

Preservation of sodium bicarbonate eye lotion BPC against contamination with *Pseudomonas aeruginosa*

R. M. E. RICHARDS AND R. J. McBRIDE

Pharmaceutical Microbiology Group, Pharmacy Department, Heriot-Watt University, Edinburgh

BPC (1968) eye lotions are sterile aqueous solutions which do not contain an antibacterial preservative and which are intended to be used undiluted in first-aid or domiciliary treatment. The fact that the eye lotion does not contain an antibacterial preservative means that the solution, after opening, is limited to being used once only in first-aid treatment or within 24 hours in domiciliary treatment. There may be a case, however, for sodium bicarbonate eye lotion BPC to be so formulated that it can be used as a multiple dose preparation for domiciliary treatment.

Pseudomonas aeruginosa (*P. aeruginosa*) was stated to be the most common contaminant of aqueous solutions and the most lethal in its effect upon the eye (Allen, 1959). A survey of the literature revealed that the first report implicating contaminated ophthalmic solutions as the cause of *P. aeruginosa* corneal ulceration was that of Garretson and Coogrove (1927). By the early 1950s it was fairly widely recognized that *P. aeruginosa* could be introduced to the eye as the result of using contaminated ophthalmic solutions (Bignard, 1951; Theodore, 1951; Theodore and Minsky, 1951; Allen, 1952; Theodore and Feinstein, 1952; Soet, 1952). The selected preservative system for ophthalmic solutions should therefore be capable of inactivating heavy contamination with *P. aeruginosa* as quickly as possible. It has been suggested that a sterilizing time of 1 hour or less is what should be expected of an antibacterial substance considered suitable for the preservation of ophthalmic solutions (Kohn, Gershenfeld, and Barr, 1963).

In an extensive review of the literature concerned with the preservation of ophthalmic solutions, however, it was concluded that "no single substance is entirely satisfactory for use as a preservative for ophthalmic solutions" (Richards, 1967a, b). It was suggested that anti-bacterial combinations offered possibilities of overcoming the problem and that the activity of such chemicals should be demonstrated against resistant bacteria.

Disodium ethylenediamine tetra-acetate (EDTA) was shown to accelerate the action of a quaternary ammonium compound against resistant strains of *P. aeruginosa* (MacGregor and Elliker, 1958). Consequently, the United States, National Formulary XII recommended the use of EDTA, with benzalkonium chloride, for the preservation of ophthalmic solutions. EDTA was shown to potentiate the activity of polymixin B. sulphate, chlorhexidine diacetate and benzalkonium chloride against *P. aeruginosa* (Brown and Richards, 1965). Nevertheless there is no advantage in using EDTA in combination with phenylmercuric nitrate (PMN) to preserve fluorescein solutions (Brown, 1968). 2-phenyl-ethanol (PEA), in combination with PMN, however, is a more effective preservative system for fluorescein sodium eye drops than the official BPC formulation, which is preserved with PMN alone (Richards, Suwanprakorn, Neawbanik, and Suraskidul, 1969).

PEA was first recommended for use as a preservative for ophthalmic solutions by Brewer, Goldstein, and McLaughlin (1953). Other workers have found PEA to have too slow an antibacterial action for use in ophthalmic solutions (Kohn and others, 1963). It has been shown, however, that PEA exerts its antibacterial effect by modifying the permeability properties of the bacterial cell (Silver and Wendt, 1967; Richards and others, 1969), and so when used in combination with other antibacterials PEA can not only exert its own antibacterial action, but also enhance the action of the antibacterial with which it is combined (Richards and others, 1969). PEA is one of the four antibacterials recommended for preserving ophthalmic solutions in the USNF XIII 1970.

In view of the foregoing it was decided to investigate the efficiency of suitable antibacterials alone and in combination with EDTA, or PEA, for preserving sodium bicarbonate solutions against contamination with *P. aeruginosa*.

Chemical incompatibilities limited the choice of "suitable" antibacterials to benzalkonium chloride, chlorocresol, and PMN. Preliminary tests showed thiomersal to have no advantage over PMN and so thiomersal was not included in the extended tests.

Material and methods

The test organism used was *P. aeruginosa* strain NCTC 6750 and the growth medium for liquid cultures was Oxoid No. 2 nutrient broth. Oxoid nutrient agar was the solid culture medium. The inactivating medium used to prevent any carry over effect of antibacterial action consisted of thio-glycollate medium USP, lecithin and polysorbate 80 (Riegelman, Vaughan, and Okumoto, 1956).

The sodium bicarbonate and the benzalkonium chloride were of BP quality and the chlorocresol, EDTA, PEA and PMN were BDH laboratory chemicals. Cell numbers were estimated by colony counts as already described (Richards and others, 1969) and stock cultures were maintained as before (Brown and Richards, 1964).

PREPARATION OF THE SODIUM BICARBONATE EYE LOTIONS

The eye lotions were prepared by mixing aliquots of concentrated solutions of sodium bicarbonate and antibacterials before diluting to volume. The pH of the resultant solutions was measured before carbon dioxide was bubbled through for 1 minute. The solutions were then autoclaved at 121°C. for 20 minutes and the pH for each solution was determined within a short interval of opening the containers. A further series of pH determinations was made 3 weeks after opening the containers. Sterilization time determinations were made on the series of solutions immediately after autoclaving and on the series of solutions which were in the containers that had been opened and then left for 3 weeks.

DETERMINATION OF STERILIZATION TIME

9.9-ml. volumes of the different formulations were placed in metal-capped sterile test tubes and left to equilibrate in a water bath at 25°C.; 0.1 ml. overnight *P. aeruginosa* culture containing approximately 6×10^8 organisms/ml. was added to each tube. These inoculated solutions were designated reaction mixtures. At intervals of 15, 30, 45, 60, 90, 120, 150, 180, 240, and 300 minutes after the addition of the inoculum, 0.5-ml. samples were taken from each reaction mixture and added to 9.5 ml. of inactivating recovery medium and incubated for 3 days at 37°C. Positive controls to test the efficiency of the recovery medium consisted of 0.1 ml. of a 1 in 100 dilution of the overnight culture added to tubes of recovery medium separately containing 0.5 ml. of the different formulations. Samples of doubtful cultures were incubated on a milk agar medium to check for possible pigment formation and clearing of casein (Brown, 1968).

To ensure uniform suspensions of bacteria a vortex mixer was used to disperse the overnight culture, to disperse the inoculum in the reaction mixtures, to resuspend the organisms before sampling the reaction mixtures, and to disperse the sample of reaction mixture throughout the inactivating recovery medium.

Results

The results of the pH determinations and the survival time determinations are collated in the Table.

Table Sterilization times for 3.5 per cent. w/v sodium bicarbonate solutions contaminated with *P. aeruginosa*

Concentration of antibacterial(s)	pH			Sterilization time (min.)			
	Before carbonating and sterilization	After sterilization	3 weeks after sterilization	At lower pH inoculum 5.4×10^8 /ml.		At higher pH inoculum 7.4×10^8 /ml.	
0.01 per cent. w/v Benzalkonium chloride ⁺	8.10	7.65	9.55	> 30	< 45	> 15	< 30
0.01 per cent. Benzalkonium ⁺ and 0.4 per cent. Phenylethanol (PEA)	8.13	7.70	9.65	> 15	< 30	< 15	
0.01 per cent. Benzalkonium ⁺ and 0.05 per cent. Sodium edetate* (EDTA)	8.05	8.10	9.70	> 30	< 45	< 15	
0.002 per cent. Phenylmercuric nitrate (PMN)	8.08	7.75	9.68	> 180	< 240	> 45	< 60
0.002 per cent. PMN and 0.4 per cent. PEA	8.12	7.55	9.65	> 45	< 60	> 15	< 30
0.02 per cent. PMN and 0.05 per cent. EDTA	8.03	7.60	9.72	> 150,	< 180	> 45	< 60
0.05 per cent. Chlorocresol	8.08	7.55	9.65	> 15	< 30	> 30	< 45
0.05 per cent. Chlorocresol and 0.4 per cent. PEA	8.10	7.55	9.62	< 15		< 15	
0.05 per cent. Chlorocresol and 0.05 per cent. EDTA	8.02	9.00	9.65	> 30	< 45	> 15	< 30
0.4 per cent. PEA	8.12	7.55	9.60	> 240	< 300	> 30	< 45
0.05 per cent. EDTA	8.10	7.55	9.65	> 300		> 300	

Controls: Duplicate controls separately containing 0.5 ml. of each formulation plus 9.5 ml. inactivating recovery broth were inoculated to a final concentration of approximately 6×10^4 overnight cells/ml. All gave growth.
⁺A fine white precipitate was noted in all tubes containing benzalkonium chloride
 *Sodium edetate—a synonym for disodium ethylenediamine tetracetate

From the pH readings it can be seen that bubbling carbon dioxide through the different formulations for 1 minute before sealing the containers was effective in lowering the pH of the solutions. The high pH, after autoclaving, of the chlorocresol and EDTA combination would indicate that the seal of the container was inadequate for that container. The pH of all formulations increased on storage. This in fact would be the case if the eye lotion was used as a multi-dose preparation. At the higher pHs the sterilization times were less than those determined at the lower pHs. The exception to this was chlorocresol alone which had a longer sterilization time at the higher pH. This was expected as the higher pH would ionize the chlorocresol and reduce its activity. The activity of benzalkonium chloride is known to increase with increasing pH but the fact that a fine white precipitate was formed in all formulations containing benzalkonium chloride indicates that benzalkonium chloride cannot be used for preserving these solutions.

PMN in combination with PEA is again shown to be a more effective preservative system than PMN alone, and PMN in combination with EDTA is again shown to be a less effective preservative system than PMN alone, thus confirming the results already mentioned (Richards and others, 1969; Brown, 1968).

Chlorocresol is shown to have a very rapid action even at the high pH. Chlorocresol in combination with PEA has the shortest sterilization time of all the possible formulations at both pHs.

CONCLUSION

These results would indicate that it is possible to adequately preserve sodium bicarbonate solutions against contaminations with *P. aeruginosa*.

Summary

The rationale behind using preservative combinations for the preservation of ophthalmic solutions is given.

Formulations of 3.5 per cent. w/v sodium bicarbonate solutions were tested for their ability to sterilize high inocula of *P. aeruginosa*.

Sodium bicarbonate 3.5 per cent. w/v solutions preserved with a 0.002 per cent. PMN/0.4 per cent. PEA combination had a sterilization of less than 1 hour.

Formulations preserved with either 0.05 per cent. chlorocresol alone or a 0.05 per cent. chlorocresol/0.05 per cent. EDTA combination sterilized in less than 45 minutes.

Solutions preserved with a 0.05 per cent. chlorocresol/0.4 per cent. PEA combination sterilized in less than 15 minutes.

Therefore the preservative systems in all these formulations quoted, when challenged with high inocula of *P. aeruginosa*, satisfied the requirement that a preservative suitable for preserving ophthalmic solutions should be capable of sterilizing contamination in less than 1 hour.

References

- ALLEN, H. F. (1959) *Trans. Amer. ophthal. Soc.*, **57**, 377
 ——— (1952) *Amer. J. Ophthal.*, **35**, no. 5, pt 2, p. 99
 BIGNELL, J. L. (1951) *Brit. J. Ophthal.*, **35**, 419
 BREWER, J. H., GOLDSTEIN, S. W., and McLAUGHLIN, C. B. (1953) *J. Amer. pharm. Ass. (Sci. Ed.)*, **42**, 584
 BROWN, M. R. W. (1968) *J. pharm. Sci.*, **57**, 389
 ——— and RICHARDS, R. M. E. (1964) *J. Pharm. Pharmacol.*, **16**, Suppl. (British Pharmaceutical Conference, Edinburgh, 1964), p. 41T
 ——— (1965) *Nature (Lond.)*, **207**, 1391
 GARRETSON, W. T., and COSGROVE, K. W. (1927) *J. Amer. med. Ass.*, **88**, 700
 KOHN, S. R., GERSHENFELD, L., and BARR, M. (1963) *J. pharm. Sci.*, **52**, 967
 MACGREGOR, D. R., and ELLIKER, P. R. (1958). *Canad. J. Microbiol.*, **4**, 499
 RICHARDS, R. M. E. (1967a) *Aust. J. Pharm.*, **48**, Sci. Suppl. 55, s86
 ——— (1967b) *Ibid.*, **48**, Sci. Suppl. 56, s96
 ——— SUWANPRAKORN, P., NEAWBANIJ, S., and SURASDIKUL, N. (1969) *J. Pharm. Pharmacol.*, **21**, 681
 RIEGELMAN, S., VAUGHAN, D. G., and OKUMOTO, M., (1956) *J. Amer. pharm. Ass. (Sci. Ed.)*, **45**, 93
 SILVER, S., and WENDT, L. (1967) *J. Bact.*, **93**, 560
 SOET, J. C. (1952) *Sight Sav. Rev.*, **22**, 202
 THEODORE, F. H. (1951) *Amer. J. Ophthal.*, **34**, 1764
 ——— and FEINSTEIN, R. R. (1952) *Ibid.*, **35**, 656
 ——— and MINSKY, H. (1951) *J. Amer. med. Ass.*, **147**, 1381