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The influence of various counter ions on the interaction of chlorhexidine with the hydrophilic contact lens polymer, poly(2-hydroxyethyl methacrylate)*

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The commercially available salts of chlorhexidine were found to interact with the hydrogel poly (2-hydroxyethyl methacrylate) to different extents, the affinity for the polymer decreasing in the order acetate, gluconate, chloride. The uptakes of the acetate and gluconate salts were almost entirely irreversible over the concentration range studied. The influence of some alternative counter ions, namely amino acids and dicarboxylic acid salts, was examined and found to generate a three-fold variation in the extent of the chlorhexidine interaction. Uptakes were greatest for counter ions that were either hydrophobic in character or bore a net negative charge. Only two compounds, glycine and monosodium oxalate, were successful in reducing the extent of sorption below that observed for chlorhexidine hydrochloride.

The manufacture of a soft contact lens first became a possibility with the introduction of the hydrophilic plastic, poly (2-hydroxyethyl methacrylate) (PHEMA), the synthesis of which was described by Wichterle & Lim in 1960. This polymer, based on a single monomer unit, 2-hydroxyethyl methacrylate (HEMA), is crosslinked with a small amount of ethylene glycol dimethacrylate and provides a lens material that contains a maximum of 39% water when fully hydrated. At the present time most daily wear contact lenses contain HEMA either as a major or minor component. There are, however, hydrogels that rely on other monomers such as vinylpyrrolidone to provide hydrophilic centres within the gel (Refojo 1976) but because of the relatively widespread occurrence of the HEMA monomer, PHEMA was chosen as the most appropriate polymer on which to base this study.

It is generally recommended that contact lenses intended for daily wear be disinfected overnight. Although it is possible to disinfect PHEMA lenses thermally, this method has disadvantages including a shortening of the lens life (Dallos & Hughes 1972) and an enhanced build up of proteinaceous deposits formed by heat denaturation on the lens surface (Cureton & Hall 1974). A cold, chemical soaking regime is simpler to use and avoids the problems mentioned above. Most solutions formulated for use in conjunction with soft, hydrophilic lenses contain chlorhexidine digluconate as the antibacterial agent in concentrations ranging from 0·002 to 0·006% w/v. In vitro studies, however, have shown that when PHEMA lenses are cycled in fresh batches of 0·01% w/v chlorhexidine digluconate solution, they repeatedly sorb preservative from the soaking medium resulting in high intralens concentrations (Richardson et al 1978, 1979). Some of the bound chlorhexidine is released from lenses both into 0·9% NaCl (saline) and a simulated tear solution (MacKeen & Green 1978; Richardson et al 1979). This release could constitute one of the sources of eye irritation. Since the extent of the interaction of chlorhexidine with PHEMA has only been quantified for the gluconate salt, we examined the sorption characteristics of PHEMA with the three commercially available salts; the acetate, the chloride and the gluconate. Their differing affinities for the polymer led us to investigate the influence of some other counter ions, notably amino acids and dicarboxylic acid salts, on the extent of the preservative : polymer interaction.

MATERIALS AND METHODS

Materials

Chlorhexidine digluconate (as a 20% solution), acetate and chloride were gifts from ICI Pharmaceuticals. The α-amino acids glycine, L-arginine

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hydrochloride, L-proline, L-hydroxyproline, L-methionine, L-phenylalanine, L-alanine and L-sodium glutamate were of Analar quality as was oxalic acid. All other amino acids and dicarboxylic acids were reagent grade. The reagents for the colorimetric assay of chlorhexidine (Holbrook 1958) were as follows: bromine and propan-2-ol (Analar, BDH Ltd) and cetrimide B.P. from ICI Ltd. Radioactively labelled chlorhexidine (hexane-1,6-^{14}C: 2·05 mCi mmol^{-1}) obtained from New England Nuclear Co. as the gluconate salt was a gift from Burton, Parsons and Co. Inc. 2,5-Diphenyloxazole (PPO), 1,4-bis-[2-(4-methyl-5-phenyloxazolyl)] benzene (dimethyl POPOP) and Triton X110 were obtained from Sigma and xylene (scintillation grade) from BDH Ltd. All other salts were Analar grade. Buttons of optical quality PHEMA were a gift from Smith & Nephew Optics Ltd.

Preparation of polymer

PHEMA buttons were powdered in a domestic coffee grinder (Moulinex: type 104–2–02), cleaned by Soxhlet extraction with water for 24 h and then dried under vacuum (21 332 Nm^{-2}) over phosphorus pentoxide at 30–40 °C. The material was reground, sieved mechanically and the 65–120 μm fraction collected. Since the affinity of chlorhexidine for PHEMA showed variation between batches, experiments of a strictly comparative nature such as the sorption isotherms of the chloride, acetate and gluconate salts (Fig. 1) were confined to a single PHEMA batch. The experiments with amino and dicarboxylic acids were carried out with a different PHEMA preparation.

pH measurements

Measurements were made at 20 °C using a Pye Unicam Model 291 pH meter and a Pye Ingold 401 Eo7 combined electrode. The pH meter was calibrated with two standard buffers, one above and one below the pH to be measured (Bates 1973). pH values were determined at the completion of each sorption experiment.

Assay procedures

Chlorhexidine was estimated by the colorimetric method of Holbrook (1958) using the protocol described by Richardson et al (1977) in which 5 ml aliquots of chlorhexidine solution of concentrations up to 0·12 mM were included. Absorbances at 480 nm were read on a Pye Unicam SP1800 spectrophotometer in 1 cm path length cells. Regression analysis of the Beer Lambert plot gave a molar extinction coefficient of 4089 (s.d. = 11·2).

Radioactively-labelled chlorhexidine solutions were assayed by addition of 10 ml of scintillation fluid consisting of PPO (3 g litre^{-1}) and dimethyl POPOP (0·2 g litre^{-1}) in a mixture of xylene and Triton X110 (3 : 1 v/v) to 10 ml samples of aqueous solution. Samples were counted for 10 min in a Philips Liquid Scintillation Analyser Model OM1. The amount of chemical quenching was found to be independent of chlorhexidine concentration, thus enabling preservative content to be determined directly from the counts recorded min^{-1}. Calibration curves relating counts min^{-1} to concentration, which were constructed for each individual sorption experiment, were linear with standard deviation to slope ratios of less than 0·5%.

Glassware

Sampling tubes for the sorption experiments, which were fitted with number three sintered glass discs, were cleaned with potassium permanganate and acidified hydrogen peroxide solutions. The discs were aged by equilibrating for at least 16 h with solutions of the appropriate chlorhexidine concentration. Before use they were removed, blown out and blotted dry.

Sorption of the acetate, chloride and gluconate salts of chlorhexidine by PHEMA

Simple, unbuffered, aqueous solutions of chlorhexidine diacetate (1·60 mM; 0·1% w/v), digluconate (2·23 mM; 0·2% w/v) and dihydrochloride (1·73 mM; 0·1% w/v) were prepared and labelled with [^{14}C]chlorhexidine isotope giving specific activities of 1·2, 0·9 and 1·3 μCi mmol^{-1} respectively. These stock solutions which constituted the maximum concentrations studied were diluted to generate a minimum 20-fold concentration range. The level of radioactivity was such that the weakest solution recorded a count of at least 200 counts min^{-1} ml^{-1} of solution.

The experimental procedure was based on that described by Richardson et al (1978). 0·2 g aliquots of PHEMA powder were incubated with 10 ml of chlorhexidine solution and shaken at 70 cycles min^{-1} in a bath thermostated at 30 ± 0·1 °C. With two chlorhexidine concentrations that differed initially by an order of magnitude (0·111 mM and 1·114 mM), maximum uptakes for the gluconate salt were found to occur within 24 h. To ensure that equilibrium had been achieved, the time course was followed for a total of 160 h. For experimental convenience, an incubation time of 24 h was adopted for all samples and the validity of this time

was also verified for the acetate and chloride at concentrations that spanned the entire experimental range. Supernatant solutions were removed via the sampling tubes. Number 3 glass sinters have a range of maximum pore diameter 15–40 μm (BS 1752, 1963) and therefore do not permit the polymer particles to pass through. Replicate assays were carried out on each sample. Where desorption was also studied, 5 ml samples were removed from the sampling tube and replaced by 5 ml of water which was then blown back into the flask through the sintered disc. The volume of solution lost by this process was estimated by weight loss to be 0·13 ml (s.d. = 0·04 ml) which was considered to be negligible. After re-incubation for 24 h, solutions were sampled and assayed as described before. The equilibration time for desorption was investigated for all three chlorhexidine salts and no further elution of preservative was found to occur over 24 h beyond the end of the normal desorption period. A more detailed desorption: time dependence experiment for the gluconate salt showed that a concentration of chlorhexidine within the polymer of 4·2 mmol kg^{-1} remained constant up to 160 h from initiating desorption.

The validity of the radiolabelling technique used to monitor the uptake of chlorhexidine acetate and chloride rests on two factors. Firstly, a small concentration of gluconate ion is introduced into the solution along with the ^{14}C-labelled chlorhexidine cation. The proportion was calculated on a molar basis to be always less than 0·1% of the total anion concentration and was therefore considered to be insignificant. Secondly, the gluconate ion must be freely interchangeable with the chloride and acetate anions in solution otherwise the isotope could remain preferentially associated with the gluconate ion resulting in the distribution of 'hot' chlorhexidine not being a true representation of its unlabelled counterpart. This point was clarified by assaying colorimetrically supernatant solutions from the chlorhexidine hydrochloride sorption: desorption experiment. The concentrations all lay within ±2% of the values derived by the radiolabelling technique.

Influence of amino and dicarboxylic acids on chlorhexidine sorption

A simple unbuffered solution of chlorhexidine hydrochloride in water (1·114 mM) was prepared in which, to allow for the bifunctional nature of the chlorhexidine cation, a bimolar equivalent (2·228 mM) of the alternative counter ion was included. In the case of the amino acids there were no additional additives but for the dicarboxylic acids a bimolar (2·228 mM) or tetramolar (4·456 mM) equivalent of sodium hydroxide was added as a 0·1 M solution so that the acid was converted to its mono- or di-sodium salt respectively. Experimental solutions were a 1 in 10 dilution of the stock solutions thus enabling the uptake of chlorhexidine from 0·1114 mM solutions to be determined. The sorption conditions were the same as already described. The amount of chlorhexidine remaining in solution was assayed colorimetrically as was its concentration in the initial solution which always lay within ±2% of the theoretical value (0·1114 mM) thus demonstrating that the various counter ions did not interfere with the colour reaction.

RESULTS AND DISCUSSION

Fig. 1 shows that the uptake of chlorhexidine from simple aqueous solution varies with the salt form, being greatest for the acetate and least for the chloride. All the interactions are characterized by separate sorption and desorption isotherms, the distinction between the two being most marked for the acetate and gluconate salts. One explanation of these phenomena is that either the sorption or the

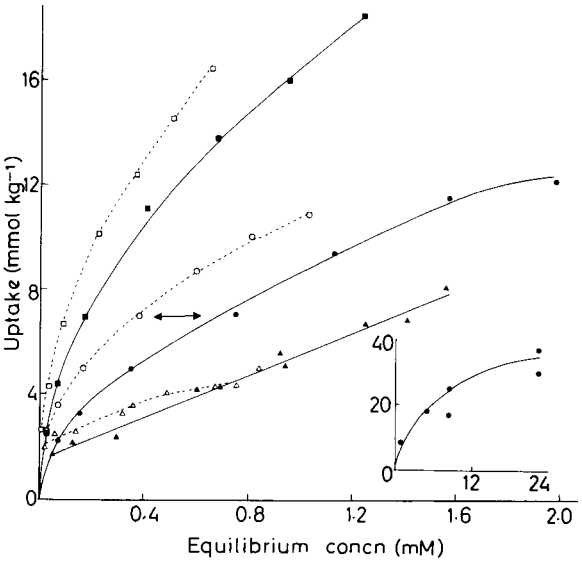


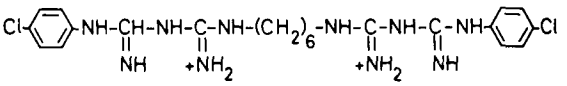
FIG. 1. Sorption (closed symbols) and desorption (open symbols) isotherms for PHEMA powder with aqueous solutions of chlorhexidine dihydrochloride (▲ △), digluconate (● ○) and diacetate (■ □) at 30 °C. Continuous lines represent sorption and broken lines desorption isotherms. Each point is the mean of at least two replicate determinations.

desorption process, or both, have not reached equilibrium. It was for this reason that the time courses for the sorption and desorption of chlorhexidine digluconate with PHEMA were followed at length (see Methods). Equilibrium was achieved within 2 h and no further changes in the concentration of chlorhexidine in the supernatant solutions were detectable over 160 h. This invariance of preservative uptake with time validates the 24 h equilibrium data presented in Fig. 1 and confirms that the difference in behaviour for the three salts is real.

In previous studies, the differing affinities of groups of chemically related substrate molecules for a particular solid absorbent have been discussed in terms of the aqueous solubility of the substrate, there being an inverse relationship between uptake and solubility (Ward & Upchurch 1965; Richardson & Meakin 1974). In this particular case, the solubility of the chlorhexidine salts decreases in the order gluconate, acetate, chloride. On the basis of solubility alone, therefore, the chloride is 'out of order' and should exhibit the greatest rather than the least affinity for PHEMA. The nature of the water contained within the hydrogel could be a factor accounting for this anomaly. Lee et al (1975) have classified the water within a 40% PHEMA gel into three types: water bound to the polymer network (50%), free or bulk water (21%) and water of intermediate properties (29%). Most water molecules, therefore, are held in an ordered arrangement probably as a result of hydrogen bonding with the polymeric hydroxyl groups. This structuring would be expected to lead to a lowering of the dielectric constant of water contained within the hydrogel. Chlorhexidine hydrochloride is the salt of a strong acid as opposed to the gluconate and acetate which are weak acid-base salts and therefore incompletely dissociated in solution. Electrically neutral molecules would be expected to partition more strongly into a less polar environment than would free ions. It is therefore possible that the acetate and gluconate salts can penetrate more effectively into the polymeric water and the polymer matrix exposing irreversible binding sites that are inaccessible to the chloride salt, thus accounting for their higher affinity for the polymer.

Each of the desorption values in Fig. 1 is linked with a corresponding sorption point, one such pair being indicated by the double-headed arrow. For uptakes less than 7 mmol kg^{-1} the interaction of chlorhexidine digluconate is completely irreversible, no preservative being eluted from the polymer during the desorption step. At higher concentrations, a certain amount of chlorhexidine is bound reversibly, the proportion eluted reaching a value of 11% at the upper limit of the experiment. The non-superimposability of sorption and desorption isotherms is uncommon for solute:plastic interactions. The irreversibility observed here would be consistent with an ion:ion interaction between the cationic chlorhexidine ion and a negatively charged site within the polymer.

Since the extent of the chlorhexidine:PHEMA interaction is dependent on the salt form of the preservative, the possibility existed of finding a chlorhexidine derivative that exhibited only minimal affinity for the polymer. Because of the low solubility of the inorganic salts of chlorhexidine (Fürst et al 1977), the exploration of additional salts was restricted to organic, and in particular carboxylic acid, derivatives. Chlorhexidine is a symmetrical molecule possessing two biguanide groups each of which is characterized by two pK_a values of 2·2 and 10·3 (Hugo & Longworth 1964). Since equimolar solutions of chlorhexidine chloride, gluconate and acetate 0·1114 mM) were found to have pH's (at 30 °C) of 6·25, 6·45 and 6·50 respectively, it was considered that chlorhexidine existed in aqueous solution as the di-cation:



thus requiring a bimolar equivalent of the anion under investigation. A single preservative concentration of 0·111 mM was selected for these experiments. This corresponds to a chlorhexidine digluconate concentration of 0·01% w/v.

Since the sorption of chlorhexidine by PHEMA is sensitive to the presence of additives, particularly salts (Richardson et al 1978), the effect of the various additives was investigated in the absence of buffers. The pH of the solutions was therefore sensitive to the carbonic acid concentration in the distilled water as well as to perturbations arising from the presence of the carboxylic acid derivatives themselves. However, the pH of the post-equilibration solutions lay between 3·9 and 7·0 (Tables 1 and 2) in which region the uptake of chlorhexidine by PHEMA shows only minimal variation (Richardson et al 1978). The ionization state of the additives was another variable. A knowledge of the ion-dissociation constants for the various amino acids (Sober 1968) as well as the experimental pH values

Table 1. Sorption of chlorhexidine from 0·1114 mM solution by PHEMA powder in the presence of bimolar equivalents of various amino acids.

Straight chain amino acids			α-Amino acids		
Amino acid	$\overset{+}{N}H_3$–$(CH_2)_x$–COO^- x =	Chlorhexidine concn in PHEMA (mmol kg^{-1})	Amino acid	$\overset{+}{N}H_3$–CH(R)–COO^- R	Chlorhexidine concn in PHEMA (mmol kg^{-1})
Glycine	1	2·21 2·27	Glycine	–H	2·21 2·27
			L-Arginine hydrochloride	$-(CH_2)_3-NH-C(=\overset{+}{N}H_2)-NH_2$	2·42 2·50
3-Aminopropionic acid	2	2·58	L-Proline	$\overset{+}{N}H_2-(CH_2)_3-CH-COO^-$	2·46
		2·82	L-Hydroxyproline	$\overset{+}{N}H_2-CH_2-CH(OH)-CH_2-CH-COO^-$	2·58
4-Aminobutanoic acid	3	2·88 3·21	L-Methionine	$-(CH_2)_2-S-CH_3$	2·90 2·94
			L-Phenylalanine	$-CH_2-C_6H_5$	2·90 3·02
			L-Alanine	$-CH_3$	3·13 3·17
			L-Sodium glutamate	$-(CH_2)_2-COO^-$	5·05 5·08

permits an estimation of the proportion of counter ion in the form $\overset{+}{N}H_3$–CH(R)–COO^- where R is in the ionization state shown in Table 1. With the exception of 4-aminobutyric acid (93%), this proportion was always at least 98%. The fraction of the various simple carboxylic acid species are shown in Table 2.

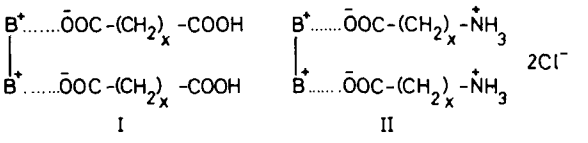
The variation in preservative uptake induced by the addition of various amino and dicarboxylic acids is extensive ranging from 1·76 to 5·12 mmol kg^{-1} (see Tables 1 and 2). One possible explanation for this phenomenon is that the different additives can compete with chlorhexidine for the polymer binding sites with varying degrees of effectiveness. We have not favoured this interpretation, however, since other sorption experiments have demonstrated a complete absence of any interaction between PHEMA and the benzoate anion (Plaut et al 1980). Taking this result together with the knowledge that PHEMA is itself negatively charged, we assumed that all simple organic anions are excluded from the polymer matrix. However, the mono- and disodium dicarboxylic acid salts must participate actively in the sorption process otherwise they would all generate the same uptake and Table 2 shows clearly that this is not the case. One possibility is that carboxylate additives, being derived from weak parent acids, exhibit some degree of association

Table 2. Experimental and theoretical values for the uptake of chlorhexidine from 0·1114 mM solution by PHEMA powder in the presence of bimolar equivalents of mono- and di-sodium carboxylate salts.

Parent acid*	$HOOC(CH_2)_xCOOH$	x =	Chlorhexidine uptake (mmol kg^{-1})	Exp. pH	Ionization† constants pK_1	pK_2	Relative concn of ionic species H_2A	HA^-	$A^=$	Theoretical uptakes. Calculated according to Equation I U_{HA^-} (mmol kg^{-1})	$U_{A^=}$ (mmol kg^{-1})
Oxalic acid	M	0	1·73 1·79	3·95	1·27	4·266	0·001	0·673	0·325	0·29	4·81
	D		3·29 3·47	4·60			0·0001	0·317	0·683		
Malonic acid	M	1	2·38 2·50	4·58	2·85	5·697	0·017	0·913	0·070	1·69	12·79
	D		5·08 5·16	5·35			0·002	0·688	0·310		
Succinic acid	M	2	3·15 3·37	4·85	4·209	5·638	0·164	0·719	0·117		
	D		4·75 5·01 5·03	5·83			0·009	0·388	0·603		
Glutaric acid	M	3	3·65 3·69	4·85	4·32	5·42	0·189	0·639	0·172		

* M refers to the mono- and D the di-sodium salt.
† Albert & Serjeant (1971). All pK_a values measured at 25 °C except for glutaric acid at 18 °C.

with the chlorhexidine biguanide groups (B). Examples of the postulated complexes are shown below:



I II

where the counter ions are monosodium dicarboxylic acid salts (I) and the zwitterionic form of the amino acids (II). Were such complex formation to be accompanied by the generation of an electrically neutral species, the penetration of the preservative into the polymer should be facilitated on account of the preferred affinity of PHEMA for unionized molecules (Plaut et al 1980).

The straight chain amino acids (Table 1) and the monosodium carboxylate salts (Table 2) both show a positive correlation between uptake and increasing chain length. In the latter case, this trend could result from an increase in the lipophilic character of the electrically neutral complexes formed between chlorhexidine and the longer chain monosodium carboxylate salts. The complexes of these higher homologues would be expected to partition more readily into the relatively hydrophobic polymer environment. Any chlorhexidine:amino acid complexes (II), however, would bear net positive charges and so the fact that the extent of their interactions with PHEMA do not differ markedly from that observed for chlorhexidine hydrochloride (2·37 mmol kg^{-1} from a 0·1114 mM solution) is not surprising. The small rise in uptake with increasing chain length probably reflects the greater hydrophobic nature of the longer chain complexes (II).

Since the α-amino acid counter ion, glycine, results in one of the lowest uptakes, the possibility exists that the chlorhexidine interaction may be further minimized by the inclusion of side chains into the glycine molecule. The sorption of chlorhexidine in the presence of different α-amino acids (Table 1) does not follow any clear trend. Although the idea of the counter ions competing with chlorhexidine for polymer binding sites has been discounted for anions, the amino acid, arginine, bears a net positive charge and could interact with the polymer in the same way as other organic cations (Plaut et al 1980). Such a mechanism, however, is not indicated since the chlorhexidine uptake in the presence of arginine is 2·46 mmol kg^{-1}, a value that does not differ significantly from that of 2·37 mmol kg^{-1} characteristic of chlorhexidine hydrochloride itself. The other α-amino acid with an ionizable side chain is glutamic acid. A chlorhexidine:glutamate complex would carry no net charge which possibly accounts for the particularly high uptake associated with it. However, there is no *a priori* reason why the glutamate moiety, being highly hydrophilic, should give rise to an uptake that is so much greater than the monosodium dicarboxylate salts, indicating that this interpretation is possibly over-simplistic.

The conversion from mono- to disodium dicarboxylic acid salts is accompanied by a dramatic increase in preservative uptake. Were complex formation to be analogous to that of structure I, the entity would be negatively charged. An alternative possibility is the formation of a circular 1:1, chlorhexidine:disodium carboxylate complex. This would be electrically neutral and should readily penetrate the polymer matrix.

The treatment of the dicarboxylate salts as 'mono' and 'di' valent is also oversimplistic owing to the relatively close proximity of the two dissociation constants of the parent acids. Table 2 shows the relative proportions of each ionic species at the experimental pH values, calculated from their acid dissociation constants (Albert & Serjeant 1971). The temperature variation between the pH values (20 °C), pK_a's (25 °C) and sorption experiments (30 °C) was not considered to be significant since the ionization constants of carboxylic acids show little variation in this temperature range (Albert & Serjeant 1971). Assuming that for each dicarboxylic acid the chlorhexidine is taken up as two separate complex forms, it is possible to express experimentally determined uptake from a 0·1114 mM chlorhexidine solution (U_{EXP}) as a additive function of the two ionic species according to equation 1.

$$U_{EXP} = U_{HA^-}\alpha_{A^-} + U_{A=}\alpha_{A=} \quad \ldots \quad (1)$$

U_{HA^-} and $U_{A=}$ represent the uptakes from 0·1114 mM chlorhexidine solution in the presence of 0·2228 mM mono- and di-valent salts respectively. α_{HA^-} and $\alpha_{A=}$ are the fractions of the relevant ionic species. It is only possible to apply this equation where the proportion of unionized acid is small (less than 2%) as is the case for oxalic and malonic acids. Table 2 contains data for oxalate and malonate at two pH's which allow simultaneous equations to be constructed and solved for U_{HA^-} and $U_{A=}$. These values, given in the last two columns of Table 2, show that the change from the mono to the bivalent anionic form of the counter

ion enhances the uptake by factors of 8 and 16 for oxalate and malonate respectively. This reinforces the trend already noted, that is, that the extent of uptake shows a positive dependence on counter ion chain length and on the degree of ionization of the anion.

The different counter ions produced a three-fold variation in the extent of the sorption of chlorhexidine by PHEMA from 1·76 to 5·12 mmol kg^{-1} illustrating that this may be an effective means of varying the preservative uptake. Only two compounds, however, glycine and mono sodium oxalate, lowered the uptake below 2·37 mmol kg^{-1} which is the level measured for sorption from a 0·1114 mM chlorhexidine hydrochloride solution. Since this reduction was only marginal, neither of these compounds present a practical alternative to the commercially available salts in formulating chlorhexidine solutions for use with PHEMA based soft contact lenses.

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