J. Pharm. Pharmac., 1977, 29, 717–722 Received June 27, 1977

Loss of antibacterial preservatives from contact lens solutions during storage*

N. E. RICHARDSON, D. J. G. DAVIES†, B. J. MEAKIN AND D. A. NORTON

Pharmaceutics Group, School of Pharmacy and Pharmacology, University of Bath, Bath, BA2 7AY, U.K.

The preservative content of 34 commercially available contact lens solutions has been determined. Over half of the solutions contained less than 90% of the stated preservative content. Storage tests conducted at 40°, using both simulated and commercially available contact lens solutions in plastics containers of the type used to present these products showed that thiomersal and chlorbutol appeared to be sorbed by these containers in contrast to benzalkonium chloride and chlorhexidine gluconate which interacted mainly by a surface adsorption process. The extent of any interactions was dependent upon the type of plastics material used to fabricate the container.

We have previously reported (Norton, Davies & others, 1974) on the antimicrobial activity of 34 commercially available contact lens solutions against common test organisms such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*. A wide variation was reported in their bactericidal activity, but it was also noted that differences in activity occurred between solutions purporting to contain similar concentrations of the same preservative. These differences could have been due to the inhibiting influence of formulatory adjuvants, but the possibility also existed that solutions purporting to contain identical concentrations might in reality contain different amounts. Therefore the 34 products were assayed for preservative content. Table 1 shows that about half of the solutions contained less than 90% of the stated concentrations. These discrepancies could have been due to bad manufacturing processes or poor quality control. Alternatively they could be due to interactions between the preservative and the plastics materials used to package these products; 32 were packaged in polyethylene bottles and 2 in polypropylene bottles, most being closed with a low density polyethylene plug and polystyrene cap. Both polyethylene and polypropylene are polymers of the long chain hydrocarbon type and consequently are considered to be highly resistant to chemical attack. However, many observations have been reported in the literature concerning the interaction of preservatives in solution with plastics containers, particularly those fabricated from polyethylene (Russell & Stock, 1966; Erikkson, 1967; Fischer & Neuwald, 1971; Friesen & Plein, 1971; Kakemi, Sezaki & others, 1971; Youssef, Sina & others, 1973). It was therefore decided to investigate these different possible explanations by carrying out a series of storage tests at 40° using both commercially available and laboratory simulated contact lens solutions.

* Work supported by the Supplies Division, D.H.S.S.
† Correspondence.

MATERIALS AND METHODS

Chlorbutol (reagent grade, Hopkin and Williams) was recrystallized from 50% ethanol before use. Chlorhexidine gluconate (20% w/v solution, ICI) was used as received. Benzalkonium chloride (50% w/v solution, Koch-light Ltd) was used as received. Thiomersal (reagent grade, BDH) was recrystallized from 95% ethanol before use.

The following materials were chosen as typical formulatory adjuvants and the assay of all of the preservatives was verified in their presence:—

1/15 M Sørensens phosphate buffer at pH 7·0 prepared from disodium hydrogen phosphate and

Table 1. *The preservative content of commercially available contact lens solutions.*

Preservative	Number of solutions assayed	Number of solutions containing more than 110% of stated concentration	Number of solutions containing within 90–110% of the stated concentration	Number of solutions containing within 50–90% of the stated concentration	Number of solutions containing less than 50% of the stated concentration
Benzalkonium chloride	14	2	11	1	0
Chlorbutol	5	1	1	2	1
Chlorhexidine gluconate	6	1	3	0	2
Thiomersal	15	1	2	8	4

potassium dihydrogen phosphate (Analar, BDH), 0·1% EDTA (Analar, BDH), 0·33% methyl cellulose 450 (Evans Medical Co.), 0·35% w/v hydroxyethylcellulose (Natrosol M, Hercules Powder Co.), 10% w/v polyethylene glycol 400 (BDH), 1·4% w/v polyvinylalcohol (Gohsenol GH17, Nippon Gohsei, British Traders & Shippers) and 0·9% sodium chloride (Analar, BDH).

Assay procedures

Chlorbutol. 2 ml of solution containing 0·5% w/v chlorbutol and 0·9% w/v sodium chloride was diluted with water to 50 ml and 2 ml of concentrated nitric acid added. The potential difference between a silver electrode and a reference mercurous sulphate electrode was recorded after successive additions of 0·02 N silver nitrate solution using a Radiometer Autoburette (type ABU1c) Titrator (type TTT1d) and Titrigraph recorder (type SBR2c). Five replicates of the end point gives a mean value of 135·8 mV (s.d. 2·05 mV). 2 ml of the chlorbutol and sodium chloride solution was then hydrolysed by refluxing with 2 ml of 20% w/v sodium hydroxide solution for 15 min and the end-point determination repeated. Five replicates give a mean value of 137 mV (s.d. 2.24 mV). The titrator was therefore set to an end-point of 136 mV and the value of titrant required to reach this end-point could then be read off directly from the Autoburette. The difference in titre before and after hydrolysis represents the amount of chloride released, each ml of 0·02 N silver nitrate being equivalent to 1·43 mg $C_4H_7OCl_3.\frac{1}{2}H_2O$. Calibration plots of the mean titre difference of two determinations against chlorbutol concentration in the sample were linear, passing through the origin, and had a mean value for the slope of 15·92. The precision of the assay process ranged from ±0·70% at a concentration of 0·3% w/v chlorbutol to ±5·3% for 0·03% w/v chlorbutol.

Chlorhexidine gluconate. 2 ml of a solution containing 0·002–0·010% w/v chlorhexidine gluconate were placed in a 25 ml volumetric flask and 5 ml of 20% w/v cetrimide solution added together with 1 ml of isopropanol to suppress frothing. 2 ml of alkaline sodium hypobromite, prepared according to the method of Holbrook (1958) which had previously been assayed and accurately diluted to give 1·5% available bromine, was then added and the solution was made up to 25 ml and left for 15 min when its absorbance was determined in a 4-cm cell at 480 nm. Two calibration plots of absorbance at 480 nm against chlorhexidine gluconate concentration in the sample were linear and had a mean value for the slope of 69·1. The precision of the assay varied from ±0·7% to ±2·8% for nominal chlorhexidine gluconate concentrations of 0·01 and 0·001% w/v respectively.

Benzalkonium chloride. 4 ml of a solution containing 0–0·01% benzalkonium chloride was placed in a 50 ml beaker, and 10 ml water, 1 ml of 50% sulphuric acid and 1 ml dimethyl yellow added. As not all contact lens solutions contain viscolysers a standard amount of polymer (2 ml of 1·4% polyvinyl alcohol [Gohsenol GH17]) was also added to all solutions before assay in order to standardize the visual end-point. After mixing, 20 ml of chloroform was added giving a resultant bright yellow colour. The contents of the beaker were stirred continually on a magnetic stirrer whilst being titrated with 0·01% w/v sodium lauryl sulphate (extra pure, BDH Ltd) in a 5-ml microburette. The end-point was indicated by a change to a darker orange colour. Two calibration plots of titre against benzalkonium chloride concentration in the sample were linear and had a mean value for the slope of 324·1. The reproducibility of the assay ranged from ±1·6 to 3·2% over the concentration range 0·002–0·006% w/v.

Thiomersal. A 2-ml sample of a solution containing 0·001–0·006% w/v thiomersal was added to a conical flask containing 5 ml of 50% sulphuric acid and 25 ml of 5% potassium permanganate solution which was then refluxed for 30 min, cooled and 4 ml of 20% w/v hydroxylammonium chloride added to remove unreacted potassium permanganate. The solution was transferred to a 50 ml flask and made up to volume with water. 25 ml of this solution was transferred to a separating funnel and 5 ml of 6 N acetic acid added. The solution was then shaken for 30 s with 10 ml of 0·002% w/v dithizone (Analar, BDH) solution in chloroform which had previously been adjusted with chloroform to give an absorbance of 1·16 at 608 nm in a 1-cm cuvette. The contents of the separating funnel were allowed to separate for 2 min and then a 3-ml sample of the chloroform layer was removed by pipette and the absorbance of a 1 cm layer determined at 608 nm. Two calibration plots of absorbance against thiomersal concentration in the sample were linear and gave a

mean value for the slope of −186·3. The concentration of thiomersal in the sample is then given by:

$$\% \text{ Concentration thiomersal in 2 ml sample} = \frac{1{\cdot}16 - \text{absorbance at 608 nm}}{186{\cdot}3}$$

The influence of formulatory adjuvants on the preservative assays

Solutions nominally containing either chlorbutol 0·4%, chlorhexidine gluconate 0·005%, benzalkonium chloride 0·006% or thiomersal 0·003% were assayed using the procedures outlined above in the presence of each of the formulatory adjuvants described previously. Replicate assays were determined for each solution and the results, given in Table 2, show that the assay procedures were not influenced by the presence of formulatory adjuvants to a significant extent. Similar experiments showed that the assay procedure for a given preservative was valid in the presence of the other preservatives.

Table 2. *Determination of the preservatives in the presence of formulatory adjuvants*

Formulatory Adjuvant	% concn relative to standard			
	Chlorbutol	Chlorhex gluc.	Benzal. Cl	Thiomersal
Methylcellulose	98·5	98·3	97·9	99·5
Hydroxyethylcellulose	99·5	99·1	98·4	98·8
Polyvinylalcohol	99·5	98·3	97·9	96·7
Polyethylene Glycol 400	98·5	100·6	98·4	96·7
Phosphate buffer pH 7·0	98·6	101·1	97·3	99·5
0·9% Sodium chloride	100·0	99·1	97·3	98·9
0·1% EDTA	97·0	101·9	95·7	101·5

Preservative loss on storage of contact lens solutions at 40°

Storage tests were conducted at 40° ± 0·2° in a controlled temperature cabinet (Fisons Electrical Equipment Ltd). To assess the extent of the interaction between unfabricated polymer resin and the preservative, 15g samples of low density polyethylene granules (ICI type XDB76) and polypropylene powder (ICI type LY542M) were stored in 100 ml of a simple aqueous solution containing either 0·004% benzalkonium chloride, 0·004% chlorhexidine gluconate, 0·004% thiomersal or 0·5% chlorbutol in glass stoppered Pyrex flasks.

Simulated contact lens solutions consisted of each preservative at the concentrations given above, in aqueous solution containing 0·1% EDTA, 0·9% sodium chloride and phosphate buffer at pH 7·0 in the absence and presence of 0·35% hydroxyethylcellulose (Natrosol M) as an example of a viscolizer. These solutions were stored in standard low density polyethylene containers of nominal volume 110 ml.

The commercially available solutions were purchased from retail outlets, opened, assayed for the preservative content and then transferred to a fresh unused container for that product obtained from the relevant manufacturer.

Two samples of each of the above solutions were withdrawn after various time intervals and the concentration of each preservative determined. The data were plotted as the percentage residual preservative concentration remaining at each time interval and are shown in Figs 1–4.

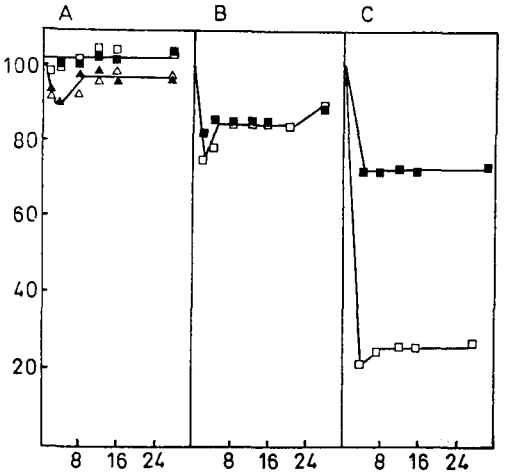

FIG. 1. The effect of storage at 40° on benzalkonium chloride concentration in contact lens solutions. A—Commercially available solutions (■□, ▲△) in new containers. B—Solutions stored in standard low density polyethylene containers: with hydroxyethylcellulose (■), without hydroxyethylcellulose, □. C—Solutions stored in glass flasks in contact with polyethylene granules (■) or polypropylene powder (□) Ordinate—% residual benzalkonium chloride concentration. Abscissa—Time (weeks).

For each preservative the data are divided into three sections showing plots for the commercial solutions in part A and the simulated solutions stored in the standard container in part B. The data for the solutions stored in contact with the polymer resins are shown in part C.

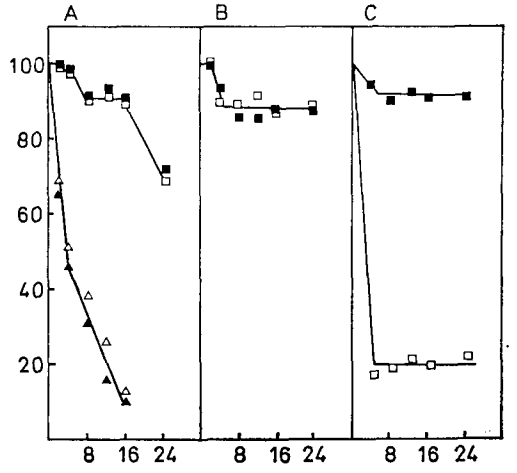

FIG. 2. The effect of storage at 40° on chlorhexidine gluconate concentration in contact lens solutions. Legend as for Fig. 1. Ordinate—% residual chlorhexidine gluconate concentration. Abscissa—Time (weeks).

DISCUSSION

It is apparent from Table 1 that the preservative content of many commercially available contact lens solutions differs markedly from that stated on the container by the manufacturer. Of solutions which showed a low concentration of preservative those containing thiomersal gave rise to the greatest concern. Only two solutions containing thiomersal were within the acceptable limits of 90–110% of the declared preservative concentration and one solution contained about 170% of the stated amount. Of the remaining twelve solutions, eight were between 50 and 90% of the declared concentration and four were below 50%; in two no thiomersal could be detected by our assay procedure. Similarly, three out of the five solutions containing chlorbutol were below 90% of their stated preservative content, one solution having only about 30%. Of the six solutions containing chlorhexidine gluconate one had about 18% and the other about 38% preservative present. In contrast, eleven of the solutions containing benzalkonium chloride were within ±10% of the stated preservative content. Two solutions contained excessive concentrations of benzalkonium chloride (130 and 150%) and one was only slightly below the acceptable range (84%).

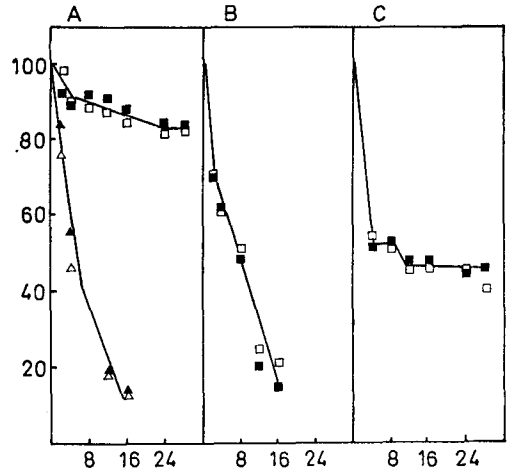

FIG. 3. The effect of storage at 40° on chlorbutol concentration in contact lens solutions. Legend as for Fig. 1. Ordinate—% residual chlorbutol concentration. Abscissa—Time (weeks).

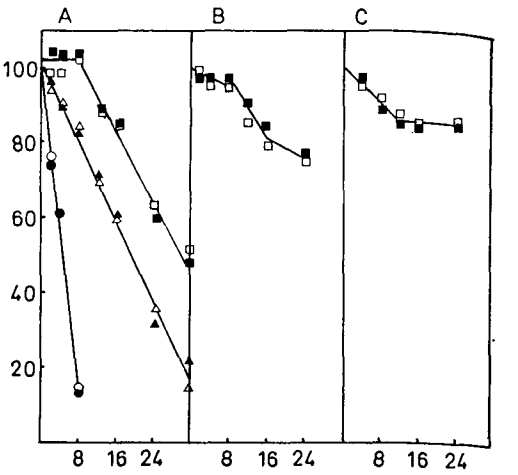

FIG. 4. The effect of storage at 40° on thiomersal concentration in contact lens solutions. A—Commercially available solutions in new containers (■□, ▲△, ○●). B—Solutions stored in standard low density polyethylene containers; with hydroxyethylcellulose (■), without hydroxyethylcellylose (□). C—Solutions stored in glass flasks in contact with polyethylene granules (■) or polypropylene powder (□). Ordinate—% residual thiomersal concentration. Abscissa—Time (weeks).

Contact lens solutions containing excessive percentages of preservative (130–170%) must presumably have arisen as a result of poor manufacturing techniques and quality control. Those with a deficiency could also be the result of interactions between the preservative and the container. However it is difficult to see why the manufacturers were not aware of such problems if adequate 'in house' storage tests and control had been carried out.

For this survey, two separate samples of each solution were obtained from various retail outlets

and consequently we had no knowledge of the preparations' age; in some instances the products had no batch number. It is interesting to note that those solutions found to contain excessively low or high concentrations of preservative often showed poor reproducibility between containers although within containers the reproducibility of preservative content was within the limits described in the assay methodology.

Examination of the data in Figs 1–4 shows that all of the four preservatives in aqueous solution may interact with both the plastics containers in which they were stored and the polymer resins although the extent of the interaction is variable. The commercially available solution containing benzalkonium chloride which was packaged in a polypropylene bottle did not lose any significant amount of preservative over the six month period studied whereas the other solution, in a low density polyethylene bottle, showed a small initial drop in preservative concentration which then became constant (Fig. 1A). Similarly, the benzalkonium chloride content of solutions stored in the standard low density polyethylene container showed an initial drop of about 15% and again did not show any further reduction in preservative concentration (Fig. 1B). This pattern of preservative loss suggests that the benzalkonium chloride is interacting with the plastics containers by a surface adsorption process which is consistent with the cationic nature of this preservative. This is also reflected in the large loss of benzalkonium chloride (70–80%) observed in the simple aqueous solution of benzalkonium chloride stored in contact with polypropylene powder which has a large surface area compared with that of the polyethylene granules where only about 30% of the preservative was removed (Fig. 1C). With the standard container, the polypropylene powder and one of the commercial solutions a minimum was noted in the graph after 4 weeks, but the reason for this is not yet understood.

Solutions containing chlorhexidine gluconate stored in the standard container and in contact with the polymer resins show similar preservative loss curves to those for benzalkonium chloride which again indicates that a surface adsorption process is operating. However, the two commercial solutions containing chlorhexidine exhibited a continuous rapid loss of preservative with the result that one of the solutions was reduced to 10% of its original concentration in only 16 weeks. Preliminary studies in our laboratories have shown that chlorhexidine is unstable at alkaline pH. The solution in question had a pH of 9·2 and calculation infers that all the loss observed could be accounted for by breakdown of the preservative rather than preservative-plastic interactions. The other commercial solution had a pH of 8·6 which may again be the reason for the apparent loss of chlorhexidine over the six months.

Fig. 3A shows that chlorbutol was also gradually lost from the two commercially available solutions containing this preservative. One solution which was packaged in a low density polyethylene container, rapidly lost chlorbutol and only 10% of the original concentration remained after 16 weeks. The other commercial solution was packaged in a polypropylene container and the observed loss rate for the chlorbutol was greatly reduced. The solutions stored in the standard container also showed a rapid continuous removal of chlorbutol. The lack of a plateau in these loss curves indicates that the chlorbutol – polymer interaction is due to a sorption process. There was no significant difference in behaviour between the polyethylene granules and polypropylene powder stored in chlorbutol solutions which both showed a fall in preservative concentration down to about 50% residual in about three months and then became constant and this is again in line with the postulated sorption mechanism which requires the extent of the interaction to be independent of the surface area at equilibrium. The appearance of a plateau in these systems is a function of the container, stoppered Pyrex glass flasks. Glass does not sorb chlorbutol and consequently when the drug-plastic interaction has reached equilibrium no further uptake is observed. In the plastics containers, the volatile nature of chlorbutol probably results in its being desorbed from the outer surface of the container into the atmosphere and an equilibrium situation will not be achieved. This permeation process involves distribution of the preservative into the plastics matrix, diffusion through the container wall, followed by desorption into the atmosphere and is described by equation 1 where P is the permeability coefficient for the process, D is the diffusion coefficient within the plastics matrix and K is the partition coefficient between polymer and water.

$$P = DK \quad .. \quad .. \quad (1)$$

Due to the chemical similarity between polyethylene and polypropylene, K would not differ greatly for the two materials. Diffusion however would be

hindered by the greater crystalline alignment of the polymer chains present in polypropylene. The permeability coefficient would thus be lower than that for polyethylene which is reflected in the much lower rate loss observed for the commercial solution packaged in a polypropylene container.

The three commercially available solutions containing thiomersal (Fig. 4A) showed rapid continuous losses of preservative and by extrapolating the data it could be predicted that all three solutions would be completely depleted of thiomersal within 40 weeks storage at 40°. These solutions were all packaged in low density polyethylene containers in which titanium dioxide was incorporated into the plastics resin as a filler. In contrast, thiomersal loss from the standard low density polyethylene containers was at a much slower rate. The loss rate curves for thiomersal from solutions stored in contact with the polypropylene powder and the polyethylene granules both show a small drop in preservative concentration of about 15% over a two month period and then become constant which is a similar pattern to that shown by chlorbutol in these systems. The loss of thiomersal is not so readily explained as this compound is the sodium salt of ethyl mercurithiosalicylate which is non-volatile having a melting point of 230°. On the basis of drug plastics interactions studies previously reported (Nasim, Meyer & Autian, 1972, Richardson & Meakin, 1974) no interactions would be expected between a carboxylate anion and a polymeric hydrocarbon. Examination of the literature suggests that thiomersal degrades to give a mixture of ethyl mercurichydroxide and di(ethyl mercuric) thiosalicylate (Trikojus, 1946). Both these materials would be expected to partition into a hydrocarbon polymer and ethyl mercuric hydroxide at least is sufficiently volatile to be lost from the container into the atmosphere. The presence of a filler such as titanium dioxide in the polyethylene would result in a much more open network of polymer chains which could then allow a greater and more rapid penetration of the thiomersal than of the unfilled plastics material and may therefore explain the more rapid loss of preservative from the commercial solution.

It is apparent therefore that chlorbutol and thiomersal may be sorbed by polyethylene and polypropylene containers which may lead to almost complete loss of preservative on storage. Both benzalkonium chloride and chlorhexidine gluconate appear to interact with these plastics by a surface adsorption process. Benzalkonium chloride showed the least interaction and the loss of this preservative from these systems is probably not microbiologically significant. Differences in preservative loss rates in these plastics containers are a function of the container, the loss rate from polypropylene being generally slower than from polyethylene. Preliminary storage studies have also been conducted at 20° and ambient temperature and the overall preservative loss pattern was the same as that which could be predicted from the data described above.

REFERENCES

Eriksson, K. (1967). *Acta pharm. suecica*, **4**, 261–264.

Fischer, H. & Neuwald, F. (1971). *Pharm. Int., Eng. Ed.*, **4**, 11–15.

Friesen, W. T. & Plein, E. M. (1971). *Am. J. Hosp. Pharm.*, **28**, 507–512.

Holbrook, A. (1958). *J. Pharm. Pharmac.*, **10**, 370–374.

Kakemi, K., Sezaki, H., Arakawa, E., Kimura, K. & Ikeda, K. (1971). *Chem. Pharm. Bull.*, **19**, (12), 2523–2529.

Nasim, K., Meyer, M. C. & Autian, J. (1972). *J. pharm. Sci.*, **61** (11), 1775–1780.

Norton, D. A., Davies, D. J. G., Richardson, N. E., Meakin, B. J. & Keall, A. (1974). *J. Pharm. Pharmac.*, **26**, 841–846.

Richardson, N. E. & Meakin, B. J. (1974). *Ibid.*, **26**, 166-174.

Russell, J. & Stock, B. H. (1966). *Aus. J. Pharm.*, **47**, (556), S37–S38.

Trikojus, V. M. (1946). *Nature*, **158** (4014), 472–473.

Youssef, M. K., Sina, A., Kassem, A. A., Ibrahim, S. A. & Attia, M. A. (1973). *Ind. J. Pharm.*, **35** (5), 155–158.