

Studies on the Formation of Electrostatic Complexes Between Benzethonium Chloride and Anionic Polymers

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Abstract □ The chemistry of a mixture of benzethonium chloride and a copolymer of methoxyethylene-maleic anhydride was investigated. This mixture was of interest because it was effective in reducing dental plaque, calculus, and gingival inflammation *in vivo*. Evidence from dialysis, pH measurements, and stoichiometry demonstrated that the benzethonium cation and the anion of the hydrolyzed copolymer formed an electrostatic complex. An emulsion was produced when a stoichiometric excess of either component was present, but this mixture coacervated at stoichiometric quantities. The stability of the complex was pH dependent, and it did not form in 50% acetone. The complex was decomposed by simulated saliva, mainly due to calcium and magnesium ions, but was unaffected by salivary proteins. Other anionic polymers also formed this type of complex.

Keyphrases □ Electrostatic complexes—reaction of benzethonium chloride and methoxyethylene-maleic anhydride copolymer □ Polyanions—complex formation with long-chain quaternary ammonium ion □ Benzethonium chloride—electrostatic complex with methoxyethylene-maleic anhydride copolymer

The antibacterial compound benzethonium chloride has been shown to be an effective inhibitor of dental plaque formation in humans (1). In a subsequent study, a mixture of benzethonium chloride and a copolymer of methoxyethylene-maleic anhydride (mol. wt. 500,000) was evaluated *in vitro* for antibacterial activity and *in vivo* (beagle dogs) for inhibition of plaque, gingivitis, and calculus formation (2-4). The results demonstrated that the combination was as effective as benzethonium chloride alone in antibacterial activity and in the reduction of plaque and gingivitis. A difference did exist, however, in the effect on calculus. Benzethonium chloride

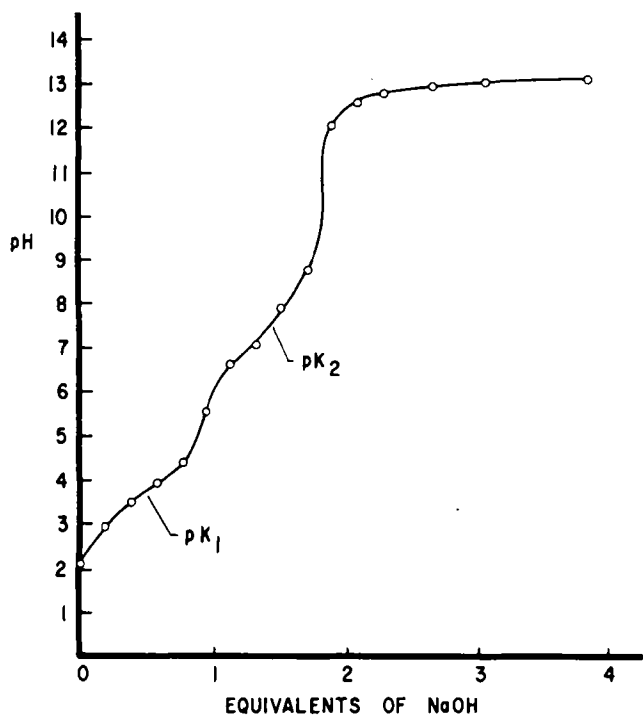


Figure 1—Titration curve of the methoxyethylene-maleic anhydride copolymer.

significantly increased calcified deposits on teeth as compared with the water control group, whereas the mixture showed significantly less calculus. Hence, the mixture was as effective as benzethonium chloride for the inhibition of plaque and gingivitis and had the advantage of inhibiting calculus formation rather than enhancing it. It was, therefore, of interest to elucidate the chemistry of the complex.

A number of investigators have studied the interactions of cations with polymers. Ackart *et al.* have investigated carboxyl-containing copolymers, such as the copolymer of ethylene-acrylic acid, and quaternary ammonium compounds, such as benzalkonium cations (5). They have suggested that the carboxyl-containing copolymer can be neutralized directly by the benzalkonium hydroxide or that the partial sodium salt of the copolymer could be ion-exchanged with benzalkonium chlorides. No evidence was presented, however, for the formation of electrostatic complexes. Rölla *et al.* have demonstrated interactions of another cationic antibacterial agent, chlorhexidine, with acidic salivary proteins (6). They hypothesized that this precipitate was an insoluble salt of the negatively charged protein and the positively charged chlorhexidine cations. Saito has studied interactions of tetra-alkyl ammonium chlorides with polyacrylic acid (7). By measuring the surface tension, he demonstrated that the quaternary compound became bound to the negatively charged polymer, neutralizing the surface and causing precipitation. Further addition of the quaternary cation coated the neutral surface of the coagulum, causing it to become charged and water soluble. Interactions of the alkaloid cation with the negatively charged ionized group of biocolloids were determined by coagulation observations and measurements of the direction of electrolytic transport by Jong and Meer (8). They demonstrated that there was an increase in interaction with increasing charge density on the colloid anion. Michaels and Miekka studied the interaction between sodium polystyrene sulfonate and polyvinylbenzyltrimethyl ammonium chloride (9). They have demonstrated that when the two were mixed in water, a precipitate formed which contained almost exactly stoichiometric proportions of the component polyions and, furthermore, contained virtually none of the sodium and chloride counterions associated with the individual polymers.

Table I—Formula for Simulated Saliva^a

Component	Weight, g/L
CaCl ₂ ·2H ₂ O ^b	0.228
MgCl ₂ ·6H ₂ O ^b	0.061
NaCl ^b	1.017
K ₂ CO ₃ ·1 1/2 H ₂ O ^b	0.603
Na ₂ HPO ₄ ·7H ₂ O ^b	0.204
NaH ₂ PO ₄ ·H ₂ O ^b	0.273
Submaxillary Mucin ^c	1.000
α-Amylase ^d	2.000

^a See Refs. 17 and 18. ^b J.T. Baker Chemical Co., Bricktown, N.J. ^c Worthington Biochemical Corp., Freehold, N.J. ^d ICN Nutritional Biochemicals, Life Science Group, Plainview, N.J.

Table II—Equilibrium Dialysis of Aqueous Mixture of Ammonia, Benzethonium Chloride, and Methoxyethylene–Maleic Anhydride Copolymer

Cation	Theoretical for 100% Electrostatic Complex	Theoretical for 0% Electrostatic Complex	Found
		<u>Inside Dialysis Bag</u>	
Benzethonium ^a	0.10% (2.2 mM)	0.05% (1.1 mM)	0.11% (2.4 mM)
Ammonium ^b	$2 \times 10^{-4}\%$ (0.1 mM)	$20 \times 10^{-4}\%$ (1.2 mM)	$2 \times 10^{-4}\%$ (0.1 mM)
		<u>Outside Dialysis Bag</u>	
Benzethonium ^a	None	0.05% (1.1 mM)	0.003% (0.06 mM)
Ammonium ^b	$3.8 \times 10^{-3}\%$ (2.2 mM)	$2.0 \times 10^{-3}\%$ (1.2 mM)	$3.1 \times 10^{-3}\%$ (1.8 mM)

^a Calculated as benzethonium chloride. ^b Calculated as ammonia.

Studies have been done on the degree of binding of negatively charged polymers with small positively charged ions (10). Increased binding at a particular polymer carboxylate site resulted in decreased negative charge density at that site. This resulted in reduced energy requirements for nearby carboxylic acid groups to ionize and, therefore, in a lowering of the pH (11). This decrease in pH has, in fact, been used to establish the magnitude of binding of counterions to polyanions (12–15).

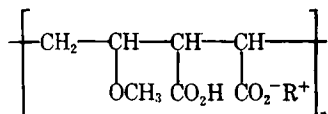
The studies described above, taken together, strongly suggest the formation of electrostatic complexes between polyanions and cations. The evidence for interaction with long-chain quaternary ammonium salts such as benzethonium chloride, however, is nonexistent. Furthermore, evidence of this kind of interaction with a type of copolymer such as methoxyethylene–maleic anhydride is lacking. Therefore, an investigation was undertaken to determine the nature of the mixture of benzethonium chloride and methoxyethylene–maleic anhydride copolymer.

EXPERIMENTAL SECTION


Preparation of Benzethonium Chloride–Polymer Mixtures in Water—Methoxyethylene–maleic anhydride copolymer¹ (100 mg) was added to deionized, distilled water at 50°C. The pH was adjusted with 3 M NH₄OH to 5.0, and the solution was diluted to 100 mL. An equal volume of a 0.2% solution of benzethonium chloride² in water was slowly added while maintaining the pH at 5.0–5.1 with 3 M NH₄OH. The final concentration was 0.0023 M NH₄⁺, 0.0022 M benzethonium chloride, and 0.0032 M methoxyethylene–maleic acid repeating units. A 5% solution of the polymer was used for titration studies with 4 M NaOH (Fig. 1). Copolymer and benzethonium chloride were used as received.

Other benzethonium chloride–polymer mixtures were prepared in the same manner, but without pH adjustment, to provide the same concentrations of repeating units as described above. The complex was also prepared in 50% acetone by mixing equal volumes of 0.2% benzethonium chloride in acetone with 0.016–0.4% aqueous solutions of the copolymer at room temperature.

Dialysis—Cellulose acetate dialysis tubing³, with a molecular weight cutoff of 12,000, was washed with distilled, deionized water. Equilibrium dialysis was performed at room temperature, with solvent inside and outside of the bag identical in composition and pH, under the following conditions: (a) equal volumes outside and inside the bag; (b) four times the volume outside as inside the bag; and (c) 19 times the volume outside as inside the bag.



I: R⁺ = NH₄⁺

II: R⁺ = (CH₃)₃CCH₂C(CH₃)₂——(OCH₂CH₂)₂N⁺(CH₃)₂CH₂C₆H₅

Advantages of the high ratio were that it allowed more unbound ions to dialyze out of the bag and there was a reduction of the Donnan effect. The disadvantage, however, was that any material of interest outside the bag was diluted. The ratio used depended on the optimum requirement for a particular experiment.

Dialysis was performed for 18–24 h with constant stirring. Before analysis of components under condition c, the volume of solution was concentrated to the volume found inside the bag.

Effect of Salivary Components—Four solutions, each containing some of the salivary components from simulated saliva (16, 17), were prepared (Table I). The solutions were: (A) calcium and magnesium salts, (B) potassium and sodium salts, (C) all saliva salts included in A and B; (D) proteins. For the salt reactions, 1 volume of these solutions or water was added to 3 volumes of 0.1% benzethonium chloride–polymer mixture and dialyzed. On the outside of the dialysis bag was a mixture of the salts with the same concentration as inside the bag. Dialysis proceeded according to condition c.

For the protein reactions, 1 volume of protein solution was added to 3 volumes of benzethonium chloride or the benzethonium chloride–polymer mixture and dialyzed according to condition a.

Analysis of Benzethonium Chloride—Benzethonium chloride was determined by anionic titration with sodium lauryl sulfate, using disulphine blue–dimidium bromide as an end point indicator (18).

Analysis of Ammonia—Ammonia was determined with an ammonium ion-selective electrode⁴.

RESULTS AND DISCUSSION

Equilibrium Dialysis—An aqueous mixture of benzethonium chloride and ammonia-adjusted hydrolyzed methoxyethylene–maleic acid copolymer contained 0.0023 M ammonia, 0.0022 M benzethonium chloride, and 0.0032 M copolymer repeating units. This mixture was equilibrium dialyzed by using dialysis condition c. If all the benzethonium cations formed an electrostatic complex with the polymer anions, which by virtue of its molecular weight (500,000) would not pass through the membrane, then one would have expected to find all the benzethonium cations (0.0022 M) and only 0.05% of the ammonium ions (0.0001 M) inside the dialysis bag. On the other hand, if there was no selective binding of the benzethonium cation to the polymer counterion, then half of the benzethonium cation (0.0011 M) and half of the ammonium cation (0.0012 M) would have remained inside the bag to maintain charge neutrality with that portion of the polymer which is ionized (0.0023 M).

The results (Table II) show that approximately all the benzethonium cations (0.0024 M) and only 0.0001 M ammonium ions remained inside the dialysis bag. Virtually no benzethonium cations and most of the ammonium ions were found outside the bag. The ammonium ion concentration outside the bag would probably have been even higher if not for the likely loss of ammonia during the concentration step. These results indicate selective electrostatic binding of the benzethonium cations to the copolymer anions, *i.e.*, formation of an electrostatic complex.

pH Drop—The pH of a 0.20% benzethonium chloride concentrate rose slightly after being diluted to 0.10%, as would be expected for a twofold dilution. Similarly, after a 0.10% ammonia-adjusted copolymer concentrate was adjusted to 0.05%, the pH also rose slightly. When the two concentrates were diluted by mixing together, however, the pH decreased to below that of the individual components (Table III). This could be due to the fact that the benzethonium cation had a higher affinity than NH₄⁺ for the polymer anion. This, in effect, reduces the amount of negative charge on the neutralized carboxylate group, permitting the adjacent carboxylic acid group to ionize, thus lowering the pH.

Stoichiometry—In Table IV, a study of precipitation conditions for the mixture is presented. It shows that when one equivalent of benzethonium chloride was mixed with one equivalent of active carboxylic acid (pK_a 3.7),

¹ Gantrez 139; GAF Corp., Wayne, N.J.

² Hyamine 1622; Rohm and Haas, Philadelphia, Pa.

³ Arthur H. Thomas Co., Philadelphia, Pa.

⁴ Orion Research, Cambridge, Mass.

Table III—pH Drop in Mixing Benzethonium Chloride and Methoxyethylene–Maleic Anhydride Copolymer

Mixture Composition	pH
0.10% Benzethonium chloride	6.38
0.05% Ammonia-adjusted methoxyethylene–maleic anhydride copolymer	5.82
0.10% Benzethonium chloride and 0.05% ammonia-adjusted methoxyethylene–maleic anhydride copolymer (from respective stock solutions)	5.37

Table IV—Stoichiometry of Precipitation of Benzethonium Chloride and Active Methoxyethylene–Maleic Acid Copolymer Carboxylic Acids

Mixture Composition	Ratio of Equivalents of Mixture to Active Carboxylic Acid	Appearance
0.1% Benzethonium chloride + 0.050% polymer	1:1.5	Emulsion
0.1% Benzethonium chloride + 0.033% polymer	1:1	Precipitate
0.1% Benzethonium chloride + 0.025% polymer	1:0.75	Emulsion
0.1% Benzethonium chloride + 0.017% polymer	1:0.50	Emulsion
0.1% Benzethonium chloride + 0.009% polymer	1:0.25	Emulsion
0.1% Benzethonium chloride	1:0	Clear Solution

a precipitate formed, but an excess of either reactant resulted in formation of an emulsion. This can be explained by formation of an electrostatic complex between the reactants, which would tend to reduce the effective charge on each of the components, thereby decreasing water solubility. At the same time, a large, hydrophobic molecule is produced, which would also tend to have minimal water solubility.

Formation of an emulsion at any other ratio is readily explainable. With an excess of copolymer, the surplus negative charge keeps the material partially suspended in water. With an excess of benzethonium chloride, the positively charged, surface-active benzethonium chloride acts as an emulsifying agent.

Other Anionic Polymers—The effect of polymer molecular weight was investigated as a possible parameter of interaction with benzethonium chloride; dialysis condition *c* was used. The methoxyethylene–maleic anhydride copolymers used all have the same structure but average molecular weights of 250,000⁵ (A), 500,000¹ (B), and 1,250,000⁶ (C), respectively. Table II shows the theoretical results expected for 100 and 0% electrostatic complexation of benzethonium chloride with copolymer B (used throughout this research). These values also apply for copolymers A and C. The observed results (Table V) show that in all three cases, approximately all of the benzethonium cation stayed inside the membrane and approximately all of the ammonium ion was released. That is, within the limits investigated, formation of an electrostatic complex proceeded to completion independent of molecular weight.

The possibility of electrostatic complex formation was also investigated for benzethonium chloride and other polycarboxylic acids. Benzethonium chloride was mixed with several different types of anionic polymers. Two changes were observed (Table VI) which are similar to those observed with benzethonium chloride and copolymer B. These changes were marked increases in turbidity and, generally, decreases in pH to below that of either of the individual components. The pH of the benzethonium chloride solution was 6.38. The pH of the polymer solutions are listed in Table VI.

These two effects are consistent with formation of an electrostatic complex. Production of turbidity was explained above. Decrease in pH by formation of an electrostatic complex is also easily explained. Methoxyethylene–maleic anhydride copolymer, polyacrylic acid, and ethylacrylate–methacrylic acid copolymer yield hydrochloric acid, a relatively strong acid, as a by-product of the reaction. (As explained above, for copolymer B the decrease in the pK_a of the less active acid should also contribute to the decrease in pH.) For the diisobutylene–maleic acid and vinyl alcohol–acrylic acid copolymers, in which sodium chloride rather than hydrochloric acid would be expected to be released during the formation of an electrostatic complex, no decrease in pH was observed. One of these polymers, the disodium salt of the copolymer of diisob-

Table V—Equilibrium Dialysis of Aqueous Mixture of Ammonia, Benzethonium Chloride, and Three Copolymers of Different Molecular Weights

Cation	Mol. Wt. 250,000	Mol. Wt. 500,000	Mol. Wt. 1,250,000
Benzethonium ^a	0.105 ^b	0.107	0.108
Ammonium	0.0002 ^b	0.0002	0.0002

^a Calculated as benzethonium chloride. ^b Inside membrane, %.

utylene–maleic acid, was further investigated by equilibrium dialysis. After equilibrium was attained, 98% of the benzethonium cation remained inside the membrane, demonstrating electrostatic complex formation. Dialysis was not attempted with the sodium salt of the vinyl alcohol–acrylic acid copolymer because of difficulties anticipated due to the precipitate.

Effect of Acetone—To elucidate the driving force of the reaction, it was of interest to determine the effect of other solvents on the benzethonium–copolymer complex. The reaction mixture was made with water–acetone (50:50) as solvent. The ratios of equivalents of benzethonium chloride–active carboxylic acid groups ranged from excess benzethonium chloride (1:0.25, for 0.1% benzethonium chloride and 0.008% copolymer) to excess carboxylic acid groups (1:6.00, for 0.1% benzethonium chloride and 0.200% copolymer). The solutions were dialyzed according to condition *b*. The degree of complexation was determined by analysis of the benzethonium cation inside and outside the membrane. The observed values were compared with theoretical values calculated *via* Donnan's equilibria.

The results (Table VII) show very good agreement between the observed ratio and that calculated for 0% complexation, indicating that, surprisingly, reaction did not occur, which is in contrast to the corresponding situation in water in which the reaction went to completion. This would suggest that a driving force of the reaction in acetone is different than in water. It has been suggested that in water, charged species (polyanions and polycations) show an increase in entropy (9). Molecules tend to occupy as much space as possible. Movement of the polymer microions is restricted due to the limited movement of the polyelectrolyte chain. Therefore, there is an "escaping tendency" of the microions. This escaping tendency is overcome by two oppositely charged polyions coming together, thereby liberating the microions from the polyelectrolyte. One would expect the reaction also to occur in aqueous acetone because the same escaping tendency should be present. However, the reaction did not occur in aqueous acetone.

This suggests a second possibility with respect to the driving force of the reaction. Benzethonium chloride forms micelles in water due to the incompatibility of the hydrophobic tails with water. The extent of this micelle formation is limited because of the repulsion among the positively charged benzethonium head groups. This can be overcome, however, by the oppositely charged field exerted by the polyanion. That is, strong attraction of the positively charged head groups on the benzethonium cation with the negatively charged field exerted by the polyanion tends to reduce the repulsion of the benzethonium cations with each other, allowing increased formation of micelles. This could be the driving force for the reaction in water. In acetone, however, there is no incompatibility between the benzethonium hydrophobic tails and the solvent and, therefore, no micelle formation. The reaction, therefore, should not, and did not, take place in acetone. Hence, a driving force for the reaction that is consistent with the facts is increased hydrophobic interactions of the long chains rather than increased freedom of the microions. Both could cause an increase in entropy.

Influence of pH—The effect of pH on the stability of aqueous benzethonium–copolymer complex was studied (Table VIII). The mixture was treated with either dilute hydrochloric acid or ammonium hydroxide to achieve the desired pH. Virtually no difference in appearance was observed at pH 4.0, 5.0, and 8.0. A decrease in pH to 3.6, however, by slow addition of hydrochloric acid led to complete clarification of the supernatant and concomitant formation of a precipitate. On the other hand, when hydrochloric acid was rapidly added to the pH 5.0 emulsion until it was at pH 2.0, a clear solution resulted without ever going through a precipitate stage. At pH 1.0, the clear solution separated into an oil and a clear supernatant.

Analysis of the dispersions or clear supernatants before dialysis showed the theoretical amount of benzethonium cation at pH 2.0, 4.0, 5.0, and 8.0, but not at pH 1.0 and 3.6. This suggests that there was a meaningful amount of benzethonium cation in the oil (pH 1.0) and solid precipitate (pH 3.6).

The mixtures of pH 2.0, 4.0, 5.0, and 8.0 were treated according to dialysis condition *b*. The results demonstrate that at pH \geq 4.0, approximately all of the benzethonium cation is retained inside the membrane; however, at pH 2.0, within experimental error, the benzethonium cation is evenly distributed on both sides of the membrane. The formation of a precipitate at pH 3.6 can be explained by the fact that protons react with any excess negatively charged

⁵ Gantrez 119; GAF Corp.

⁶ Gantrez 169; GAF Corp.

Table VI—Comparison of pH and Appearance of Polymers Before and After Addition of Benzethonium Chloride

Polymer	pH of Polymer		Appearance of Polymer	
	Without Benzethonium Chloride	With Benzethonium Chloride	Without Benzethonium Chloride	With Benzethonium Chloride
Methoxyethylene-maleic anhydride	3.45	2.78	Clear Solution	Cloudy + Precipitate
Polyacrylic acid ^a	3.89	3.20	Clear Solution	Cloudy
Ethylacrylate-methacrylic acid copolymer ^a	5.15	3.47	Clear Solution	Cloudy
Diisobutylene-maleic acid disodium salt copolymer ^a	10.23	10.30	Clear Solution	Cloudy
Vinyl alcohol-acrylic acid sodium salt copolymer ^a	9.40	8.75	Clear Solution	Cloudy + Precipitate

^a Rohm and Haas, Philadelphia, Pa.

Table VII—Benzethonium-Copolymer Complexation in Aqueous Acetone Studied by Equilibrium Dialysis

Complex Composition	Benzethonium Cation Inside-Benzethonium Cation Outside		
	Theoretical		Observed
	100% Complexation	0% Complexation	
0.1% Benzethonium chloride	—	1.0	1.0
0.1% Benzethonium chloride and 0.008% methoxyethylene-maleic anhydride	2.0	1.6	1.5
0.1% Benzethonium chloride and 0.017% methoxyethylene-maleic anhydride	3.6	2.2	2.4
0.1% Benzethonium chloride and 0.025% methoxyethylene-maleic anhydride	6.0	2.8	2.8
0.1% Benzethonium chloride and 0.033% methoxyethylene-maleic anhydride	11.2	3.3	3.5
0.1% Benzethonium chloride and 0.050% methoxyethylene-maleic anhydride	∞	4.2	3.9
0.1% Benzethonium chloride and 0.200% methoxyethylene-maleic anhydride	∞	12.2	7.8

Table VIII—pH Effect on Stability of the Benzethonium-Copolymer Complex

pH	Appearance	Benzethonium Cation, % ^a		
		Before Dialysis	After Dialysis	Outside Tube
1.0	Clear supernatant and oil	0.011 ^b	—	—
2.0	Clear solution	0.103	0.024	0.019
3.6	Clear supernatant and solid precipitate	0.010 ^b	—	—
4.0	Dispersion	0.100	0.116	0.001
5.0	Dispersion	0.105	0.101	0.001
8.0	Dispersion	0.104	0.096	<0.001

^a Calculated as benzethonium chloride. ^b Virtually only the supernatants were analyzed.

groups on the complex, thereby making the complex less water soluble. Formation of a clear solution at pH 2.0, in which the benzethonium cation was approximately evenly distributed inside and outside of the membrane, can be explained by the fact that the hydrogen and chloride ions of the hydrochloric acid successfully compete with the reactants for the charged sites on the complex. Hence, at pH 2.0, the benzethonium-polymer complex dissociated to form the soluble protonated polymer and soluble benzethonium chloride, and the benzethonium chloride passed through the dialysis bag. At pH 1.0, the same situation as at pH 2.0 existed, except that benzethonium chloride was insoluble at low pH. The results of the studies at pH 4-8 demonstrated that at these pH values, stable emulsions formed.

Effect of Saliva—Since the ultimate use of the benzethonium-copolymer complex was for a mouth rinse or dentifrice, it was important to determine whether it remained intact in the presence of saliva. Saliva contains mainly salts and glycoproteins. Due to experimental difficulties in working with whole saliva containing both salts and proteins, simulated saliva was made containing either one or the other.

Salivary Salts—After it was shown in a preliminary experiment that, indeed, the complex dissociated considerably in the presence of saliva salts under

exaggerated conditions (in terms of contact time and concentrations of salts), it was of interest to determine which salts in the saliva were responsible for this dissociation. The reaction of the benzethonium-copolymer complex with salivary salts was studied by equilibrium dialysis condition *c*. The composition inside the dialysis tube roughly simulated in-use conditions, *i.e.*, dilution of a mouth wash by 25% with the saliva salt solution. This saliva salt solution had approximately the same composition as found in saliva. Benzethonium chloride and the benzethonium-copolymer complex diluted by 25% with water, were used as controls.

The data (Table IX) show that the 0.075% benzethonium chloride was not held back by the dialysis membrane and was diluted ~20-fold by the dialysis solution. The 0.075% benzethonium-copolymer control was essentially maintained completely (0.079%) inside the membrane. In the presence of either the monovalent or divalent salts, little effect was apparent, with final concentrations of 0.071 and 0.064%, respectively. But when the monovalent and divalent salts were combined, a marked dissociation of the complex resulted. It appeared that a combination of calcium and magnesium salts and high ionic strength had a synergistic effect, giving a significant dissociation of the benzethonium-copolymer complex.

The contribution of calcium and magnesium ions was also studied by atomic absorption (Table X). The data show that the number of moles of calcium and magnesium ions which became bound to the copolymer was approximately equal to the sum of the carboxylate groups which contained sodium and am-

Table IX—Effect of Salivary Salts on Decomposition of Benzethonium-Copolymer Complex^a

Reaction Mixture	Benzethonium Cation Inside of Bag After Dialysis, % ^b
Benzethonium chloride ^a	0.004
Benzethonium-copolymer complex	0.079
Benzethonium-copolymer complex and potassium and sodium salts	0.071
Benzethonium-copolymer complex and calcium and magnesium salts	0.064
Benzethonium-copolymer complex and all saliva salts	0.023

^a 0.075% with respect to benzethonium chloride. ^b Calculated as benzethonium chloride.

Table X—Relationship Between Available Copolymer Carboxylates and Binding of Calcium and Magnesium Ions

	Available Carboxylate ^a , mol × 10 ⁻⁵ /100 mL	Bound Ca and Mg, mol × 10 ⁻⁵ /100 mL
Before salt reaction	8	—
After salt reaction	19	20

^a Available carboxylate groups before the reaction are those containing sodium or ammonium counterions; available carboxylate groups after the reaction include the ones with sodium or ammonium counterions as well as those in which the benzethonium cations were displaced.

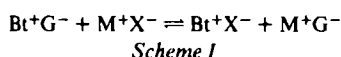
Table XI—Effect of Salivary Protein on Stability of the Benzethonium-Copolymer Complex

Mixture Composition	Benzethonium Cation After Dialysis, % ^a	
	Inside Bag	Outside Bag
Benzethonium chloride	0.038	0.038
Benzethonium chloride and mucin	0.034	0.032
Benzethonium chloride and α -amylase	0.038	0.038
Benzethonium-copolymer complex	0.071	0.000
Benzethonium-copolymer complex and mucin	0.074	0.001
Benzethonium-copolymer complex and α -amylase	0.071	0.002

^a Calculated as benzethonium chloride.

monium ions and those groups which were freed of benzethonium cations. This suggests a direct relationship between the amount of benzethonium cation released from the polymer and the amount of divalent cations taken up.

The dissociation of the complex in the presence of saliva salts can be represented by Scheme I:



where Bt represents benzethonium, G represents the copolymer, M represents the saliva salt cations, and X represents the saliva salt anions.

These results provide a reasonable explanation for the mode of action of the benzethonium-copolymer complex in the oral cavity (2). Simply, the calcium-magnesium ions of the saliva displace the benzethonium cation in the benzethonium-copolymer electrostatic complex. Slow release of the benzethonium cations by the saliva salts explains the low plaque and gingivitis values since benzethonium chloride is an active antimicrobial compound. Complexation of calcium ions from the saliva by the copolymer explains the low calculus value.

Salivary Proteins—The stability of the benzethonium-copolymer complex with another type of salivary component, the proteins, was also studied by equilibrium dialysis (condition a). Benzethonium chloride and the benzethonium-copolymer complex were mixed with salivary proteins and dialyzed. The results (Table XI) show that the benzethonium cations from benzethonium chloride solutions were approximately evenly distributed inside and outside the dialysis bags, not being affected by the proteins, and the benzethonium cation from the benzethonium-copolymer emulsions remained inside the bag, not being released by the proteins.

If a complex formed between the proteins and the benzethonium cation, then the benzethonium cation would have been trapped with the protein inside the bag, since the molecular weight cutoff of the membrane is 12,000. The fact that in the benzethonium chloride-protein cells, the benzethonium cation was not trapped indicated that the salivary proteins had little or no affinity for the benzethonium cation. If the proteins formed some sort of complex with the copolymer, thereby dissociating the benzethonium-copolymer complex,

then the benzethonium cation would have been found in the dialysate. It was not. Hence, although the complex dissociated in the presence of salivary salts, it did not dissociate in the presence of saliva proteins.

CONCLUSIONS

A mixture of benzethonium chloride and methoxyethylene-maleic anhydride copolymer formed an electrostatic complex of the benzethonium cation and the anion of the hydrolyzed polymer, as determined by dialysis, pH, and stoichiometric evidence. An emulsion of the complex was stable in the presence of an excess of either constituent and at pH > 4. The complex was unstable at pH < 4, precipitated at stoichiometric quantities, and did not form in 50% acetone. It was decomposed by simulated saliva because of calcium and magnesium ions in saliva and was unaffected by saliva proteins.

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