigating product as described the Garabedian et al technique has achieved some degree of acceptance and success, there are some limitations as a result thereof. First, the long-term stability and maintenance of acceptable pH values is difficult to achieve in accordance with the method and techniques described in the Garabedian et al patents. Second, in order to steam sterilize the first, basic solution, it is necessary to place the glutathione in the second, acidic package, because glutathione cannot stand steam sterilizing. Third, since the sodium bicarbonate is in the first package, the package must be glass which is subject to breakage, because it is difficult to maintain the stability of sodium bicarbonate in solution in a polymeric container. This occurs because sodium bicarbonate will not remain stable in a polypropylene bottle as a result of the transmission of vapors through the wall of the bottle.

Summary of the Present Invention

In order to overcome the problems described hereinabove and offer an improved formulating and packaging technique, the present invention contemplates a two-part intraocular or tissue irrigating system. In first embodiment set forth in the parent application, the invention encompass a first part which includes a stable, sterile pre-packaged acidic solution containing at least the calcium ions and magnesium ions. The second part includes a lyophilized powder containing at least the sodium bicarbonate and glutathione. The disodium hydrogen phosphate is preferably included with the second part (powder). The potassium ions and dextrose may be provided in either the first or second part. When the first and second parts are mixed together, there is formed an extremely satisfactory irrigating solution. Preferably, the powder and solution should be aseptically mixed to maintain the sterility thereof.

More specifically, in the proposed irrigating product, the larger solution (on the order of 500mls) contains all the chlorides, i.e., sodium, potassium, calcium and magnesium, in a polypropylene bottle that is terminally steam-sterilized according to conventional processes. The second or smaller part is a lyophilized powder which includes sodium bicarbonate, disodium hydrogen phosphate, dextrose and glutathione disulphide. The second part is sterile-filtered before being aseptically filled into either a glass vial or a small polypropylene bottle for lyophilization. It also has been found preferable to include the disodium hydrogen phosphate with the sodium bicarbonate. Thus the calcium and magnesium salts are placed in the large bottle. Both the bicarbonate and the glutathione are stabilized by the lyophilizing (freeze-drying) process.

In addition, a special technique has been developed to eliminate any breakdown that could occur before the solution containing bicarbonate and glutathione is freeze

dried. According to this improved technique, the solution containing the sodium bicarbonate is first frozen, then the glutathione is frozen onto the surface of the frozen sodium bicarbonate solution, then both frozen components are lyophilized (freeze-dried).

The large solution is preferably placed in a 500ml polypropylene bottle and the lyophilized powders are placed in a small 50ml glass or polypropylene vial. The two components are mixed aseptically through a transfer spike. One end of the spike is inserted through the stopper in the small vial. With the bottle containing the larger amount of solution in the upright position, the small vial is then inverted and the other end of the spike is inserted through the stopper into the large bottle. The large bottle, being polypropylene, is then squeezed which forces a small amount of fluid into the vial which promptly dissolves the lyophilized powder. When the polypropylene bottle is released, the fluid and powder dissolved therein will return to the large bottle. This process is repeated several times to flush all the contents of the vial into the bottle and to thoroughly mix the contents of the two containers.

In an alternative embodiment, which is the subject of this continuation-in-part, the present invention contemplates an intraocular or tissue irrigating system for mixing with a conventional I.V. solution containing a portion of the sodium salts. The invention includes a lyophilized powder containing at least the sodium bicarbonate and glutathione. The disodium hydrogen phosphate, calcium salts and magnesium salts, potassium salts and dextrose, and the remaining sodium salts are preferably included with the second part (powder). When the conventional I.V. solution and the specially formulated lyophilized powder are mixed together, there is formed an extremely satisfactory irrigating solution without the necessity of having two packages on hand.

It is, therefore, an object of the present invention to provide an improved formulating and packaging technique for the manufacture of glutathione/bicarbonate/ Ringer solution type products.

Another object of the present invention is to provide an enhanced balanced salt solution of the type described in which the sodium bicarbonate and glutathione are packaged together in a powder form.

Still another object of the present invention is to provide a lyophilized powder which can be mixed with a conventional I.V. solution to produce the enhanced balanced salt solution.

Other objects and a fuller understanding of the invention will become apparent upon reading the following detailed description along with the accompanying drawings.

Brief Description of the Drawings

Figures 1 and 1A illustrate a two-part packaging system in which the components of the irrigating solution of the present invention are prepackaged and stored; and

Figure 2 is a schematic block diagram illustrative of the procedural steps involved in formulating the irrigating solution of present invention.

Detailed Description of the Preferred Embodiments

The present invention is directed generally to an improved technique for packaging a tissue irrigating solution of the type which includes glutathione/ bicarbonate/Ringer solution, whereby the components are initially packaged in a two-component system. The composition and concentration of the two components of the system are such that they remain stable, even when stored for long periods of time. The two components are separately sterilized, then aseptically mixed so that the ultimate solution is completely sterile and available for use in surgery during the ensuing 24 hours. The mixed solution has been found to be extremely useful for maintaining the appropriate environment and preventing

cell damage during surgical procedures, particularly procedures such as intraocular surgery.

The desired irrigating solution, when mixed, preferably contains the following components in the amount indicated:

Ingredients for Enhanced Balanced Salt Solution

	mg/ml
Sodium chloride (NaCl)	7.14
Potassium chloride (KCl)	0.38
Calcium chloride ($CaCl_2 \cdot 2H_2O$)	0.154
Magnesium chloride ($MgCl_2 \cdot 6H_2O$)	0.20
Dextrose	0.92
Sodium carbonate ($NaHCO_3$)	2.1
Disodium hydrogen phosphate (Na_2HPO_4)	0.42
Glutathione disulphide	0.184

As has been described above, the irrigating solution identified hereinabove is supplied in two parts that will be mixed by the end user immediately prior to usage and will have usable life, when mixed, of 6-24 hours. One part is a mixed salt solution shown below in Formula 1 and the second part is a powder containing the components shown in Formula 2. Formula 2 dissolves readily in Formula 1 and forms a clear solution consisting of the components in the amounts shown above. When packaged and before mixing the Formula 1 and Formula 2 powder contain the following ingredients in the indicated amounts.

Formula 1 (500ml Solution)

Ingredients	mg/ml
Sodium chloride (NaCl)	7.14
Potassium chloride (KCl)	0.38
Calcium chloride ($CaCl_2 \cdot 2H_2O$)	0.154
Magnesium chloride ($MgCl_2 \cdot 6H_2O$)	0.20
Dextrose	0.92

Formula 2 (Powder)

Ingredients	Each Vial Contains
$NaHCO_3$	1,081 mg
Na_2HPO_4	216 mg
Glutathione disulphide	95 mg

Looking now at the drawing, Formula 1 is placed in the large polypropylene container or bottle (500ml) 10 and Formula 2 is placed in the small container or vial (50ml) 12. A mixing spike 14 is provided and utilized in the following manner. The mixed salt solution is carried in container 10 and the lyophilized powder in container 12. One end of the spike 14 is placed through the rubber stopper 13 in vial 12. The vial 12 and spike 14 are then inverted and the other end of the spike is placed through the rubber stopper 11 in the cap of bottle 10. The bottle 10 is preferably formed of a resilient polypropylene material, so that when it is squeezed, a portion of the fluid is forced up into the small vial 12, where it mixes with and dissolves the powder therein. When the bottle 10 is released, the fluid then flows back down through spike 14 into the bottle 10. When this process is repeated several times, the powder is fully mixed, dissolved, and transferred into the large container 10. The small vial and spike are then disposed of, and the large container is ready for use in the operative procedure.

In the alternative embodiment, the present invention contemplates an intraocular or tissue irrigating system for mixing with an I.V. solution. The invention includes a lyophilized powder containing at least the sodium bicarbonate and glutathione. The disodium hydrogen phosphate, calcium salts and magnesium salts, sodium salts, potassium salts and dextrose are preferably included with the lyophilized powder. When the I.V. solution and lyophilized powder are mixed together, there is formed an extremely satisfactory irrigating solution.

As has been described above, the alternative embodiment of the irrigating solution identified hereinabove is supplied in powdered form that will be mixed by the end user with a conventional I.V. solution immediately prior to usage and will have usable life, when mixed, of 6-24 hours. Preferably, one part is the

I.V. solution shown below in Formula 1 and the second part will be the powder containing the components shown in Formula 2. Formula 2 dissolves readily in Formula 1 and forms a clear solution consisting of the components in the amounts shown above. When packaged and before mixing the Formula 1 and Formula 2 powder contain the following ingredients in the indicated amounts, wherein the I.V. solution of the mix comprises ingredients substantially in the following relation:

Formula 1 (500ml I.V. Solution)

Ingredients	mg/ml
Sodium chloride (NaCl)	4.5

and wherein said lyophilized powder comprises ingredients substantially in the following relation:

Formula 2 (Powder)

Ingredients	Each Vial Contains
Sodium chloride (NaCl)	1,320 mg
Potassium chloride (KCl)	190 mg
Calcium chloride ($CaCl_2 \cdot 2H_2O$)	77 mg
Magnesium chloride ($MgCl_2 \cdot 6H_2O$)	100 mg
Dextrose	490 mg
$NaHCO_3$	1,152 mg
Na_2HPO_4	208 mg
Glutathione disulphide	97 mg

In the ensuing examples, there are explained several experiments which were conducted in order to determine the preferred manner for formulating the irrigating solution of the present inventions. In preparation for experiments 1-6, the following solutions A and B were prepared.

Solution A

Ingredients	Amounts
Distilled water	10 liters
NaCl	71.4 gms
KCl	3.79 gms

$CaCl_2$	1.54 gms
$MgCl_2$	2.00 gms

Divide Solution A into four equal lots, 1A-4A, each lot equaling approximately 2.5 liters, and adjust the pH of each lot with 1N HCl as follows:

	Target	Actual
Lot 1A	pH 3.0	pH 2.9
Lot 2A	pH 4.0	pH 4.1
Lot 3A	pH 5.0	pH 5.5
Lot 4A	pH 6.0	pH 6.9

Fill nine 250ml plastic bottles from each pH lot, cap and sterilize. Retain the remaining 250ml of each lot of Solution A for further observation.

Solution B

Ingredients	Quantities
Distilled water	1 liter
$NaHCO_3$	42 gms
Na_2HPO_4	8.32 gms
Dextrose	18.4 gms
Glutathione	3.68 gms

Dissolve the Na_2HPO_4 and the dextrose in the one liter of distilled water. Check the pH (8.8). Add glutathione and check the pH again (7.38). Adjust the pH to 7.9-8.0 using 1 N. NaOH solution. Add $NaHCO_3$. Check pH (7.86). Divide the solution into three equal lots and adjust the pH as follows:

Lot 1B	pH	7.9-8.0
Lot 2B	pH	7.7
Lot 3B	pH	7.4

Each of the above lots should be filled into 30ml glass vials there being 13.25ml in each of 18 vials of each pH. Freeze dry each vial as soon as possible after mixing.

EXPERIMENT 1

Samples of the freeze-dried Solution B were received and the following tests were performed. First 13.1mls of water was added to each of two vials of each of the three

different types of pH samples and the pH checked immediately and after one hour.

Table 1

Original pH	Sample No.	Reconstituted pH
7.6	(1)	8.24
	(2)	8.23
After 1 hour ----	(2)	8.26
7.73	(1)	8.27
	(2)	8.28
After 1 hour ----	(2)	8.30
7.86	(1)	8.28
	(2)	8.28
After 1 hour ----	(2)	8.30

Preliminary tests excluded the pH 5 and pH 6 of Solution A as being too high initially. Thereafter, the pH 3 and pH 4 samples of Solution A were used to mix with the three pH levels of freeze-dried Solution B.

Table 2

Mix	Immediate	Three Hour
2.9/7.86	7.68	7.91
4.1/7.86	8.10	8.26
2.9/7.73	7.58	7.84
4.1/7.73	8.04	8.23
2.9/7.6	7.47	7.69
4.1/7.6	*8.26	8.32

*This is not a true result as a solution in water from Sample 1 had to be used.

In conclusion as to Experiment 1, it was determined that the pH of Solution B could not be reduced below about 7.9 before freeze-drying. The HCl used to reduce the pH appears to liberate CO_2 from the bicarbonate and eventually all free CO_2 is lost on freeze-drying. It would, therefore, probably be necessary to have a very low pH (2.0 or less) in Solution A to counteract the buffering effect of the phosphate and the bicarbonate.

EXPERIMENT 2

A fresh batch of Solution A and Solution B were prepared, and the pH of Solution B was adjusted to 7.89 before the $NaHCO_3$ was added. After the addition of $NaHCO_3$, the pH of Solution B was 7.98. The pH of Solution A was adjusted down to 2.5 by using 0.5 N HCl. When Solutions A and B were mixed in the correct proportions (10ml to 0.5ml), the pH of the initial mixture was 7.3. After 24 hours, this had risen to pH 8.2 and, thus, did not meet the necessary criteria of pH 7.4.

EXPERIMENT 3

The purpose of Experiments 3 and 4 are to determine by comparison in Experiment 5 what is the best technique for freeze-drying Solution B. In Experiment 3, 18.4 gms of dextrose was dissolved in 1 liter of distilled water. 900ml of the distilled water/dextrose solution had dissolved in it 8.3 gms of Na_2HPO_4 and 42.0 gms of $NaHCO_3$ to form a Solution B 1 having a pH of 8.15.

Using 12mls of the remaining 100mls of the dextrose solution, dissolve therein .442 gms of glutathione disulphide to form Solution B-2 having a pH of 2.63.

Pipette 11.9mls of Solution B-1 into each of twelve 30ml vials, cap and place in a freezer. Cool Solution B-2 to about the freezing point, and when Solution B-1 is frozen and thoroughly chilled, add 1.3mls of Solution B-2 to each of nine vials of Solution B-1 and return all twelve immediately to the freezer. The second layer of Solution B-2 froze almost immediately on contact with the first layer, which was expected and intended. Retain in the freezer one of the nine vials of the combination solution B-1 and B-2 and one of the three vials of Solution B-1 only. Freeze-dry the remainder for further tests in Experiment 5.

EXPERIMENT 4

Dissolve 18.4gms of dextrose in 1 liter of distilled water. Into 900mls of the dextrose solution, dissolve therein 42gms of $NaHCO_3$ to form Solution B-1 with a pH of 8.03. Fill 11.9mls of Solution B-1 into each of twelve

vials, stopper and freeze. To the remaining 100mls of the dextrose solution, add 8.32gms of Na_2HPO_4. When completely dissolved (pH 8-9.5), transfer 12mls to another container and add .442gms glutathione and dissolve forming a Solution B-2 at pH 7.36. Cool Solution B-2 to about the freezing point and, when Solution B-1 is frozen and thoroughly chilled, add 1.3mls of Solution B-2 to each of nine vials and return all twelve immediately to the freezer. In the lab experiment, the second layer again froze almost immediately on contact with the first layer as was intended. Retain in the freezer one of the nine vials of the combination solution B-1 and B-2 and one of three vials of Solution B-1 only. Freeze-dry the remainder for further tests in Experiment 5.

EXPERIMENT 5

The freeze-dried samples from Experiments 3 and 4 were received and the following tests were performed on them. First, the four retained frozen samples were thawed and four equivalent freeze-dried samples were reconstituted with distilled water. The pH of all eight samples was measured with the following results:

Table I

	Sample	Frozen(A)pH	Freeze-dried(B)pH
Exp. 4, Solution 1	(1)	8.24	8.32
Exp. 4, Solution 1 & Glutathione	(2)	7.99	8.23
Exp. 5, Solution 1	(3)	8.15	8.26
Exp. 5, Solution 1 & Glutathione & Na_2HPO_4	(4)	8.10	8.33

It was observed that Sample 4-B did not dissolve as rapidly as the other samples, presumably because of "caking" of the phosphate in its anhydrous state.

Next, three samples each of the freeze-dried complete solution (Samples 2 and 4) were reconstituted

with exactly 12mls of distilled water and the final pH checked.

Table II

	Sample	pH		
		1	2	3
Exp. 4, Solution 1 & Glutathione	(2)	8.26	8.21	8.27
Exp. 5, Solution 1 & Glutathione & Na_2HPO_4	(4)	8.28	8.25	8.27

Mixture 2 went easily into solution whereas Mixture 4 took five minutes to completely dissolve.

In conclusion as to Experiment 5, in both Experiments 3 and 4, there was an increase in the final pH after freeze-drying which indicates some slight loss of CO_2 during the process. The fact that the samples without glutathione showed a loss, although somewhat smaller, shows that the bicarbonate is inherently unstable -- as is well known -- and it will probably be necessary to match the Solution 1 to the freeze-dried component in each lot in a production environment in order to produce a consistent pH in the final mixture. There was no marked difference in the apparent bicarbonate stability between the two experiments. In view of the solution difficulty with Experiment 4 material, it seems reasonable to concentrate on the Experiment 3 approach, i.e., using the $NaHCO_3$ and Na_2HPO_4 in Solution B-1 with only the glutathione in Solution B-2. This should help with any possible instability of the glutathione in an alkaline pH.

EXPERIMENT 6

The purpose of this experiment was to now determine whether a Solution A could be formulated with a Solution B-1 from Experiment 3 successfully. Again, it should be kept in mind the purpose of the line of experiments (Exp. 1-6) is to determine whether a solution having a final pH

of approximately 7.4 when Solution B is dissolved into it can be attained and whether such pH will remain stable.

A 250ml bottle of Solution A from Experiment 1 at a pH of 2.9 was used. A vial of freeze-dried Solution B (actually Solution B-1 from Experiment 3) was added and the pH checked at 7.65. A small amount (0.2mls) of 0.5 N HCl was added resulting in a pH of 7.54. A further small amount (0.2ml) of 0.5 N HCl was added providing a pH of 7.4. Thereafter, 0.4mls of .5 N HCl was added to a bottle (265mls) of pH 2.9 Solution A giving a pH of 2.58. When a vial of Solution B (actually Solution B-1 from Experiment 3) was added to this solution, there resulted a final mixture having a pH of 7.41. Four additional bottles of Solution A initially having a pH of 4.1 (Lot 2A) were similarly adjusted to pH values around 2.6 with the following results when mixed with vials of Solution B from either Experiment 3 or Experiment 4.

Table I

Final pH of Solution 1 + vial	pH After			
	5 min.	1 hr.	3 hr.	24 hr.
2.53 + Solution B (Exp. 3)	7.2	7.22	7.32	7.4
2.60 + Solution B (Exp. 3)	7.36	7.33	7.36	7.42
2.65 + Solution B (Exp. 4)	7.45	7.4	7.4	7.51
2.80 + Solution B (Exp. 4)	7.65	7.59	7.67	7.73

After 48 hours, there were obvious signs of degradation with deposits forming and in the case of the two higher pH values a strong smell of H_2S presumably from glutathione degradation. The two lower pH solutions appeared more stable, with less deposit and no odor.

In conclusion:

1. The bicarbonate and phosphate in the first solution with glutathione only in the smaller second portion is the choice because it affords a better chance of stability for the glutathione with the lower pH and there is no problem of phosphate solubility when it is reconstituted.

2. A final pH of about 7.3 can be achieved by adjusting the chloride solution (Solution A) to about pH 2.6. The higher pH of the final solution appears to be less stable and it appears preferable to hold the final pH to or below 7.4.

EXPERIMENT 7

A pilot batch of the enhanced balanced salt solution in accordance with the above invention was produced to confirm the conclusion set forth in the experiments above. A 15 liter batch of Solution A was prepared as follows:

14 liters of WFI (distilled water which has been tested and qualified for injection) were measured into a graduated container and 107.1gms of NaCl was dissolved therein.

1 liter of WFI had dissolved therein 22.7gms KCl; 9.24gms $CaCl_2$; and 12.00gms of $MgCl_2$.

250mls of the potassium/calcium/magnesium solution was added to the 14 liters of the NaCl solution and the volume was made up to 15 liters by the addition of further WFI. The initial pH of Solution A was 6.03 and was lowered by the addition of 1 N HCl as follows:

9mls HCl pH 3.02
+4mls HCl pH 2.80
+6mls HCl pH 2.67
+6mls HCl pH 2.57

At this point, and after thorough mixing, the solution was filled into 250ml plastic bottles, stoppered, capped and sterilized with Dispersa Balansalt.

Solution B was prepared for freeze-drying in such a way that 10mls of the B-1 solution was frozen and then 1ml of the B-2 solution was added on top of the surface of the B-1 solution. Solution B-1 was prepared by dissolving 22.0gms of dextrose in 1 liter WFI. Approximately 800mls of this solution was transferred and had dissolved in it 50gms of $NaHCO_3$ and 9.9gms of Na_2HPO_4.

Additional amount of the dextrose WFI was added to make up 900mls and equally distributed throughout 30ml vials at 10ml per vial and frozen. This made 90 samples.

90mls of the remaining dextrose solution was transferred to a flask and in it was dissolved 4.4gms of glutathione disulphide. This glutathione disulphide solution was chilled to near freezing and 1ml thereof was added to each of the 90 frozen 10ml aliquots of the bicarbonate solution. The vials were immediately returned to the freezer and they were subsequently freeze-dried without allowing the material to thaw. Five frozen samples were retained as control samples and 85 were sent for freeze-drying.

After freeze-drying, five vials were taken and the contents mixed with each of the five bottles of Solution A and the pH of the mixtures were measured immediately and after 1, 5, 24, 48 and 72 hours, all at room temperature. The following results were obtained:

Sample	pH Value				
2mm	1 Hr.	5 Hrs.	24 Hrs.	48 Hrs.	72 Hrs.
1 7.35	7.40	7.50	7.50	7.40	7.60
2 7.40	7.40	7.45	7.50	7.40	7.60
3 7.45	7.40	7.45	7.45	7.40	7.60
4 7.40	7.40	7.40	7.45	7.50	7.60
5 7.40	7.40	7.50	7.50	7.55	7.65

From the preliminary pH measurements, it appears that the process used gives reproducible results and that the mixed product is at least as stable as any other glutathione/ bicarbonate/Ringer solution product on the market.

Experiment 8

Based on the above experiments, it was further realized that a "powder only" mix could be prepared which could subsequently be mixed with an I.V. solution to form a final solution suitable for an intraocular or tissue irrigating solution. Calculations based on the formulation of the preferred two-part system, yielded the

following results for a lyophilized powder suitable for mixing with a standard 500ml 4.5 wt% sodium chloride I.V. solution, wherein the I.V. solution of the mix comprises ingredients substantially in the following relation:

Formula 1 (500ml I.V. Solution)

Ingredients	mg/ml
Sodium chloride (NaCl)	4.5

and wherein said lyophilized powder comprises ingredients substantially in the following relation:

Formula 2 (Powder)

Ingredients	Each Vial Contains
Sodium chloride (NaCl)	1,320 mg
Potassium chloride (KCl)	190 mg
Calcium chloride ($CaCl_2 \cdot 2H_2O$)	77 mg
Magnesium chloride ($MgCl_2 \cdot 6H_2O$)	100 mg
Dextrose	490 mg
$NaHCO_3$	1,152 mg
Na_2HPO_4	208 mg
Glutathione disulphide	97 mg

While preferred embodiments of the present invention have been described in detail here and above, it is apparent that various changes and modifications might be made without departing from the scope of the invention which is set forth in the accompanying claims. For example, other I.V. solutions, such as Ringer's Injection or Lactated Ringer's Injection could be chosen and an appropriate lyophilized powder produced which would result in an equivalent final solution.

I Claim:

1. A mix capable of separate packaging and storage for subsequent mixture with an I.V. solution to form a tissue irrigating solution comprising:

a) a lyophilized powder containing sodium bicarbonate and glutathione;

b) calcium salts, magnesium salts, sodium salts, potassium salts, and dextrose, each being added to at least one of said I.V. solution and lyophilized powder;

c) said I.V. solution and lyophilized powder exhibiting the characteristics of extended shelf life and when mixed together form a solution for irrigating body tissues during surgery.

2. The mix according to Claim 1 wherein said I.V. solution comprises a portion of said sodium salts.

3. The mix according to Claim 1 wherein said lyophilized powder further comprises disodium hydrogen phosphate.

4. The mix according to Claim 1 wherein said I.V. solution is pre-sterilized prior to mixing.

5. The mix according to Claim 1 wherein said lyophilized powder is packaged in a polypropylene bottle.

6. The mix according to Claim 3 wherein said final solution, when said I.V. solution and lyophilized powder are mixed, comprises ingredients substantially in the following relation:

Ingredients for Enhanced Balanced Salt Solution	mg/ml
Sodium chloride	7.14
Potassium chloride (KCl)	0.38
Calcium chloride ($CaCl_2 \cdot 2H_2O$)	0.154
Magnesium chloride ($MgCl_2 \cdot 6H_2O$)	0.20
Dextrose	0.92
Sodium bicarbonate ($NaHCO_3$)	2.1
Disodium hydrogen phosphate (Na_2HPO_4)	0.42
Glutathione disulphide	0.184

7. The mix according to Claim 3 wherein said I.V. solution of said mix comprises ingredients substantially in the following relation:

Formula 1 (500ml I.V. Solution)

Ingredients	mg/ml
Sodium chloride (NaCl)	4.5

and wherein said lyophilized powder comprises ingredients substantially in the following relation:

Formula 2 (Powder)

Ingredients	Each Vial Contains
Sodium chloride (NaCl)	1,320 mg
Potassium chloride (KCl)	190 mg
Calcium chloride ($CaCl_2 \cdot 2H_2O$)	77 mg
Magnesium chloride ($MgCl_2 \cdot 6H_2O$)	100 mg
Dextrose	490 mg
$NaHCO_3$	1,152 mg
Na_2HPO_4	208 mg
Glutathione disulphide	97 mg

8. The mix according to Claim 7 wherein the pH of the final solution, when mixed, is no greater than 7.8.

9. A tissue irrigating product for subsequent mixture with an I.V. solution to form a tissue irrigating solution comprising:

a) a lyophilized powder containing sodium bicarbonate and glutathione;

b) calcium salts, magnesium salts, sodium salts, potassium salts, and dextrose, each being added to at least one of said I.V. solution and lyophilized powder;

c) means for aseptically intermixing said I.V. solution and said lyophilized powder;

d) said I.V. solution and lyophilized powder, when mixed together, forming a solution for irrigating body tissues during surgery.

10. The irrigating product according to Claim 9 wherein said I.V. solution comprises a portion of said sodium salts.

11. The irrigating product according to Claim 9 wherein said lyophilized powder further comprises disodium hydrogen phosphate.

12. The irrigating product according to Claim 9 wherein said I.V. solution is pre-sterilized prior to mixing.

13. The irrigating product according to Claim 9 wherein said lyophilized powder is packaged in a polypropylene bottle and there is further included a double-ended mixing spike.

14. The irrigating product according to Claim 11 wherein said solution, when said I.V. solution and lyophilized powder are mixed, comprises ingredients substantially in the following relation:

Ingredients for Enhanced Balanced Salt Solution	mg/ml
Sodium chloride	7.14
Potassium chloride (KCl)	0.38
Calcium chloride ($CaCl_2 \cdot 2H_2O$)	0.154
Magnesium chloride ($MgCl_2 \cdot 6H_2O$)	0.20
Dextrose	0.92
Sodium bicarbonate ($NaHCO_3$)	2.1
Disodium hydrogen phosphate (Na_2HPO_4)	0.42
Glutathione disulphide	0.184

15. The irrigating product according to Claim 11 wherein said I.V. solution comprises ingredients substantially in the following relation:

Formula 1 (500ml I.V. Solution)

Ingredients	mg/ml
Sodium chloride (NaCl)	4.5

and wherein said lyophilized powder comprises ingredients substantially in the following relation:

Formula 2 (Powder)

Ingredients	Each Vial Contains

Sodium chloride (NaCl)	1,320 mg
Potassium chloride (KCl)	190 mg
Calcium chloride ($CaCl_2 \cdot 2H_2O$)	77 mg
Magnesium chloride ($MgCl_2 \cdot 6H_2O$)	100 mg
Dextrose	490 mg
$NaHCO_3$	1,152 mg
Na_2HPO_4	208 mg
Glutathione disulphide	97 mg

16. The irrigating product according to Claim 15 wherein the pH of said final solution is no greater than 7.8.

17. A method for preparing a prepackaged tissue irrigating product for subsequent mixture with an I.V. solution to form a tissue irrigating solution comprising the steps of:

a) preparing an aqueous solution having dissolved therein at least sodium bicarbonate and glutathione;

b) lyophilizing the solution of step (a);

c) packaging the lyophilized powder of step (b).

18. The method according to Claim 17 wherein sodium salts, potassium salts, and dextrose are also introduced into the aqueous solution of step (a).

19. The method according to Claim 17 wherein the I.V. solution is pre-sterilized prior to mixing.

20. The method according to Claim 18 wherein step (a) includes forming a first solution of said sodium bicarbonate and disodium hydrogen phosphate and a second solution containing said glutathione.

21. The method according to Claim 20 wherein step (b) includes freezing said first solution, then introducing said glutathione second solution onto the frozen surface of the first solution whereby the second glutathione solution freezes, then freeze-drying the

resulting combination of the frozen first solution and frozen second solution.

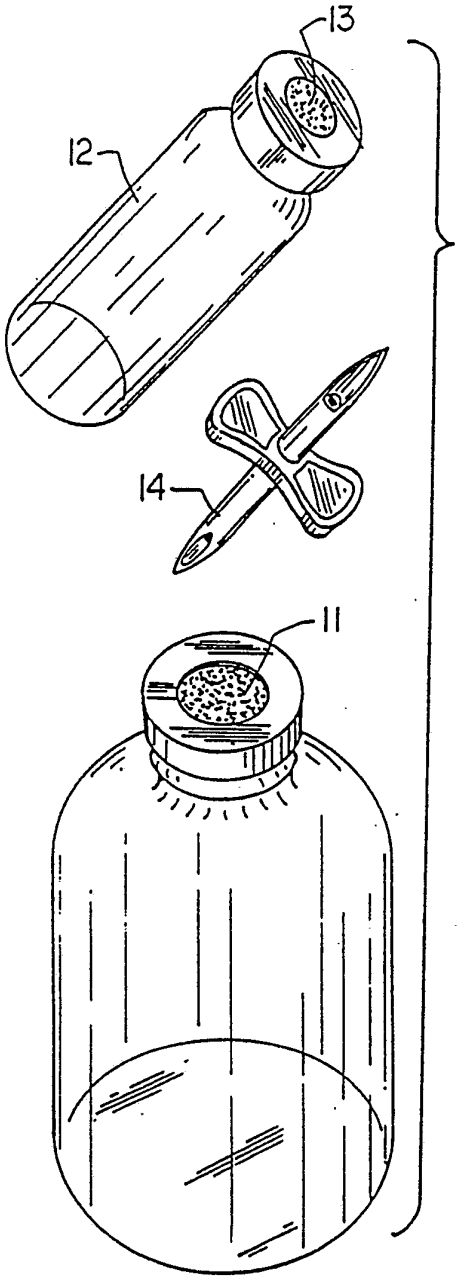


FIG. 1

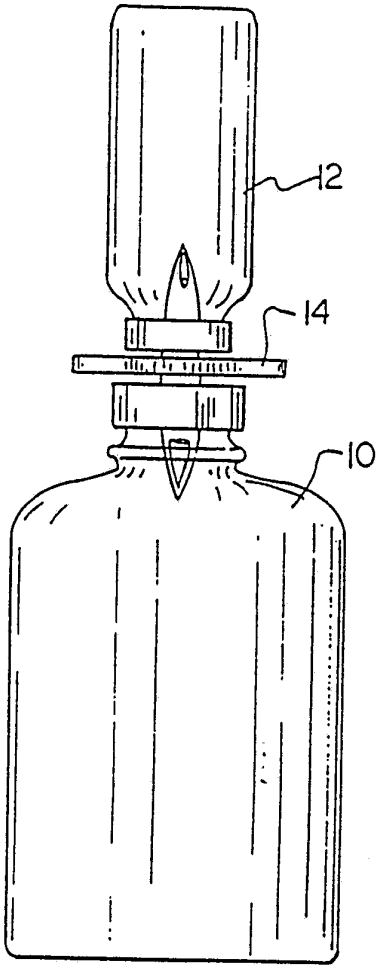
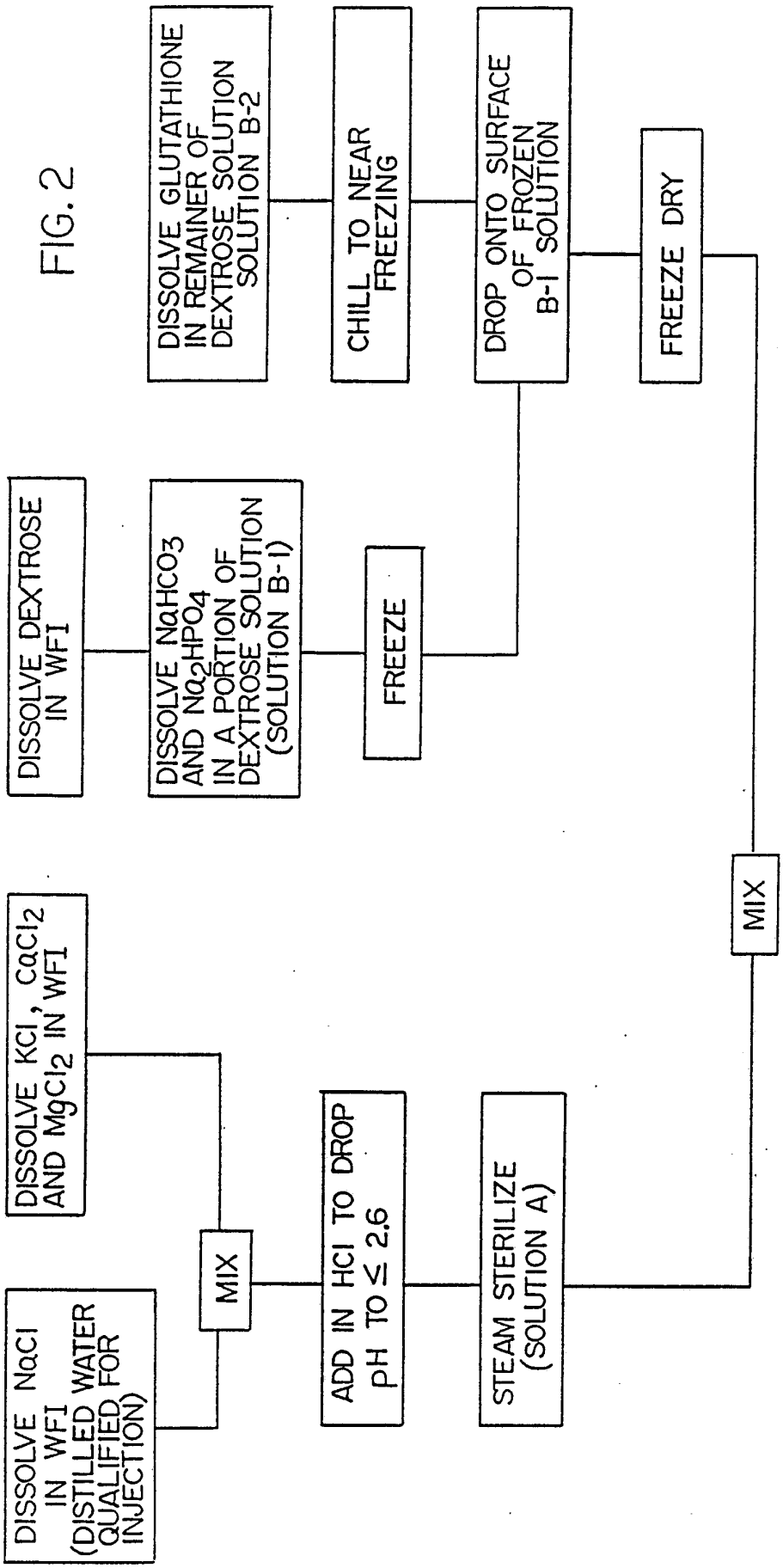


FIG. 1A

FIG. 2


DISSOLVE NaCl IN WFI (DISTILLED WATER QUALIFIED FOR INJECTION)
DISSOLVE KCl, $CaCl_2$ AND $MgCl_2$ IN WFI
MIX
ADD IN HCl TO DROP pH TO ≤ 2.6
STEAM STERILIZE (SOLUTION A)
DISSOLVE DEXTROSE IN WFI
DISSOLVE $NaHCO_3$ AND Na_2HPO_4 IN A PORTION OF DEXTROSE SOLUTION (SOLUTION B-1)
FREEZE
DISSOLVE GLUTATHIONE IN REMAINER OF DEXTROSE SOLUTION SOLUTION B-2
CHILL TO NEAR FREEZING
DROP ONTO SURFACE OF FROZEN B-1 SOLUTION
FREEZE DRY
MIX

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 93/01408

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 A61K37/02; //(A61K37/02,33:42,33:14,33:00,31:70)

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	A61K ; A61M

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
X	US,A,4 975 419 (ENTRAVISION, INC) 4 December 1990 cited in the application see claims 1-6	1-6

° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document 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

"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

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
26 MAY 1993	11. 06. 93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	LEHERTE C.F.M.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

US 9301408
SA 70590

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 26/05/93

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US-A-4975419	04-12-90	EP-A,B	0358369	14-03-90
		JP-A-	2115128	27-04-90
		US-A-	5104663	14-04-92