

METHOD FOR STERILISING AQUEOUS SALINE SOLUTION

5 The present invention relates to a method of sterilising and packaging aqueous saline solutions, and especially isotonic saline solutions to provide long term storage stability and biocidal activity.

10 Isotonic saline solutions are used, for example, in the washing and cleaning of "soft" contact lenses and it is already known to package such solutions in an aerosol container with a suitable propellant. The solution can then be easily dispensed onto the contact lens when the lens needs cleaning. In such cases it is essential that the aerosol saline product be free of bacteria, and to this end the industry presently packs the saline solution and propellant into the container and the entire unit is then transported to a suitable radiation facility so that the contents of the package can then be
15 sterilised by irradiation of the entire package. This operation is costly.

Moreover, saline solutions sterilised in this way are merely sterile and do not possess any residual biocidal activity.

20 The lack of biocidal activity in an isotonic saline solution that has been sterilized by radiation creates a particular problem in that such solutions cannot be used to sterilize, as opposed to merely clean, "soft" contact lenses. "Soft" lenses, which normally consist of a copolymer of mainly a hydrophilic monomer, such as a 2-hydroxyethyl methacrylate, have approximately thirty percent (30%) by weight of
25 water, or more, and thus the lens itself offers a vehicle or environment suitable for the propagation of various kinds of bacteria which may cause serious injury to the human eye and surrounding tissue.

30 Therefore, soft contact lenses have to be sterilised by boiling the lens in water for a predetermined period of time. Although this method is very effective for sterilisation, it has, at the same time, the following defects:

35 (1) The protein components of lachrymal liquid remaining adhered to the lens become fixed to the surface of the lens during boiling and as the protein is thermally degenerated. This not only spoils the optical properties of the lens but also makes the lens feel very uncomfortable when replaced in the eye.

(2) The slightly cross-linked hydrophillic copolymer of 2-hydroxethyl methacrylate used in the manufacture of water-containing soft contact lenses deteriorates in the course of repeated boiling treatments, so that the lens becomes discoloured and/or the rated configuration changes, thus greatly shortening the life of the lens.

(3) The sterilisers used generally require an AC power source which is an inconvenience when travelling, or in outdoor environments such as when the user is at a camp site or the like.

(4) The technique is not suitable for non-water-containing contact lens made of polyethyl methacrylate or silicon rubber.

As a means of avoiding the difficulties involved in the sterilization of contact lenses by boiling, various sterilizing liquids have been proposed, such as thimerosal and chlorohexidine. However, such methods have drawbacks in that the sterilizing liquid tends to be absorbed into the lens and to cause anaphylactic inflammation of the ocular tissue.

Contact lenses may also be sterilised using a 3% sodium hydrogen peroxide solution, and a catalyst such as platinum, which causes the decomposition of the sodium hydrogen peroxide into water and oxygen with consequent sterilisation of the lens. However, this method also has drawbacks in that it takes a long time and involves a complicated procedure. That method is therefore impractical.

In accordance with the present invention it has been found that sterile saline solutions with long term stability and with long term biocidal activity can be prepared by subjecting the saline solution to electrolytic sterilisation and packaging the electrolytically sterilised solution under superatmospheric pressure in an aerosol container. Such solutions are found to retain their biocidal activity in the aerosol package for periods approaching four months, and thus provide an ideal medium for the cleansing and sterilisation of contact lenses.

In one aspect therefore the present invention provides a method for the preparation of sterile, biocidal saline solutions having long

term stability and long term retention of biocidal activity, which comprises electrolytically sterilising an aqueous saline solution by subjecting the solution to electrolysis in an electrolytic cell, and packaging the electrolytically sterilised solution, under superatmospheric pressure, in an aerosol container equipped with a dispensing valve permitting the dispensing of the sterilised solution from the container under pressure.

In a second aspect, the present invention provides a pressurised aerosol container equipped with a dispensing valve for dispensing the liquid contents of the container under pressure of a propellant gas packaged under pressure in the container, wherein the liquid contents of the container comprise an electrolytically sterilised, biocidal aqueous saline solution, the said sterilised saline solution retaining its biocidal activity in the container over a protracted period of time.

The sterilised saline solution is preferably an isotonic saline solution (0.9% NaCl).

The electrolytic sterilisation of aqueous saline solutions and electrolytic cells for this purpose are described in United States Patents 3,443,055 and 3,616,355 and to which reference should be made. The '355 patent discloses the electrolysis of chloride containing solutions at higher voltages than those disclosed in the '055 patent.

Other electrolytic cells suitable for sterilizing saline solutions, e.g. isotonic solutions, are described in United States Patents 3,479,275 and 3,547,600.

In practising the present invention the saline solution, preferably an isotonic saline solution is flowed through a suitable electrolytic cell and directly into a contiguous aerosol container, and the container then sealed by crimping a closure (mounting cup) bearing a manually-actuable valve onto the open end or bead of the container followed by pressurisation of the container with the propellant.

It has been found that suitable sterilisation conditions and parameters to provide sterile, biocidal saline solution capable of retaining its bactericidal/bacteriostatic properties over a protracted period include passing the saline solution through a electrolytic cell comprising two 2" x 3" (5 x 7.5 cm) electrodes spaced 0.27" (35 mm) apart, and at a flow rate of one litre in 35 seconds (29 mL/sec).

Sterilisation is effected by passing a 5.0 amp current through the saline solution at an applied voltage of 7.8 volts.

The solutions referred to hereinafter had two passes through the electrolytic cell under the above conditions unless otherwise stated.

5 The experimental data illustrating the prolonged stability and biocidal activity of the aerosol packaged, electrolytically sterilised saline solutions is set forth hereafter.

10 Solutions of sodium chloride in distilled water (isotonic saline solution) were prepared and treated in several different ways before being packaged in an aerosol container fitted with an aerosol valve. Several different microorganisms were used in this study and each strain of microorganism was added to a different container of saline solution. Some of the solutions were then passed through an electrolytic cell and packaged into aerosol containers. Samples from
15 each can were sprayed immediately after filling into the aerosol container and plated onto agar media. The plates were observed for bacterial growth after 24, 48 and 72 hours. Stored samples were also sprayed onto additional agar media and observed according to the above time schedule. Additionally, samples which were originally
20 contaminated with microorganisms and found to be sterile after passing through the electrolytic cell were re-contaminated with microorganisms after standing about three (3) weeks. There were then sprayed as above and according to the same schedule.

25 The results were obtained to date indicate the following:

- 25 1. Non electrolytically sterilised isotonic saline solution will support the growth of microorganisms
- 30 2. Isotonic saline solutions packaged in an aerosol container will maintain sterility if the product is initially sterile.
- 35 3. Isotonic saline solutions containing microorganisms can be sterilised by passing the solution through an electrolytic cell.
4. Isotonic saline solutions passed through an electrolytic cell, and added to an aerosol container containing microorganisms, is capable of killing the microorganisms and rendering the solution, can, valve and nitrogen

propellant sterile.

5. After about three (3) weeks, microorganisms added to an aerosol can filled with the electrolytically sterilised saline solution are killed within a 24 hour period.

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Experimental

I Preparation of Isotonic Saline Solutions (0.9% w/v)

A. Formulation

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All solutions were prepared by taking 9.0 grams of Sodium Chloride, U.S.P. and adding it to sufficient distilled water to make 1000 ml. of solution.

B. Packaging

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All aerosols were prepared by adding 200 ml. of the saline solution (treated or non-treated) to a 2.089 x ⁶7.5" (5.3 x ^{16.5}~~14.0~~ cm) internally lined aluminium aerosol container and sealing the can with standard aerosol valve (Precision Valve Co.). Nitrogen gas was then added to the filled container at a pressure of 100 psig (689.5 kPa). A suitable actuator was then added to the

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valve.

C. Microorganisms Used

The following microorganisms were used in this study:

1. Escherichia Coli - is a gram-negative non-spore forming bacillus. It is found as normal flora in the intestinal tract. Some pathogenic strains of E. Coli have the ability to invade the intestinal mucosa and produce enterotoxins. The symptoms seen are fever, chills, abdominal pain and dysentery.
 - The bacteria grows very well on MacConkey agar and nutrient agar (pink colonies observed).
 - The bacteria grows best at an incubation 35-37°C.
 - The bacteria grows well in a saline environment.
 - One could observe growth within a 24-48 hour period.
2. Pseudomonas Aeruginosa - is a gram-negative obligate aerobe rod. It is commonly found in soil, water, sewage and (in a small percentage) in the intestinal tract.
 - The bacteria grows well on MacConkey agar and nutrient agar. The organism is known to produce a bluish pigment named pyocyanin and a greenish fluorescent pigment known as fluorescein.
 - The bacteria grows best at an incubation of 35-37°C and grows well in a saline environment. Growth was observed within a 48-72 hour period.

These organisms are opportunistic, infecting individuals who are debilitated, burned or immunosuppressed.

3. Salmonella - This organism is commonly found in spoiled foods and is responsible for food poisoning.

D. Media Used

Two types of media were used to determine if bacterial growth was taking place. When no growth was noted, then the product was deemed to be sterile. Petri dishes containing the appropriate growth media were sprayed with the saline solution which had been treated in different ways. The following types of media were used:

- A. MacConkey Agar was used to enhance the growth of gram-negative bacteria.
- B. Columbia CNA Agar was used to enhance the growth of gram-positive bacteria.

II. Results

The following combinations of saline solution, microorganisms and treatment through the electrolytic cell were studied to date:

- A. Non-treated Saline Solution (Control)
- B. Non-treated Saline Solution + Microorganism
- C. Saline Solution + Electrolytic Cell
- D. Saline Solution + Microorganism + Electrolytic Cell
- E. Saline Solution + Electrolytic Cell + Aerosol Can Contaminated with Microorganisms

Tables I and II indicate the results obtained to date.

TABLE I

Sterility of Saline Solution (0.9%)

Treatment	Organism Added		Electrolytic Cell	MacConkey Media	CNA Media	Microbial Growth* 37°C			
	To Solution	To Can				24 HRS	48 HRS	72 HRS	Repeat
Distilled Water	-----	-----	NO	YES	YES	NG	NG	NG	NG
Saline Solution (Non-Aerosol)	-----	-----	NO	YES	YES	NG	NG	NG	NG
Saline Solution (Non-Aerosol)	-----	-----	YES	YES	YES	NG	NG	NG	NG
Saline Solution (Aerosol)	-----	-----	NO	YES	YES	NG	NG	NG	NG
Saline Solution (Aerosol)	-----	-----	YES	YES	YES	NG	NG	NG	NG
NG = No Growth									

*Even though no growth was noted, this is not an indication that growth will not occur if organisms are present. It just happened that no organisms were present in these samples.

TABLE II

Sterility of Saline Solution (0.9%) - With Microorganisms Added

Treatment	Organism Added		Electro-lytic Cell	MacConkey Media	CNA Media	Microbial Growth 37°C			
	To Solution	To Can				Time			Repeat after 3 weeks
						24 HRS	48 HRS	72 HRS	
Saline Solution (Aerosol)	E. Coll	---	NO	YES	YES	+++	+++	+++	+++
Saline Solution (Aerosol)	E. Coll	---	YES	YES	YES	NG	NG	NG	NG
Saline Solution (Aerosol)	---	E. Coll	NO	YES	YES	+++	+++	+++	+++
Saline Solution (Aerosol)	---	E. Coll	YES	YES	YES	NG	NG	NG	NG
Saline Solution (Aerosol)	Pseudomonas	---	NO	YES	YES	+++	+++	+++	+++
Saline Solution (Aerosol)	Pseudomonas	---	YES	YES	YES	NG	NG	NG	NG
Saline Solution (Aerosol)	---	Pseudomonas	NO	YES	YES	+++	+++	+++	+++
Saline Solution (Aerosol)	---	Pseudomonas	YES	YES	YES	NG	NG	NG	NG
Saline Solution (Aerosol)	Salmonella	---	NO	YES	YES	+++	+++	+++	+++
Saline Solution (Aerosol)	Salmonella	---	YES	YES	YES	NG	NG	NG	NG
Saline Solution (Aerosol)	---	Salmonella	NO	YES	YES	+++	+++	+++	+++
Saline Solution (Aerosol)	---	Salmonella	YES	YES	YES	NG	NG	NG	NG
+++ = Abundant Growth : NG = No Growth									

+++ = Abundant Growth
: NG = No Growth

Streptococcus F alkalies were also studied in a manner similar to the other organisms. Samples of organism were added to isotonic saline solution packages in an aerosol can. The samples packaged without passing through the electrolytic cell showed a great amount of bacterial growth while the samples passed through the cell and then packaged in an aerosol container showed no signs of growth.

The bactericidal/bacteriostatic properties of the isotonic saline solution is believed to be attributable to the formation of a combination of ozone, hydrogen peroxide and sodium hypochlorite.

In the past, passing saline solution through an electrolytic cell has produced a solution having a short time span of sterilization capability. However, it was unexpected to discover that saline solution passed through an electrolytic cell and promptly packaged in a pressurized vessel, such as an aerosol container, would retain the sterilization capability over a protracted period. Samples prepared according to the above have been found to retain their sterilization capability for a period approaching four months.

Chemical and Physical Studies

Drop in NaCl Content After Electrolysis

	mg	ml	mg	ml	mg	ml
	Ag	NaCl	Ag	NaCl	Ag	NaCl
NaCl						
First Day						
____/ml						
Not treated	15.62	9.1225	15.60	9.1196	15.70	9.1450
	15.60		15.61		15.60	
Passed	15.40	9.0290	15.35	8.9705	15.30	8.9296

Through Cell	15.50		15.35		15.26	
2nd Day	15.40	8.9998	15.30	9.2050	15.22	8.9004
	15.40		15.30		15.24	
6th Day	15.35	8.9559	15.30	8.9267	15.20	8.8829
	15.30		15.25		15.20	
12th Day	15.35	8.9764	15.23	8.9121	15.20	8.8887
	15.37		15.27		15.22	
18th Day	15.37	8.9822	15.21	8.9033	15.20	8.8829
	15.37		15.26		15.20	
24th Day	15.36	8.9822	15.24	8.9121	15.20	8.8829
	15.36		15.26		15.20	

The drop in sodium chloride content is consistent with the formation of sodium hypochlorite. The sterilization capability is affected by the duration of time that the saline solution is subjected to electrolysis; also suggesting that an increase in voltage may also affect the sterilization capability. This is demonstrated by the following data:

	<u>Can #1</u>	<u>Can #2</u>	<u>Average</u>
Before Cell	9.0465	9.0407	9.0436
After 1 Pass	8.9413	8.9413	8.9413
2 Passes	8.8829	8.8946	8.8887
3 Passes	8.8595	8.8712	8.8654
4 Passes	8.8244	8.8303	8.8274
6 Passes	8.7660	8.7660	8.7660
10 Passes	8.6900	8.6783	8.6842

While the method of the invention has been described in terms of "electrolyzing" the saline solution outside the aerosol container, it should be understood that the pressure (aerosol) container, per se, may function as an electrolytic cell by the appropriate disposition of electrodes and electrical connection as part of the container.

Further, the method of the invention has been specifically described with reference to nitrogen as the propellant. Other propellants, both liquified gas and compressed gas may be utilized. In this connection, the method has been described in terms of adding propellant through pressure filling nitrogen gas. However, if other than compressed gases are employed as the propellant, other systems, such as, for example, under-the-cup filling of propellant may be employed.

Additionally, while the invention has been illustrated by describing an aerosol container wherein the propellant is disposed in the container in contact with the saline solution, it should be understood (and it is presently viewed by the inventor to be the best form for an aerosol container having sterilized saline solution) that the saline solution may be packed in an aerosol container of the so-called "piston" type. In the "piston" type aerosol container, the product to be dispensed and the propellant are separated by a piston which moves along the longitudinal axis of the container in response to the pressure generated by the propellant on one side of the piston. Actuation of the valve on the product side of the piston provides an opening for the product to egress from the container. Closing the valve terminates flow of product and movement of piston.

CLAIMS

1. A method for the preparation of sterile, biocidal saline solutions having long term stability and long term retention of biocidal activity, which comprises electrolytically sterilising an aqueous saline solution by subjecting the solution to electrolysis in an electrolytic cell, and packaging the electrolytically sterilised solution, under superatmospheric pressure, in an aerosol container equipped with a dispensing valve permitting the dispensing of the sterilised solution from the container under pressure.
2. A method according to Claim 1, wherein the saline solution is an isotonic saline solution.
3. A method according to Claim 1 or 2, wherein the saline solution is sterilised prior to packaging in the aerosol container.
4. A method according to Claim 1 or 2, wherein the electrolysis is effected with said solution in said container.
5. A method according to any one of Claims 1-4, wherein the electrolytic sterilization is effected at an applied voltage of about 7.8 volts.
6. A method according to any one of Claims 1-5, wherein the sterilised solution is packaged in said container under the direct pressure of a pressurised propellant gas.
7. A method according to any one of Claims 1-5, wherein the sterilised solution is packaged in said container on one side of a piston sealingly and slidingly engaging in the container and operating under the applied pressure of a pressurised propellant gas packaged in the container on the opposite side of the piston.
8. A method according to Claim 6 or 7, wherein the pressurised propellant gas is nitrogen.



9. Sterilised, biocidal saline solutions when prepared and packaged by a method claimed in any and of Claim 1-8.

5 10. A sterilised, biocidal saline solution according to Claim 9 which is an isotonic saline solution.

10 11. A pressurised aerosol container equipped with a dispensing valve for dispensing the liquid contents of the container under pressure of a propellant gas packaged under pressure in the container, wherein the liquid contents of the container comprise an electrolytically sterilised, biocidal aqueous saline solution, the said sterilised saline solution retaining its biocidal activity in the container over a protracted period of time.

15 12. A pressurised aerosol container according to claim 11, wherein the saline solution retains its biocidal activity for a period approaching four months.

20 13. A pressurised aerosol container according to claim 11 or 12, wherein the said solution is an isotonic saline solution.

25 14. A pressurised aerosol container according to any one of claims 11-13, wherein the sterilised, biocidal saline solution is packaged in the container under direct pressure from the pressurised propellant gas.

30 15. A pressurised aerosol container according to any one of claims 11-13, wherein the sterilised, biocidal saline solution is packaged in the container on one side of a piston slidingly and sealingly engaging in the container, and the pressurised propellant gas is packaged in the opposite side.

16. A pressurised aerosol container according to any one of claims 11-15, wherein the propellant gas is nitrogen.

