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The interaction of preservatives with polyhydroxyethylmethacrylate (polyHEMA)*

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

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

The interaction of the four most commonly used preservatives in contact lens solutions (chlorbutol, thiomersal, chlorhexidine gluconate and benzalkonium chloride) with polyhydroxyethylmethacrylate (polyHEMA), has been examined. Benzalkonium chloride and chlorhexidine gluconate show typical high affinity type isotherms. The interaction of benzalkonium chloride with polyHEMA from aqueous solution was reversible whereas that of chlorhexidine was only reversible in the presence of electrolyte or surfactant. Chlorbutol showed a typical reversible linear isotherm. Thiomersal does not interact with polyHEMA above pH 5·0. The extent of chlorhexidine—polyHEMA interactions is increased by the presence of formulatory adjuvants such as electrolyte and hydrophilic polymers. PolyHEMA lenses that apparently have been equilibrated with chlorhexidine gluconate will, on the addition of fresh preservative solution, bind further quantities of chlorhexidine above that which would be predicted from the sorption isotherm.

Contact lenses can be broadly classified into two types, hard and soft, depending upon their ability to absorb water. Most soft lenses at present available are manufactured from polyhydroxyethylmethacrylate (polyHEMA) cross-linked with other materials. They are hydrophilic being capable of absorbing up to 40% of water (Wyckoff, 1972). In the hydrated state they are pliable and easier for the eye to tolerate than hard lenses. However during use they sorb protein material on their surfaces and they may also become contaminated by microorganisms. Thus it is generally recommended that lenses are cleaned and disinfected daily and routines have been developed to achieve these aims. The disinfection process may involve boiling, the use of hydrogen peroxide, or soaking in a solution containing an antibacterial agent. There is a danger with antibacterial agents that they may be carried over into the eye where they may cause irritancy or toxicity (Phares, 1972; Sibley & Yung, 1973; Browne, Anderson & Charvez, 1974; Dreifus & Wobmann, 1975; Kaspar, 1976). Because of the toxicity of some of these agents most contact lens solutions contain only chlorbutol, benzalkonium chloride, chlorhexidine gluconate or thiomersal, either alone or in combination. We have investigated the interaction of these four preservatives with polyHEMA in aqueous solution. Most soft contact lens solutions contain either chlorhexidine gluconate or thiomersal and the interaction of these with polyHEMA has been examined in more detail.

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† Correspondence.

MATERIALS AND METHODS

Material

Chlorbutol, chlorhexidine gluconate, benzalkonium chloride and thiomersal were as described previously (Richardson, Davies & others, 1977). Hydroxyethylcellulose (Natrosol M, Hercules Powder Co.), polyvinyl alcohol (Gohsenol GH17, Nippon Goshei, supplied by British Traders and Shippers Ltd.,) polyethylene glycol 400 (BDH Ltd.), and Tween 80 (Honeywill-Atlas), were used as received. Buffer salts and potassium chloride were of Analar quality. Water was freshly distilled from an all glass still.

Polyhydroxyethylmethacrylate (polyHEMA) in both sheet form and as lenses was a gift from Smith & Nephew Research Ltd. The sheets were milled, mechanically sieved and the particle sizes $<120\,\mu m$ collected and used in subsequent experiments. Water extractive was determined by shaking 0·2 g powdered polyHEMA with 10 ml water for 24 h at 30°. The ultraviolet spectrum of the filtered solutions showed a high absorbance over the range 190–230 nm which was probably due to the release of ions from the polymer. Above 230 nm the spectrum showed a low constant absorption of about 0·01–0·015 optical density units which did not interfere with any of the assay procedures used. The density measured by air displacement was 1·357 g cm^{-3}. PolyHEMA lenses were used as received.

pH measurements

These were made at the temperature of the sorption experiments using a Radiometer type 27 pH meter fitted with a PHA 925a type scale expander and a

Pye Ingold 405 combined glass calomel electrode. Calibration was with two standard buffers, one above and one below the pH to be measured (Bates, 1973).

Assay procedure

Ultraviolet and colorimetric analysis was carried out using a Pye-Unicam SP500 spectrophotometer. Chlorhexidine gluconate solutions of concentration 0·01–0·1% and thiomersal solutions were diluted appropriately in 0·1 and 0·5 M hydrochloric acid respectively. Beer Lambert plots gave the molar absorption coefficients as 18530·6 for chlorhexidine gluconate at 250 nm, and 2751·0 for thiomersal at 310 nm. Below 0·01% w/v, chlorhexidine gluconate was determined colorimetrically (Richardson & others, 1977). Chlorbutol was assayed potentiometrically (Richardson & others, 1977). Benzalkonium chloride was determined using a modified method of Few & Ottewill (1956). A 2 ml sample of benzalkonium chloride solution (0·001–0·01% w/v) was shaken for 2 min in a 25 ml flask with 5 ml 0·00365% w/v Orange II in 0·1 M sodium chloride and 10 ml Analar chloroform. The flask was covered with aluminium foil to exclude light, and after 1 h to allow for the phases to separate, the absorbance of the chloroform layer was determined at 490 nm. Replicate calibration plots gave values for the slope of 113·9 (s.d. 2·3) and 109·7 (s.d. 2·5) and for the intercept of −0·041 (s.d. 0·012) and −0·030 (s.d. 0·013). The intercept in both cases was negative and did not span the origin within two standard deviations. Such a negative intercept may be the result of adsorption of the cationic preservative onto the glassware used. The concentration of benzalkonium chloride could then be obtained from equation 1 where 111·8 and −0·035 were the mean values for the slope and intercept respectively.

$$\text{Absorbance } 490\text{nm} = 111{\cdot}8 \text{ Concentration benzalkonium chloride} - 0{\cdot}035 \quad \ldots\ldots (1)$$

pKa determination. The pKa of thiomersal was determined as 3·143 at 30° and ionic strength 0·5 M by the spectroscopic method of Albert & Serjeant (1971).

Determination of sorption

The method used was that of Richardson & Meakin (1974) which enabled both equilibration and sampling to be carried out under isothermal conditions. Samples of polyHEMA powder or lenses (0·2 g), accurately weighed, were placed in a series of dry conical flasks fitted with ground glass stoppers and 10 ml of the appropriate concentration of preservative solution added. The flasks were then stoppered and shaken at 70 cycles min^{-1} in a thermostatted bath (±0·1°) until equilibrium was established when the supernatant was sampled through a No. 3 sintered glass filter and assayed. Determination of equilibration times were carried out using the highest anticipated concentration of each preservative in aqueous solution at 30° and samples taken at known intervals. Thiomersal was not taken up by polyHEMA powder over 24 h and with the other agents, maximum uptake was always established within 8 h. A standard shaking time of 24 h was therefore adopted for all subsequent sorption experiments to ensure that equilibrium was established.

Sorption-desorption isotherms at 30°. The sorption isotherm was determined for each preservative in simple aqueous solution and Figs 1–3 show the isotherms obtained with 0·2 g polyHEMA powder for chlorhexidine gluconate, benzalkonium chloride and chlorbutol respectively. The desorption isotherms were determined by removing 5 ml of the supernatant after equilibrium had been established and adding 5 ml of distilled water to the sinter sample tube which was then blown back into the flask. After allowing 24 h for re-equilibrium, a sample of supernatant was drawn off and assayed. This procedure was only carried out at selected preservative concentrations and the results are shown in Figs 1–3.

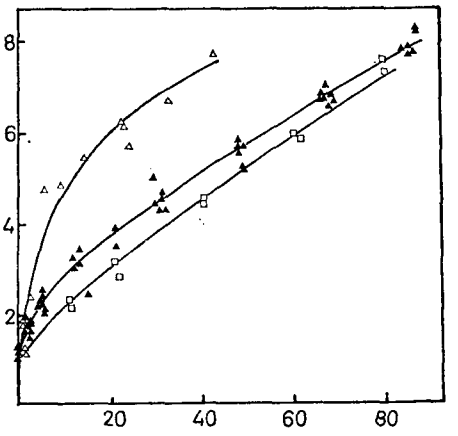

FIG. 1. The uptake of chlorhexidine gluconate (g kg^{-1}) (ordinate) by polyHEMA in powder form from aqueous solution at 30°. ▲ sorption < 120 μm powder; △ Desorption < 120 μm powder; □ sorption 420–590 μm powder. Abscissa: Equilibrium concentration of chlorhexidine gluconate in the aqueous phase (% w/v × 10^3).

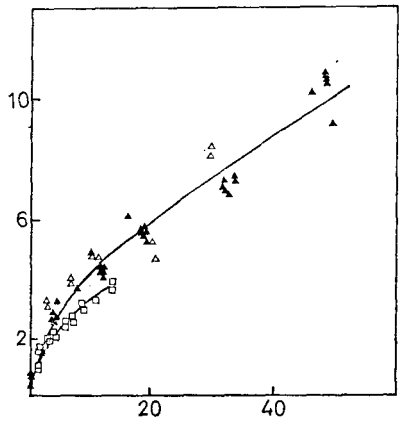

FIG. 2. The uptake of benzalkonium chloride (g kg^{-1}) (ordinate) by polyHEMA in powder form from aqueous solution at 30°. Symbols as for Fig. 1. Abscissa: Equilibrium concentration of benzalkonium chloride in the aqueous phase (% w/v × 10^3).

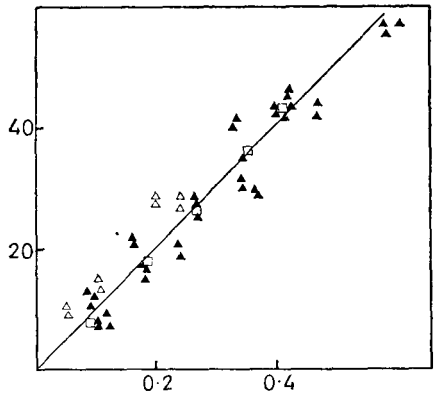

FIG. 3. The uptake of chlorbutol (g kg^{-1}) (ordinate) by polyHEMA in powder form from aqueous solution at 30° symbols as for Fig. 1. Ordinate: Equilibrium concentration of chlorbutol in the aqueous phase (% w/v).

The desorption isotherms for benzalkonium chloride and chlorbutol lie close to the sorption isotherms, but chlorhexidine gluconate is not desorbed from the polymer with the result that the desorption isotherm lies well above the sorption isotherm (Fig. 1).

The influence of surface area on sorption. Sorption isotherms for chlorbutol, chlorhexidine gluconate and benzalkonium chloride from aqueous solution were determined using polyHEMA powder of sieve size 420–590 μm. The isotherms for chlorhexidine gluconate, benzalkonium chloride and chlorbutol are shown in Figs 1–3 respectively.

The influence of formulatory adjuvants on sorption of chlorhexidine gluconate. The uptake of preservative from solutions nominally containing 0·01% w/v chlorhexidine gluconate was determined at 30° in the presence of 0·2–1·0% w/v potassium chloride, 1·4% w/v polyvinylalcohol (Gohensol GH17), 1·4% w/v hydroxyethylcellulose (Natrosol M) and 10% w/v polyethylene glycol 400. The findings are summarized in Table 1 and they show that uptake increases in the presence of both electrolyte and water soluble polymers. The necessary controls showed that the water-soluble polymers did not influence the assay procedure. This data is shown in Fig. 4.

The influence of pH on sorption of chlorhexidine gluconate. Uptakes were determined from buffered solutions of nominal chlorhexidine gluconate concentration 0·005% w/v over the pH range 0·81 to 10·83 at a constant ionic strength of 0·5 M and at 30°. Uptake values in Table 2 show that sorption increases with pH up to about pH 4·1 and then becomes constant before showing a final small increase above pH 8·0. The data shown is in Fig. 4.

Influence of pH on the sorption of thiomersal. The uptake from 0·004% w/v thiomersal by polyHEMA powder was determined from buffered solutions over

Table 1. *The influence of formulatory adjuvants on the sorption of chlorhexidine gluconate from aqueous solution by polyHEMA powder at* 30°.

	Effect of hydrophilic polymers				Effect of potassium chloride		
Polymer	Initial concn (% w/v × 10^3)	Equilibrium concn (% w/v × 10^3)	Uptake chlorhexidine gluconate (g kg^{-1})	Potassium chloride concn (% w/v)	Initial concn (% w/v × 10^3)	Equilibrium concn (% w/v × 10^3)	Uptake chlorhexidine gluconate (g kg^{-1})
Polyvinyl alcohol 1·4%	9·87	2·85	3·51	nil	9·92	5·65	2·13
	9·87	2·86	3·51		9·92	5·85	2·04
Hydroxyethyl cellulose 1·4%	10·40	3·47	3·47	0·2	10·25	2·49	3·88
	10·40	3·43	3·49		10·25	2·33	3·96
Polyethylene glycol 10%	9·83	2·90	3·47	0·5	10·00	1·91	4·04
	9·83	2·85	3·49		10·00	1·91	4·04
Water	9·92	5·65	2·13	1·0	10·20	1·87	4·16
	9·92	5·85	2·04		10·20	1·64	4·27

Table 2. *The influence of pH and solution volume on the uptake of chlorhexidine gluconate by polyHEMA powder.*

Effect of solution volume				Effect of pH (ionic strength 0·5 M, 30°)			
Solution volume in contact with polymer (ml)	Initial concn (% w/v × 10^3)	Equilibrium concn (% w/v × 10^3)	Uptake chlorhexidine gluconate (g kg^{-1})	pH	Initial concn (% w/v × 10^3)	Equilibrium concn (% w/v × 10^3)	Uptake chlorhexidine gluconate (g kg^{-1})
5		4·22	1·44	0·81	4·67	4·67	0·00
5		4·51	1·37	0·81	4·67	4·67	0·00
10		5·81	2·09	2·21	4·48	1·87	1·31
10		5·98	2·00	2·21	4·48	1·68	1·44
25	9·98	6·86	3·90	2·95	4·67	0·99	1·84
25		6·95	3·99	2·95	4·67	1·15	1·76
50		7·63	5·86	4·12	4·89	0·46	2·22
50		7·61	5·91	4·12	4·89	0·54	2·18
100		8·68	6·50	6·05	4·94	0·42	2·26
100		8·73	6·25	6·05	4·94	0·50	2·22
				8·02	4·98	0·54	2·22
				8·02	4·98	0·46	2·26
				8·91	5·00	0	2·50
				8·91	5·00	0	2·50
				10·00	5·00	0	2·50
				10·00	5·00	0	2·50
				10·83	5·00	0	2·50
				10·83	5·00	0	2·50

the pH range 0·4 to 6·0 at a constant ionic strength of 0·5 M and at 30°. Table 3 shows that uptake increases below pH 5 rising to a maximum below pH 2·0.

The influence of solution volume on sorption of chlorhexidine gluconate. Uptake from solutions nominally containing 0·01% w/v chlorhexidine gluconate were determined using volumes of preservative solution in contact with the polymer of 5 to 100 ml. The results in Table 2 show that the polymer preservative interaction increases with rise in the solution volume present.

The interaction of chlorhexidine gluconate with polyHEMA lenses

Individual lens weights varied and therefore the lenses were broken into small pieces so that the standard 0·2 g samples could be obtained. Uptake of chlorhexidine gluconate by the lens material was however extremely variable although equilibration appeared to be complete within 24 h. The sorption isotherm of chlorhexidine gluconate by polyHEMA lens material was determined from aqueous solution after 24 h equilibration and is similar to that obtained using the powdered polyHEMA (Fig. 5). In addition, four polyHEMA lenses (approximate weight 0·2 g) were placed in 10 ml of 0·01% w/v chlorhexidine gluconate solution and equilibrated for 24 h at 30°. 5 ml of the supernatant was removed and assayed for preservative content and a further 5 ml of fresh

Table 3. *The influence of pH on the uptake of thiomersal by polyHEMA powder at constant ionic strength of* 0·5 M *and at* 30°. (*Initial preservative concentration* 0·004% *w/v.*)

pH	Uptake thiomersal g kg^{-1}
0·4	1·58
1·1	1·60
2·2	1·70
2·6	1·54
3·0	1·54
3·4	1·47
3·6	1·39
4·2	1·03
4·6	0·30
5·2	0·08
6·0	0·00

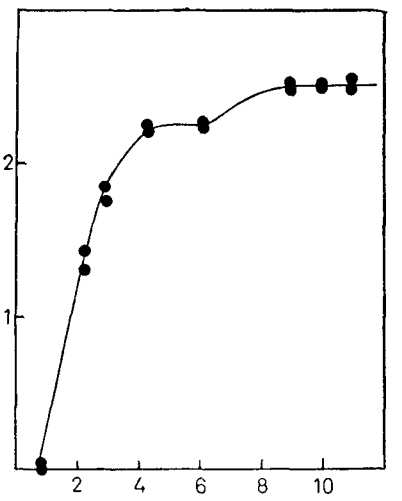

FIG. 4. The influence of pH (abscissa) on the uptake from 0·005% w/v chlorhexidine gluconate solution (g kg^{-1}) (ordinate) at a constant ionic strength of 0·5 M by polyHEMA powder at 30°.

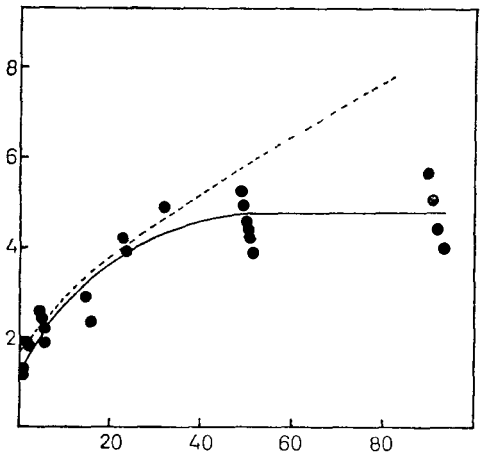

FIG. 5. The uptake of chlorhexidine gluconate (g kg^{-1}) (ordinate) by polyHEMA lenses from aqueous solutions at 30°. · · · · powder, ● lenses. Abscissa as for Fig. 1.

0·01% w/v chlorhexidine gluconate solution added to the lenses. The system was re-equilibrated for 24 h and the procedure outlined above repeated. The cycling was continued until the lenses became saturated with preservative. The results for two separate determinations are given in Fig. 6 and show that the lenses became saturated only after about 20 cycles of adding fresh preservative. The four lenses having a saturation concentration of 11·4 mg chlorhexidine gluconate per gramme of polyHEMA were removed from the preservative solution and surplus liquid removed using a paper tissue, and then one lens was placed into 10 ml of each of the following: water, 0·9% w/v sodium chloride, 10% w/v polyethylene glycol 400 and 0·1% w/v Tween 80.

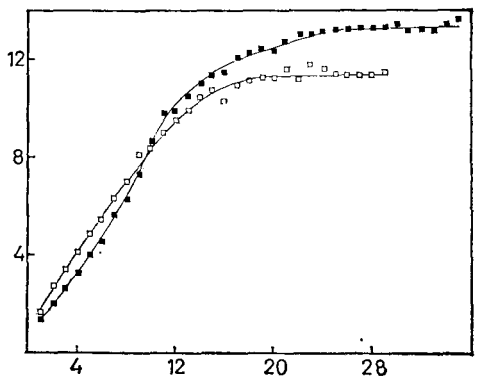

FIG. 6. The cyclic uptake of chlorhexidine gluconate (g kg^{-1}) (ordinate) by polyHEMA lenses from aqueous solutions at 30°. ■ Determination, I □ Determination II. Abscissa: Cycle number.

They were then shaken for 24 h at 30°, the supernatant removed and assayed (spectrophotometrically) for chlorhexidine gluconate. 10 ml of fresh solvent was then added and the procedure repeated. After six such cycles the spectra from water and 10% w/v polyethylene glycol 400 did not show the characteristic absorption spectrum of chlorhexidine and were similar to those obtained for the water extractive of polyHEMA. The spectra from solutions of normal saline and 0·1% Tween 80 did show the characteristic spectrum of chlorhexidine. The amount of preservative released during each cycle was determined and the findings are in Table 4.

Table 4. *Chlorhexidine gluconate release data from saturated polyHEMA lenses at* 30°.

0·9% w/v sodium chloride		0·1% w/v Tween 80	
Cycle No.	mg chlorhexidine gluconate released	Cycle No.	mg chlorhexidine gluconate released
1	0·060	1	0·176
2	0·005	2	0·108
3	0·029	3	0·144
4	0·046	4	0·078
5	0·081	5	0·076
6	0·024	6	0·000

DISCUSSION

Of the four preservatives studied, only thiomersal did not interact with polyHEMA powder from simple aqueous solution. Chlorhexidine gluconate and benzalkonium chloride show typical high affinity (H1 type) isotherms (Figs 1 and 2) in the classification of Giles, MacEwan & others (1960). This type of behaviour is usually associated with surface adsorption processes where the solute has such a high affinity for the adsorbent that it is completely removed from solution at low concentrations resulting in the initial portion of the isotherm being vertical. A plateau, indicating saturation of the polymer is not evident although this may occur at higher equilibrium concentrations. On a molar basis, about five times as much benzalkonium chloride is absorbed by the polymer as chlorhexidine gluconate. The extent of the interaction of the preservatives with polyHEMA decreased only by a factor of about 0·2 when the sieve size increased from <120 to 420–590 μm. Assuming the particles are spherical this represents about a 20 fold change in surface area and suggests that the preservative is penetrating the polymer and adsorbing onto an internal surface which is much

greater in area than that of the external surface. In contrast to benzalkonium chloride, the interaction with chlorhexidine gluconate is non reversible.

The sorption isotherm for chlorbutol is linear having a slope of 89·6 (s.d. Slope 4·8) and an intercept of 3·6 (s.d. intercept 1·4). Such a linear isotherm is characteristic of the C1 or partition type isotherm described by Giles & others (1960) and indicates that the interaction is not a surface process but that chlorbutol is penetrating the polymer matrix, as the number of available sorption sites remains constant and independent of the amount previously sorbed. Fig. 3 also shows that, as would be expected with partition behaviour, the surface area of the polymer does not influence the extent of the interaction and that the interaction is reversible.

The addition of electrolyte, in the form of potassium chloride, results in a marked increase in the sorption of chlorhexidine gluconate (Table 1). Chlorhexidine in water is predominantly in the mono-cationic form and it may be that the potassium ions are competing for anionic adsorption sites in the polymer. However, the electrolyte could be exerting its action through effects on the solubility of the preservative (Richardson & Meakin, 1974). The presence of hydrophilic polymers such as polyvinylalcohol, hydroxyethylcellulose and polyethylene glycol 400 would be expected to enhance the affinity of the preservative for the aqueous phase and reduce the polymer-preservative interaction. The findings in Table 1 shows this is not so as the extent of the interaction is nearly doubled. Similarly, a large rise in uptake of chlorhexidine is observed when the volume of preservative solution in contact with the polymer is increased (Table 2). At present, no satisfactory explanation can be offered for these results.

The influence of pH at ionic strength 0·5 M and 30° on the binding of chlorhexidine gluconate to polyHEMA is shown in Fig. 4. Below pH 1 there is no uptake, but this rises with pH up to about pH 4·0. Above pH 8 all of the chlorhexidine is removed from the 0·005% w/v solution. Hugo & Longworth (1964) quote values for the pKa of chlorhexidine of 10·3 and 2·2 for the formation of the mono- and di-cation respectively. It is apparent therefore that chlorhexidine has a high interaction with polyHEMA when in the mono-cationic form. However, this high level of interaction is still observed when some of the chlorhexidine exists in the unionized form and it may be therefore that polyHEMA may interact with chlorhexidine in both its mono-cationic and unionized forms.

The uptake of chlorhexidine gluconate by polyHEMA lens material (Fig. 5) shows a similar sorption isotherm to that determined using the powdered material except that the isotherm forms a plateau at higher equilibrium concentrations. When fresh chlorhexidine gluconate is repeatedly added to polyHEMA lenses, Fig. 6 clearly shows that the lenses become saturated with chlorhexidine after about 20 cycles and take up between 7 and 10 times the amount of preservative they bound after the apparent initial 'equilibration'. Thus the sorption experiments for chlorhexidine described above represent results determined at a 'Pseudo-equilibrium state'. This phenomenon was found by Kaspar (1976) and is not readily explained. Desorption of chlorhexidine from saturated lenses into water and 10% polyethylene glycol 400 was not detected by the assay technique used. However, chlorhexidine was released into normal saline and 0·1% Tween 80 (Table 4). The release into normal saline after 6 desorption cycles totalled 0·245 mg which represents about 40% of the preservative present in the lens at saturation. All of the chlorhexidine gluconate is released into 0·1% Tween 80 in five desorption cycles. It is possible therefore that in practice tears may leach out chlorhexidine from soft contact lenses that have been stored in preservative solutions with subsequent eye irritancy.

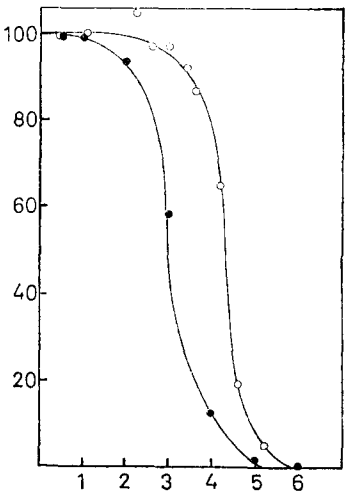

FIG. 7. The influence of pH (abscissa) on the uptake from 0·004% w/v thiomersal solution at a constant ionic strength of 0·5 M by polyHEMA powder at 30°. ● Percentage unionized thiomersal, ○ Percentage maximum uptake thiomersal. Ordinate: Percentage maximum thiomersal uptake or mole percent free thiomersal.

The influence of pH at ionic strength 0·5 M and 30° on the uptake of thiomersal by polyHEMA powder is shown in Fig. 7. Above pH 5·0 binding is low, but rises to a maximum at about pH 3·0. The mole per cent free acid, determined from the Henderson-Hasselbach equation is also plotted against pH in Fig. 7 and this shows that both ionized and unionized thiomersal appear to be bound by the polyHEMA. However, no uptake occurs until some thiomersal is present in the unionized form i.e. below pH 5·0. The displacement of the thiomersal uptake curve from the ionization curve may be a result of using single-point determinations rather than determining the individual sorption isotherm at each pH and reading off the uptake at a fixed equilibrium concentration of preservative. But some soft contact lens solutions containing thiomersal are formulated at pH 4–5 and therefore some preservative may be bound by soft lenses.

In summary, all four preservatives commonly used in contact lens solutions interact with polyHEMA to an extent that is dependent upon such factors as pH, the presence of formulatory adjuvants and the volume of solution in contact with the polymer. Chlorhexidine, which is probably the most frequently used preservative in soft lens solutions, may be concentrated in polyHEMA lenses on repeated soaking in such solutions and could be subsequently released when inserted into the eye. Thiomersal, the other preservative most frequently used in soft lens solutions, does not interact above pH 5 with polyHEMA and with solutions formulated above this value will not constitute a problem. However below pH 5·0 an interaction does occur under laboratory conditions and those commercial solutions formulated below pH 5 may bind thiomersal to soft lenses during soaking and cleaning and this might subsequently be released into the eye. There would seem a need with soft contact lenses and their associated solutions to make a proper evaluation of possible irritant and toxic reactions since at present there is a paucity of reliable published information on this topic.

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