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The mechanism of interaction between chlorhexidine digluconate and poly(2-hydroxyethyl methacrylate)

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The extent of the interaction between chlorhexidine digluconate and poly(2-hydroxyethyl methacrylate), (PHEMA), is independent of temperature between 22–50 °C which is consistent with an ion-ion interaction mechanism. Different contact lens materials exhibit different affinities for chlorhexidine digluconate, the extent of uptake correlating in rank order with the number of free carboxylic acid sites in the polymers. Esterification of the carboxyl groups with diazomethane, resulted in a reduction in the affinity of the treated polymers for chlorhexidine to a near basal level. The uptake of chlorhexidine in soaking solution experiments involving lenses made from PHEMA and the more ionic material, poly(2-hydroxyethyl methacrylate - co-isobutyl methacrylate - co - methacrylic acid), was consistent with their carboxylate content. However, the fraction of bound disinfectant released was lower from the terpolymer, suggesting there are differences in bonding strengths between chlorhexidine and different contact lens hydrogels.

Chlorhexidine is frequently incorporated as a preservative into soaking solutions designed to disinfect hydrophilic contact lenses. Although its uptake by the lens material poly(2-hydroxyethyl methacrylate), (PHEMA), has been investigated (Richardson et al 1978, 1980; Plaut et al 1980a, b), the mechanism of the interaction has not been reported.

The unusual affinity of PHEMA for positively charged ions other than chlorhexidine (Plaut et al 1980b) suggested that an ion-ion interaction might be occurring, implying the presence of negatively charged binding sites within the polymer, a phenomenon not readily explained in terms of the simple PHEMA structure. However, methacrylic acid is a known impurity of the monomer, 2-hydroxyethyl methacrylate (HEMA) (Fort & Polyzoidis 1976) and would provide the necessary sites as carboxylate residues if it were incorporated into the hydrogel during polymerization. We have investigated this hypothesis.

The influence of temperature was determined to obtain a measure of the sorption enthalpy, as ion-ion interactions are associated with small or zero enthalpy changes (Inczedy 1966). The effect of reducing the carboxylate content of PHEMA and some other hydrophilic lens materials was also examined. Samples of these polymers were treated with diazomethane which selectively methylates acidic protons (Fieser & Fieser 1967) and thus reduces the number of carboxylate sites. Sorption isotherms for chlorhexidine were then compared for both untreated and methylated lens materials.

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MATERIALS AND METHODS

Materials

The following materials were gifts from the companies indicated. The hydrophilic polymers were supplied dehydrated unless otherwise stated; the quoted water content is that for the hydrated polymer. HEMA monomer and PHEMA (38% water content) as buttons, lenses and thin film, the latter hydrated in distilled water (Smith and Nephew Optics, U K); poly (2-hydroxyethyl methacrylate-co-isobutyl methacrylate - co - methacrylic acid), (P-(HEMA-IBMA-MA), 43% water content), as buttons and lenses (Burton, Parsons and Co Inc., U.S.A.); poly(*N*-vinylpyrrolidone-co-methyl methacrylate), (P(VP-MMA), 70% water content) as flakes, (Contact Lens Manufacturing Co., U.K.); poly(methyl methacrylate), (PMMA) as Perspex CQ (I.C.I.), (Kelvin Lenses, U.K.).

N-Methyl-*N*-nitrosotoluene-4-sulphonamide, acetic acid and diethyl ether (all reagent grade), acetic anhydride, benzoic acid, butan-1-ol, potassium hydroxide and pyridine, (all AR grade) were obtained from BDH, 95% and absolute ethanol (B.P. grade) were obtained from James Burroughs. Chlorhexidine gluconate, both 'cold' and radiolabelled ('hot') were as described previously (Plaut et al 1980a).

Preparation and treatment of materials

(a) *Powdered polymers.* Buttons or flakes were ground in a domestic mill (Moulinex type 104–2–02),

cleaned by Soxhlet extraction, dried, reground and the 65–120 μm size fraction collected as described for PHEMA (Plaut et al 1980a). For PMMA the Soxhlet extraction step was omitted.

(b) *Lenses.* These were cleaned by Soxhlet extraction with water for 2 h and dried over phosphorus pentoxide in a vacuum oven at 40 °C.

(c) *Methylated polymers.* Freshly distilled ethereal solutions of diazomethane were prepared according to De Boer & Backer (1954). 25 g of PHEMA or P(HEMA-IBMA-MA) powder was suspended in 100 ml absolute ethanol, ethereal diazomethane (10 ml) was added in aliquots to just maintain a pale yellow colour, the suspension being kept at room temperature (10 °C) in a fume cupboard. When the colour finally faded (30 to 40 min), the polymer was separated by filtration, the filtrate being collected in aqueous acetic acid (10% v/v) to destroy residual diazomethane. The polymer was resuspended in absolute ethanol (100 ml) and subjected to two further diazomethane treatments. In this way, the volume of ether was never allowed to exceed 10% of the total solvent volume as the swelling of PHEMA would be expected to decrease with an increasing proportion of ether. The methylated polymer was washed with absolute ethanol and dried over phosphorus pentoxide at 30 to 40 °C in a vacuum oven. The polymer was reground, sieved mechanically and the 65–120 μm fraction collected.

Analytical methods

(a) *Chlorhexidine assay.* Aliquots (1 ml) of radioactively-labelled chlorhexidine solution were assayed as described previously (Plaut et al 1980a), by addition of 10 ml of scintillation fluid. This consisted of 2,5-diphenyloxazole (3 g litre^{-1}) and 1,4-bis[2-(4-methyl-5-phenyl-oxazolyl)] benzene (0·2 g litre^{-1}) in xylene and Triton X110 (3:1 v/v). Samples were counted for 10 min in a Phillips Liquid Scintillation Analyser Model OM1. Standard curves relating counts min^{-1} to concentration were constructed for each individual sorption experiment.

(b) *Carboxyl end group titration.* An ethanolic solution of potassium hydroxide (approximately 0·008 M) was prepared daily and standardized against benzoic acid as follows. A stream of nitrogen was bubbled through 20 ml of 0·008 M benzoic acid in absolute ethanol for 5 min to displace carbon dioxide. The solution was then titrated with ethanolic potassium hydroxide using phenolphthalein (0·05 ml of a 1% solution in propan-2-ol). 20 ml aliquots of absolute ethanol were similarly titrated to allow solvent blank corrections to be made.

Approximately 1·0 g samples of powdered polymer were suspended in 20 ml absolute ethanol in glass stoppered flasks and titrated under nitrogen with ethanolic potassium hydroxide. Once a pale pink colour was obtained, flasks were stoppered and left to allow diffusion of the hydroxide ions to the acid sites. The solvent-polymer mixture was flushed with nitrogen and the titration continued to a stable end point. This generally took several hours and the flasks were left stoppered overnight to ensure neutralization had been achieved as evidenced by a persistent pink colour. The carboxyl contents of both methylated and non-methylated polymers are shown in Table 1.

Table 1. Carboxyl group content of hydrogel materials

Material	Carboxyl content (mmol kg^{-1} with s.d.) Untreated	Methylated
P(HEMA-IBMA-MA)	132·0 (1·00)	1·8 (0·03)
PHEMA (batch 1)	34·6 (0·25)	5·1 (0·20)
PHEMA (batch 2)	30·3 (0·15)	—
PHEMA (batch 3)	—	3·4 (0·10)
P(VP-MMA)	10·4 (0·50)	—
HEMA monomer	2·2	—

(c) *Hydroxyl end group titration.* Water (3 ml) was added to pyridine (1 litre); this was used to prepare pyridine–acetic anhydride mixtures (100:30 v/v). Pyridine–acetic anhydride solution (10 ml) was added to PHEMA powder (1·0 g) and the samples acetylated by heating under air reflux for 20 min. After the mixture had cooled for 2 min, and excess acetic anhydride hydrolysed by the addition of distilled water (10 ml) and reheating to boiling, it was cooled again and the condenser rinsed with 25 ml pyridine, 25 ml butan-1-ol added, and the acetic acid content determined by titration with 1·0 M ethanolic potassium hydroxide against phenolphthalein. Blank determinations were carried out in the absence of PHEMA and the hydroxyl content determined from the difference between the blank and sample titrations.

Sorption-desorption studies with powdered polymers

(a) *General.* Full details have been given by Plaut et al (1980a). Glassware was cleaned and then stored in the appropriate concentration of chlorhexidine digluconate before use. Aliquots (10 ml) of chlorhexidine digluconate solution were added to 0·2 g polymer powder and the flasks shaken at 70 cycles min^{-1} in a water bath maintained at the

required temperature (±0·1 °C). Samples were removed for analysis after 24 h using sampling tubes fitted with no 3 sintered glass disks. Where desorption was studied, 5 ml samples were removed and replaced by 5 ml of water. The systems were then reequilibrated for a further 24 h before re-assay.

(b) *Effect of temperature on the sorption of chlorhexidine digluconate by PHEMA.* A simple aqueous stock solution of chlorhexidine digluconate (1·114 mM, 0·1% w/v) was prepared and ^{14}C-radioactively labelled chlorhexidine added to give a specific activity of 6 μCi mmol^{-1}. Dilutions were prepared to cover concentrations from 0·045 to 1·114 mM. The experiment was conducted at 22·0°, 29·6°, 39·2° and 49·6 °C and the sorption isotherms are shown in Fig. 2.

(c) *Sorption of chlorhexidine digluconate by P-(HEMA-IBMA-MA), P(VP-MMA), PMMA and methylated polymers at 30* °C. Labelled stock solutions containing 2·229 mM (0·2% w/v) chlorhexidine digluconate were prepared as required; specific activities ranged from 2·5–4·0 μCi mmol^{-1}. Appropriate dilutions were made to cover the required concentration ranges. Uptake by PMMA was studied over the range 0·045–0·530 mM, P(HEMA-IBMA-MA) and P(VP-MMA) over the range 0·022–2·229 mM and the methylated materials over the range 0·011–1·300 mM. The sorption isotherms are shown in Figs 3 and 4.

Permeability of PHEMA to chlorhexidine digluconate

The permeability of PHEMA to chlorhexidine digluconate was studied using a double-chamber glass cell each compartment having a nominal capacity of 50 ml. The whole cell was immersed in a water bath at 30 °C and the donor and receptor solutions stirred by means of PTFE coated fleas and underwater magnetic stirrers. The initial concentration of chlorhexidine digluconate in the donor cell was 2·229 mM (specific activity 9·7 μCi mmol^{-1}), the receptor cell containing distilled water. The concentration of preservative in both compartments was monitored throughout the experiment, 1 ml samples being removed and assayed for radio-labelled chlorhexidine.

The thickness of the film was measured at the edge by a travelling microscope. The mean of eight readings was 0·32 mm (s.d. = 0·05), the large coefficient of variation (16%) reflecting the uneven thickness of the polymer film. The data are presented in Fig. 1 as a Barrer Plot.

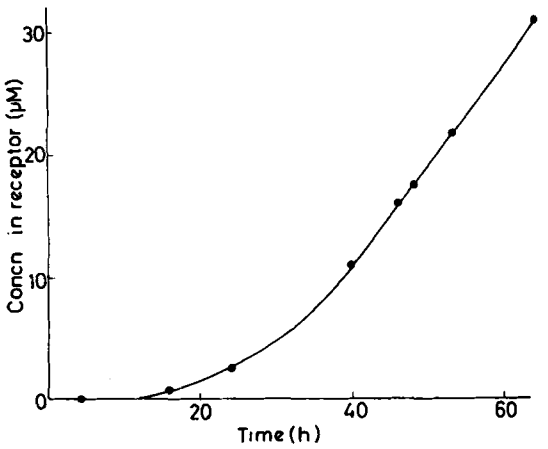

FIG. 1. Barrer plot for the permeability of PHEMA film to chlorhexidine digluconate in simple aqueous solution at 30 °C. Ordinate: Chlorhexidine digluconate concentration in receptor compartment (M × 10^6). Abscissa: Time (h).

Cycling studies: the uptake and release of chlorhexidine digluconate by hydrogel lenses

The dry weights of five PHEMA (30·7 to 58·0 mg) and four P(HEMA-IBMA-MA) (23·9 to 30·0 mg) lenses were determined before hydration in 0·9% NaCl (saline). The soaking solution consisted of 0·0068% w/v chlorhexidine digluconate (0·078 mM) with a specific activity of 546 μCi mmol^{-1} in Kolthoff's borax-phosphate buffer (Documenta Geigy 1962) containing 0·9% sodium chloride. The lenses, blotted dry, were immersed in 2 ml of soaking solution contained in 10 ml glass stoppered tubes and incubated at 30 °C. After 16 h (or 64 h every fifth day), 1 ml samples were withdrawn and assayed for radio-labelled chlorhexidine. Five 1 ml aliquots of the soaking solution were always assayed alongside the experimental samples to monitor any fluctuations in the efficiency of the scintillation counter. The mean value was used to calculate the concentration of preservative remaining in solution thus enabling the amount of chlorhexidine sorbed per lens to be determined. The lenses were then removed from the soaking solution, blotted dry and transferred to 4 ml of 0·9% sodium chloride solution contained in glass stoppered tubes at 30 °C. The amount of free chlorhexidine 'carried over' had previously been shown to be negligible (Richardson et al 1980). After 8 h, 1 ml samples were removed, assayed, and the amount of preservative eluted was calculated. The first eight uptake-elution cycles are illustrated in the inset of Fig. 5. The data in the main graph represent the mean

uptakes per lens at the end of each completed cycle for a total of 28 cycles. Average values for the amount of chlorhexidine digluconate eluted per lens are shown in Fig. 6.

DISCUSSION

Fig. 1 shows that PHEMA is permeable to chlorhexidine digluconate; its appearance in the receptor compartment of the permeability cell as a function of time follows classical Barrer behaviour (Barrer 1951; Crank & Park 1968). Presumably the charged substrate molecules diffuse through the water filled pores of the hydrogel. Extrapolation of the linear phase to the intercept on the abscissa (τ) allows the diffusion coefficient (D) for the process to be calculated from equation 1, where l is the thickness of the polymer film.

$$D = \frac{l^2}{6\tau} \quad .. \quad .. \quad (1)$$

Use of the mean film thickness of 0·32 mm generates a diffusion coefficient of $1{\cdot}8 \times 10^{-13}\ m^2\ s^{-1}$ at 30 °C. This value is of the same order of magnitude ($\sim 10^{-13}\ m^2\ s^{-1}$) as that which can be predicted from the diffusion data of Ratner & Miller (1973) for solutes of differing molecular weight in PHEMA.

Fig. 2 shows the effect of temperature on the extent of the chlorhexidine digluconate-PHEMA interaction. The four isotherms are essentially superposable, the scatter at the top end being within the assay error. This implies that the standard enthalpy associated with the sorption process is zero over the temperature range 20–50 °C (see eqn 2) and is strongly indicative of an interaction mediated by electrostatic bonding. Inczedy (1966) has reported that the standard enthalpies of ion exchange interactions are generally zero or very small, lying within the range $-1{\cdot}0$ to 10 kJ mol^{-1}. The presence of strong ionic binding forces is also consistent with the predominately irreversible nature of the chlorhexidine-PHEMA interaction in simple aqueous solution (Plaut et al 1980a). The driving power behind such ionic interactions is derived from the entropy changes associated with the disruption of the hydration shells of the ions. For this system, the mean standard entropy of interaction over the temperature range 20–50 °C was calculated to be 21·0 J $mol^{-1}\ K^{-1}$. This value was obtained by inserting the sorption coefficient (K), taken at an arbitrarily chosen equilibrium concentration of 0·557 mM (0·05% w/v) chlorhexidine digluconate, into the Van't Hoff equation (eqn 2).

$$\log_e K = \frac{-\Delta H^\circ_{sorp}}{RT} + \frac{\Delta S^\circ_{sorp}}{R} \quad .. \quad (2)$$

The sorption coefficient (K) is the ratio of the uptake to equilibrium concentration, ΔH°_{sorp} and ΔS°_{sorp} are the standard enthalpy and entropy of the interaction respectively, T is the temperature and R the gas constant.

Sorption processes that have been attributed to ionic bonding are generally characterized by so called 'high affinity' isotherms (Giles et al 1974). In these cases, an initial, almost vertical curve leads immediately into a well-defined plateau that corresponds to complete coverage of the adsorbent surface by the solute. However the sorption of chlorhexidine digluconate by PHEMA cannot be merely a surface phenomenon as evidenced by the permeability of PHEMA to chlorhexidine (Fig. 1). The isotherm in Fig. 2 is probably a composite, the initial steep portion representing a 'high affinity' uptake at the surface, superimposed on which is an interaction within the polymer matrix.

The four different contact lens polymers investigated, had different affinities for chlorhexidine (Fig. 3). With PMMA we had found that there was a small uptake of the disinfectant at the polymer surface, 1·5 mmol kg^{-1} at maximum (Richardson et al 1980). The present data, however, were derived from an experiment where all the glassware had been pre-equilibrated in solutions of appropriate

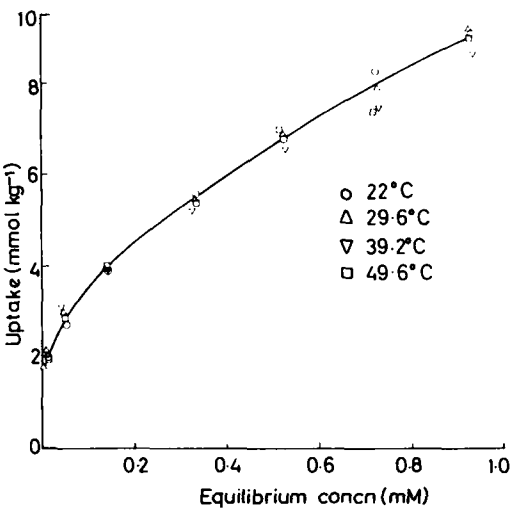

FIG. 2. Effect of temperature on the sorption of chlorhexidine digluconate from simple aqueous solution by PHEMA powder (each point is the mean of at least two replicates). Ordinate: Uptake of chlorhexidine digluconate (mmol kg^{-1}). Abscissa: Equilibrium concentration of chlorhexidine digluconate (mM).

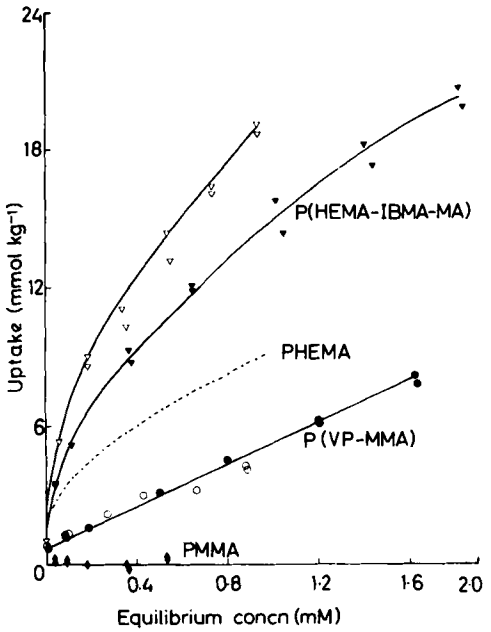

FIG. 3. Sorption of chlorhexidine digluconate from simple aqueous solution at 30 °C by powdered contact lens materials. (Key: ◆ PMMA; ●○ P(VP-MMA); ▼▽ P(HEMA-IBMA-MA); closed symbols are sorption data and open symbols are desorption data; the broken line is the PHEMA isotherm from Fig. 2). Ordinate: Uptake of chlorhexidine digluconate (mmol kg^{-1}). Abscissa: Equilibrium concentration of chlorhexidine digluconate (mM).

chlorhexidine concentration and the absence of detectable uptake suggests that the earlier experiment had monitored the sorption of chlorhexidine onto glass surfaces.

The sorption characteristics of the three hydrogels show some interesting differences. The isotherm for P(VP-MMA), a material that does not contain HEMA, is linear and reversible after a small 'high affinity' step at very low chlorhexidine concentration. It has a lower affinity on a dry weight basis, than either of the two HEMA containing polymers. Both PHEMA and P(HEMA-IBMA-MA) generate curvilinear, non-reversible isotherms (Fig. 3 and Plaut et al 1980b) although the latter substance has the higher chlorhexidine digluconate affinity.

Examination of Table 1 shows that there is a rank order correlation between the carboxyl content of the hydrogels and their affinity for chlorhexidine digluconate, and also that there is a carboxyl content variation between different samples of PHEMA.

To substantiate the role of the carboxyl group, samples of PHEMA and P(HEMA-IBMA-MA) were treated with ethereal solutions of diazomethane. Under our conditions, this reagent is unreactive towards alcoholic hydroxyl groups and should not therefore modify the HEMA hydrogel residues (Fieser & Fieser 1967). This was confirmed by determination of the polymer hydroxyl group content, the methylated samples of PHEMA being found to have 99% of their native hydroxyl content. The esterification of the methacrylic acid residues results in the formation of methyl methacrylate groupings which do not interact with chlorhexidine as evidenced by the lack of affinity of PMMA for the preservative (Fig. 3). The methylation reaction has a dramatic effect on the extent of the preservative uptake for both PHEMA and P(HEMA-IMBA-MA) (Fig. 4). The magnitude of the residual

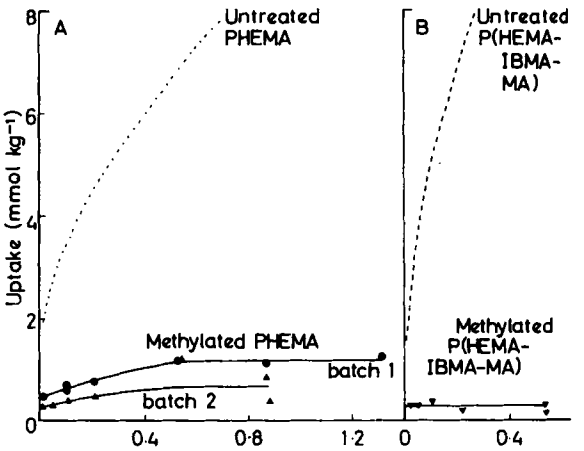

FIG. 4. Effect of polymer methylation on the sorption isotherms of chlorhexidine digluconate from simple aqueous solution at 30 °C. A; sorption by PHEMA. B; sorption by P(HEMA-IBMA-MMA). The sorption isotherms for the untreated materials, shown by the broken lines are taken from Figs 2 and 3. Ordinate: Uptake of chlorhexidine digluconate (mmol kg^{-1}). Abscissa: Equilibrium concentration of chlorhexidine digluconate (mM).

interaction again appears to be related to the number of unreacted acid residues, the concentrations of which are listed in Table 1. These experiments confirm the role of carboxyl groups in providing anionic binding sites for the cationic chlorhexidine molecules during their sorption from simple aqueous solution.

The PHEMA preparations analysed were prepared from HEMA monomer without further purification. Table 1 shows there is a fifteen fold difference between the carboxyl content of PHEMA and HEMA monomer. This large discrepancy indicates that most acidic residues are generated in situ

during polymerization. Thus the development of a 'methacrylate free' polymer is likely to be more involved than the simple purification of the starting material.

Overall, the data show that free carboxyl groups play an important role in the accumulation of chlorhexidine digluconate from simple aqueous solution by HEMA-containing lens polymers, and the implication is that the higher the carboxyl content, the greater is the extent of interaction. To test this more practically, the relative performances of lenses of PHEMA and P(HEMA-IBMA-MA) were assessed in a soaking-elution cycle test. The PHEMA lenses, with an average dry weight of 45·8 mg (s.d. 10·1 mg) were much heavier than the P(HEMA-IBMA-MA) lenses, dry weight 28·1 mg (s.d. 2·8 mg). Soaking was in 2 ml of a buffered saline - chlorhexidine digluconate system and elution was into 4 ml of saline as chlorhexidine is not eluted from PHEMA into water (Richardson et al 1980). The cumulative uptakes are illustrated in Fig. 5, the

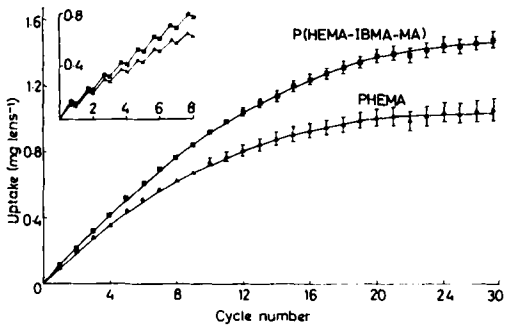

FIG. 5. Effect of soaking and elution on the uptake of chlorhexidine digluconate by PHEMA and P(HEMA-IBMA-MA) lenses at 30 °C. (Lenses soaked in 2 ml 0·0068% chlorhexidine digluconate formulated soaking solution and eluted into 4 ml normal saline; standard error bars are for four (■) and five (▲) individual lens experiments. Inset graph shows the uptake and release data for the first eight cycles. Ordinate: Uptake of chlorhexidine digluconate (mg per lens). Abscissa: Cycle number.

smaller P(HEMA-IBMA-MA) lenses achieving an uptake of about 1·5 mg per lens after 28 cycles which is 40% greater than the average 1·05 mg per lens obtained with the PHEMA lenses. On a unit dry weight basis this difference is accentuated and the affinity of the terpolymer lenses for chlorhexidine is 2·3 times greater than that for PHEMA lenses. This compares with a four times higher carboxylate content in the terpolymer as determined on powdered lens button blanks (Table 1).

Fig. 6 shows that small amounts of chlorhexidine are released into saline. This probably stems from

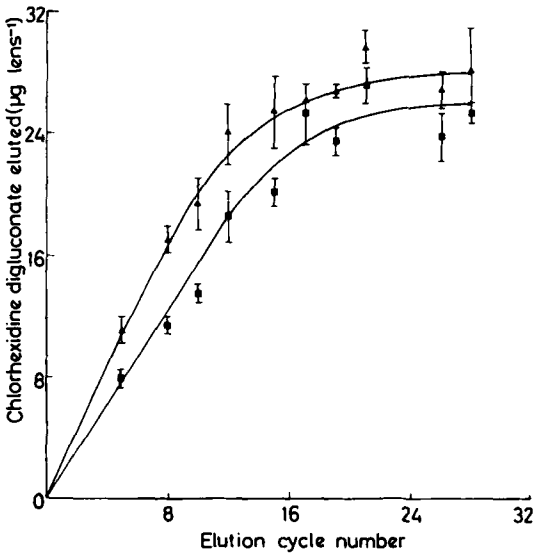

FIG. 6. Release of chlorhexidine digluconate from soaked PHEMA and P(HEMA-IBMA-MA) lenses into 4 ml saline at 30 °C. (Standard error bars are for four (■) and five (▲) individual lens experiments.) Ordinate: Chlorhexidine digluconate eluted (μg per lens). Abscissa: Elution cycle number.

competition at the anionic polymeric binding sites by sodium ions, which will not happen when the eluting fluid is water alone. Generally, the release from the PHEMA lenses which have the lower uptake, is greater than that from the P(HEMA-IBMA-MA) lenses although towards the end of the experiment when the lenses appear to be approaching saturation, the amount of chlorhexidine eluted per lens (~ 27 μg) is virtually the same for both lens types (Fig. 6). However, if these quantities are expressed in terms of unit dry lens weight, they are different by a factor of 1·5 being 1·02 mmol kg^{-1} for P(HEMA-IBMA-MA) and 0·68 mmol kg^{-1} for PHEMA. There is less difference between the relative release (1·5 times) than the relative uptake (2·3 times) on a dry lens weight basis. This is further evidenced from calculations based on the data given in Figs 5 and 6 which shows that the fraction of bound chlorhexidine released is essentially constant and independent of the total amount bound for both materials. The mean fraction released from the P(HEMA-IBMA-MA) lenses (1·71%, s.d. 0·18) is less than that from the PHEMA lenses (2·75%, s.d. 0·14). One possible explanation for this is that the chlorhexidine bonding to the more ionic terpolymer is stronger than to PHEMA.

CONCLUSION

In all, these results support our initial hypothesis that the interaction between hydrophilic contact lens polymers and chlorhexidine digluconate in simple aqueous solution, is associated with the presence of free carboxyl groups in the polymer matrix. Such a conclusion is also consistent with our previous studies into the effect of pH on the interaction of chlorhexidine with PHEMA (Richardson et al 1978), which showed that the level of interaction fell rapidly below pH 4·0 to zero at pH 0·8. The 'average' pK_a value for a polymeric carboxyl group would be expected to lie between pH 3 and 4, and at pH 1·0, any carboxylic acid residues would be almost entirely in their non-ionised form.

However, in vivo, it is the amount of chlorhexidine released during contact lens wear that is of importance. Although the data show that P(HEMA-IBMA-MA) has a higher carboxyl content and therefore a greater affinity for chlorhexidine digluconate than PHEMA, the former releases a lower fraction of the bound material under the conditions studied here, with the consequence that the amount of chlorhexidine released per lens is similar for both lens types.

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