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Research Article

Mechanisms of Mucoadhesion of Poly(acrylic Acid) Hydrogels

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It has been proposed that mucoadhesives which adhere to the gastric mucus layer may be used to prolong gastric retention time of oral dosage forms. Preliminary studies, using acrylic hydrogels, have established that the density of carboxyl groups on the polymer chain is important for mucoadhesion. To understand the role(s) of the carboxyl groups in mucoadhesion, acrylic acid–acrylamide random copolymers [P(AA-co-AM)] were synthesized, and the adhesion strength of the cross-linked polymers to the gastric mucus layer was examined as a function of the pH, initial concentration of the cross-linking agent, degree of swelling, and carboxyl-group density. From the study on mucoadhesion of various P(AA-co-AM), it was found that at least 80% of the vinyl groups of the polymer must possess carboxyl groups in the protonated form. The dependence of mucoadhesion on pH and carboxyl-group density suggests that mucoadhesion occurs through hydrogen bonding. In addition, the density of the cross-linking agent significantly affects mucoadhesion. As the density of the cross-linking agent is lowered, the mucoadhesive strength increases, although the density of carboxyl groups in the test surface area is reduced. It is concluded that for mucoadhesion to occur, polymers must have functional groups that are able to form hydrogen bonds above the critical concentration (80% for vinyl polymers), and the polymer chains should be flexible enough to form as many hydrogen bonds as possible.

KEY WORDS: bioadhesion; mucoadhesives; mucin, poly(acrylic acid); hydrogels; hydrogen bonding.

INTRODUCTION

Recently, an alternative approach to retard gastric emptying using mucoadhesives has been described (1,2). It has been suggested that mucoadhesives may remain attached to the gastric mucus layer until they are removed spontaneously from the surface by various factors including natural mucin turnover. Therefore, the study of mucoadhesives may provide approaches that enable not only prolongation of the total residence time but also control of the retention time of oral dosage forms in the stomach and perhaps elsewhere in the gastrointestinal (GI) tract. In earlier studies, a large number of polymers were examined as to their bioadhesive/mucoadhesive potential in order to derive meaningful information on the structural requirements for attachment (1–3). It was observed that polyanions with a high charge density, such as polyacrylic acid (PAA), were good mucoadhesives. Although the charge density appears to be an important factor in mucoadhesion, its role in the mechanism of attachment has not been systematically explored. Since this mucoadhesion occurs in the gastric environment, i.e., acidic pH, carboxyl groups interact with mucin molecules in their protonated form. This suggests that the interaction may be due to hydrogen bonding. In order to examine systematically the role of carboxyl groups and their involvement in the formation of hydrogen bonds with mucin molecules, it was necessary to synthesize polymers varying in the density of carboxyl groups. Copolymerization of acrylic acid with another uncharged monomer is an effective way of altering the carboxyl-group density of acrylic acid residues. Acrylamide was chosen in this study for the following reasons: first, the structure and molecular weight of acrylamide are close to those of acrylic acid; second, acrylamide is very water soluble; and third, preliminary studies established that cross-linked polyacrylamide did not adhere to the gastric mucus layer. Thus, any interaction between acrylic acid–acrylamide copolymer and the mucus layer can be expected to result from the carboxyl groups of the acrylic acid residues. The charge density of synthesized copolymers was determined by potentiometric titration (4–6), and the structure of the copolymers was inferred from reactivity ratios (7).

MATERIALS AND METHODS

Polymer Synthesis

Acrylic acid was purchased from Aldrich and acrylamide was from Bio-Rad (electrophoresis grade). Acrylic acid was vacuum distilled before use to remove inhibitors and acrylamide was used as received. Polymerization was carried out at 60°C with ammonium persulfate as an initiator (8). 2,5-Dimethyl-1,5-hexadiene (DMDH; Aldrich) was

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added as a cross-linking agent. To make copolymers of acrylic acid and acrylamide [P(AA-co-AM)], comonomers were mixed to make the desired initial feed composition. The mixture was flushed with nitrogen gas for 5 min and degassed by vacuum aspiration. For the reactivity ratio measurement, polymerization was carried out only for 5–10 min to produce copolymers at low conversions. Polymerization was stopped by pouring the reaction mixture into an excess amount of organic solvent (more than 100 times by volume) containing hydroquinone. Acetone was used for the isolation of P(AA-co-AM), and diethyl ether for PAA. For the preparation of polymers and copolymers at 100% conversion, polymerization was continued for 2 hr and the product was poured into an organic solvent to cause precipitation. Collected polymers were reprecipitated twice. Before final precipitation, the pH of the polymer solution was adjusted to pH 1 using a 3 *N* HCl aqueous solution to convert all the carboxyl groups to the protonated form. The precipitated polymers were dried in a vacuum oven until constant weight.

For cross-linked polymers, DMHD was added as a cross-linking agent. Cross-linked polymers were made in sheet form using two polyethylene plates. The concentrations of monomers and cross-linking agents were varied as described in Table I. Cross-linked polymers were purified by extensive washing in dionized distilled water to remove unreacted monomers, cross-linking agent, and initiators. The purified polymers were kept in the pH 1 solution containing 0.02% sodium azide until use. For convenience, each copolymer is described by the ratio of comonomers in the reaction feed rather than the actual mole fraction.

Potentiometric Titration of Soluble Polymers

Potentiometric titrations were performed on monomers, low-conversion linear polymers, full-conversion linear polymers, and full-conversion cross-linked polymers. Both acrylic acid and acrylamide are very water soluble and thus no difficulty was encountered in dissolving P(AA-co-AM) in water. Most linear polymer titrations were done using 30–50 mg of polymer/100 ml of distilled deionized water (Barnstead, Nanopure II). The titration was also carried out under conditions of varying ionic strength, which was adjusted by NaCl to determine the effect of ionic strength. Polymers were titrated using a pH meter (Orion, Model 601A) equipped with a Ross pH electrode (semimicro combination electrode, Orion). Sodium hydroxide (0.02 and 0.05 *N*, Fisher certified standard solutions) was used as titrant, and no special precautions were made against atmospheric CO_2.

Table I. Synthesis of Copolymers with Various Monomer Ratios and Cross-Linking Densities

Molar ratio		Cross-linking density
Acrylic acid	Acrylamide	(%)[a]
10	0	0.1, 0.5, 1.0, 2.0
8	2	0.5
6	4	0.5
5	5	0.1, 0.5, 2.0
3	7	0.5
1	9	0.5
0	10	0.5

[a] Weight percent of 2,5-dimethyl-1,5-hexadiene per monomer in the feed.

The degree of dissociation α was calculated from the electroneutrality condition,

$$\alpha = ([\mathrm{Na}^+] + [\mathrm{H}^+] - [\mathrm{OH}^-])//([-\mathrm{COO}^-] + [-\mathrm{COOH}]) \tag{1}$$

where $[\mathrm{Na}^+]$ is the concentration of sodium ions resulting from added NaOH, $[\mathrm{H}^+]$ and $[\mathrm{OH}^-]$ are the concentrations of hydrogen and hydroxyl ions, respectively, and $[-\mathrm{COO}^-] + [-\mathrm{COOH}]$ is the concentration of polyacids in monomeric equivalents per liter. The concentrations of $[\mathrm{H}^+]$ and $[\mathrm{OH}^-]$ are calculated from the measured pH values neglecting corrections for the activity coefficient. Once α was determined, the data were plotted according to the extended Henderson–Hasselbach equation:

$$\mathrm{pH} = \mathrm{p}K_{\mathrm{app}} - \ln(1 - \alpha)/\alpha \tag{2}$$

From the linear regression analysis of the α values ranging from 0.3 to 0.7, the value of $\mathrm{p}K_a$ at α at $\alpha = \frac{1}{2}$ was easily calculated for each titration curve.

Potentiometric Titration of Cross-Linked Polymers

The conventional titration method used for linear polymers could not be used for cross-linked polymers because of slow diffusion of the titrant through the network. In this study, both the discontinuous and the back titration methods were used. In discontinuous titration, the dried polymer sample was divided into several fractions of the same weight (100 mg). These were placed in a polyethylene bottle filled with 200 ml of distilled deionized water, which was flushed with nitrogen for 5 min, and increasing amounts of titrant were added to each polymer sample. Air in the bottles was replaced with nitrogen and the bottles were tightly capped. After equilibrium swelling was reached at room temperature for 14 days, the pH of the solution was measured. When the measured pH was plotted against the added amount of NaOH, a complete titration curve was obtained. This is essentially the same method used for linear polymers, except for the different time scale. In the back titration method, a known amount of cross-linked polymer (usually 100 mg) was placed in a plastic bottle containing deionized distilled water (200 ml). An excess amount of titrant was added and purged with nitrogen, tightly capped, and agitated on a shaker for 4 days. The pH was measured intermittently and after the pH became constant, a known aliquot (10 ml) of solution was withdrawn from the bottle and titrated using HCl. From this titration, the total number of equivalents consumed for neutralization of carboxyl groups could be calculated. Thus, the total number of carboxyl groups in the cross-linked polymers could be computed. Standard HCl was added to neutralize NaOH in the supernate and one-half of the equivalent inside the gel. After the gel was equilibrated again, the final pH was measured. This pH represented the $\mathrm{p}K_a$ of the gel at $\alpha = \frac{1}{2}$.

Reactivity Ratios

Information on the distribution of the sequence of each monomer and the composition of a copolymer in terms of each comonomer in the reaction mixture can be obtained by measuring reactivity ratios (7). Values for the reactivity ratios of acrylic acid and acrylamide were available in the literature. The values range from 0.36 to 1.73 and 0.05 to 1.38 for acrylic acid and acrylamide, respectively (8–10). Thus, it was difficult to use literature values and the reactivity ratios were measured independently in this study using the following equation:

$$\frac{f_1(1 - 2F_1)}{(1 - f_1)F_1} = \gamma_2 + \frac{f_1^2(F_1 - 1)}{(1 - f_1)^2F_1}\gamma_1 \tag{3}$$

where f_1 and f_2 are the mole fractions of the monomers M_1 and M_2 in the reaction feed ($f_1 + f_1 = 1$), and F_1 and F_2 represent the mole fractions of monomers M_1 and M_2 in the copolymer which is formed from the mixture ($F_1 + F_2 = 1$). From the titration data of the low-conversion linear polymers, the amounts of carboxyl groups in the copolymer was determined. This value was converted into the mole fraction of acrylic acid (F_1 value). Using Eq. (3), it is possible to calculate r_1 and r_2 from f_1 and F_1 in a straightforward fashion. From the r_1r_2 product, the copolymer structure can be inferred (7).

Equilibrium Swelling

The purified cross-linked polymers in sheet form were dried in the oven at 90°C until they reached constant weights. Swelling of the cross-linked polymers was monitored at 37°C by weighing the swollen gels. The swelling ratio *(q)* was calculated from the following equation:

$$q = \text{wet weight/dry weight} = W/W_o \tag{4}$$

The wet weight was obtained after the gel was manually blotted dry. This presented no difficulty when the initial concentration of cross-linking agent was above 1% or when an acidic medium was used. Below this cross-linking concentration, however, the gel was extremely difficult to handle at pH values above 5. In such cases, the swollen gel was retained in the nylon mesh and the weight was measured after blotting. The weight of the nylon mesh was computed in the final calculation. Swelling of most polymers reached an equilibrium state after 4 hr in an acidic condition and 8 hr at pH levels above 5. The swelling of various copolymers was measured at various pH values and ionic strengths in order to compare it with mucoadhesion data.

In Vitro Mucoadhesion Test

The force required to separate a polymer specimen from freshly excised rabbit stomach tissue was measured using a surface tensiometer (Model 215, Autotensiomat, Fisher) adapted for mucoadhesion measurements. The details of this apparatus were described earlier (2,3).

RESULTS

Titration of Acrylic Acid–Acrylamide Copolymers [P(AA-co-AM)]

The standard titration curves for linear PAA at different ionic strengths are shown in Fig. 1. In the figure, the titration curve for acrylic acid is also presented. As Arnold (11) pointed out, the pK_a of PAA rises smoothly with increasing ionization, and this is expected on purely electrostatic grounds. As the ionic strength of the solution was increased, the titration curves shifted to a lower region and showed well-defined curves. The sensitivity of the titration curves to the ionic atmosphere reflects interactions between fixed charges and counterions. Counterion binding should lead to a reduction in ΔG^{i}_{el} and the ionization of carboxyl groups should be easier. The easier deprotonation is shown by a shift of the titration curves to the lower pH region in the presence of added salt. The titration curves for other copolymers (Fig. 2) showed the same trend as that of PAA. It is seen that P(AA-co-AM) does not exhibit any conformational change during titration.

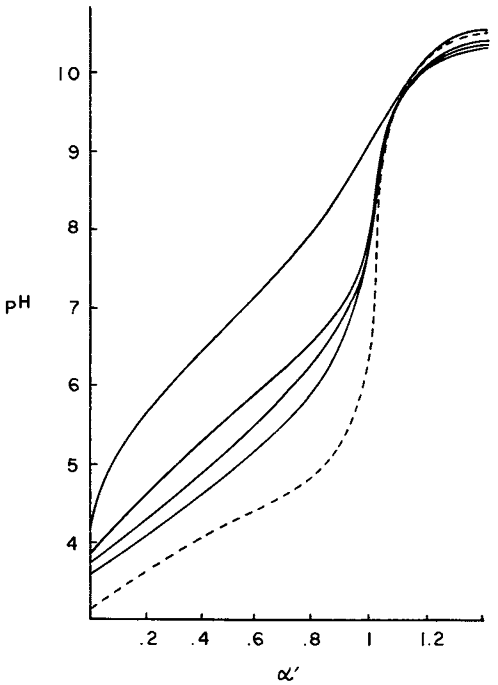

Fig. 1. Potentiometric titrations of poly(acrylic acid) at various ionic strengths. The concentrations of NaCl are 0, 0.05, 0.15, and 0.5 *M* from the top. Potentiometric titration of acrylic acid at zero ionic strength is shown by the dashed line. α' represents the added NaOH concentration divided by the carboxylic acid concentration of the polymer. α' = 1.0 indicates the end point of titration.

The pK_a of PAA was calculated to be 6.72 at zero ionic strength and it reduced to 4.88 at the ionic strength of 0.5 *M*. The literature pK_a values of PAA vary from 5.35 to 7.2 (5,6,12,13). Using Eq. (2), pK_a values at $\alpha = 0.5$ for various copolymers were calculated as plotted in Fig. 3. The pK_a of copolymers was monotonically increased as the F_1 value of the copolymers was increased up to 0.7. Above this value, no difference in pK_a was observed. The same trend was observed as the ionic strength was changed.

The pK_a values of the cross-linked copolymers are plotted in Fig. 4. A few points can be made by comparing the results of Figs. 3 and 4. The pK_a of cross-linked acrylic acid is 6.76, which is about the same as that of soluble PAA. As seen with the soluble polymers, the pK_a values increased

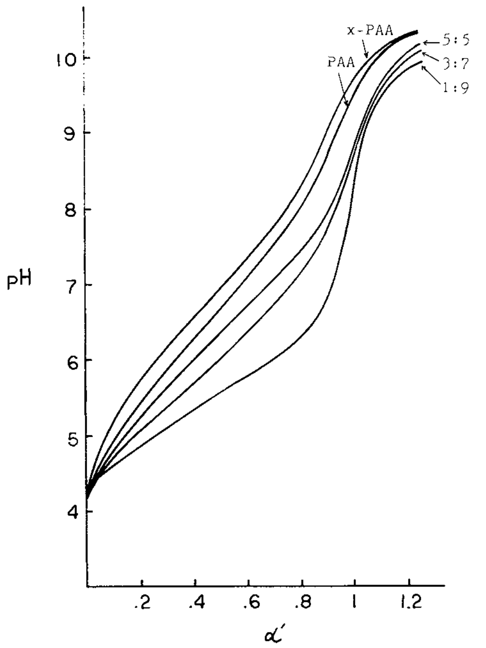

Fig. 2. Titration curves of linear P(AA-co-AM). Ratios indicate the comonomer composition (acrylic acid:acrylamide) in the feed. Curves for PAA and cross-linked PAA (x-PAA) are shown for comparison. α' represents the added NaOH concentration divided by the carboxylic acid concentration of the polymer.

as the content of carboxyl groups increased. The pK_a values of cross-linked polymers are consistently, although slightly, larger than those of linear polymers. Unlike the soluble PAA, the pK_a of the copolymers was monotonically increased as the F_1 value of the copolymers was increased only up to 0.5. In addition, the pK_a of cross-linked P(AA-co-AM) with f_1 = 0.1 at zero ionic strength (5.85) is significantly larger than 5.47, which is the pK_a of soluble P(AA-co-AM) with f_1 = 0.1 at the same condition. The slope of pK_a vs F_1 for cross-linked P(AA-co-AM) in Fig. 4 is less steep than that of soluble P(AA-co-AM) in Fig. 3. It appears that the deprotonation from the cross-linked P(AA-co-AM) may be different from that for soluble P(AA-co-AM). It may be possible, however, that the protons that are released from the carboxylic acid residues are trapped inside the gel and that entrapment makes further deprotonation more difficult (14). Titration curves for cross-linked PAA were also obtained by the discontinuous titration method, and the computed pK_a values were the same as those obtained by the back titration method. In any case, the titration data have provided information on the exact charge density of various copolymers in either soluble or cross-linked form. This allowed for investigation of the exact relationship between charge density and mucoadhesion.

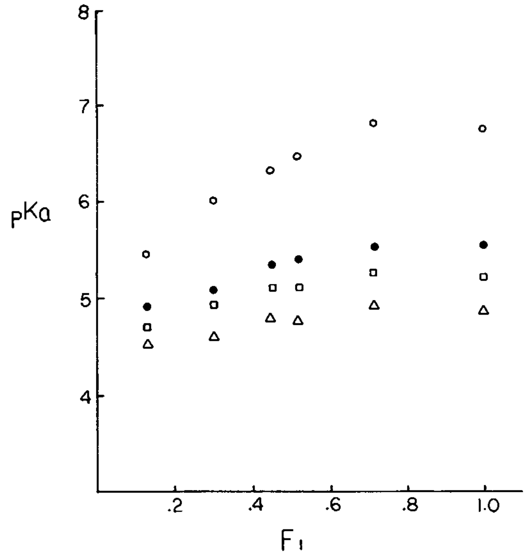

Fig. 3. pK_a (at the degree of dissociation α = ½) of linear P(AA-co-AM) with different acrylic acid contents at various ionic strengths. F_1 is the mole fraction of acrylic acid in the linear copolymer. The concentrations of NaCl are 0 *M* (○), 0.05 *M* (●), 0.15 *M* (□), and 0.5 *M* (△).

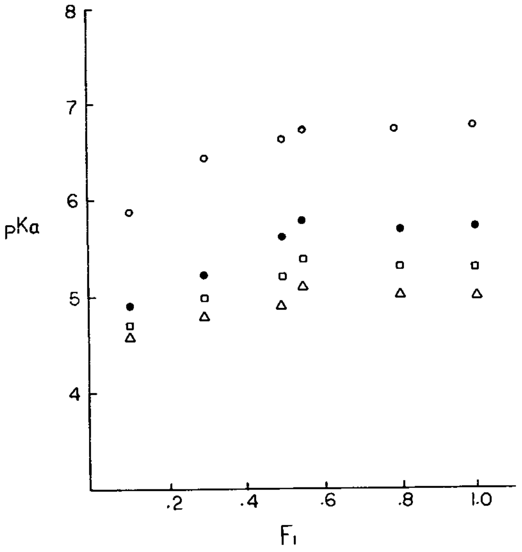

Fig. 4. pK_a (at the degree of dissociation α = ½) of cross-linked P(AA-co-AM) with different acrylic acid contents at various ionic strengths. F_1 is the mole fraction of acrylic acid in the cross-linked copolymer. The concentrations of NaCl are 0 *M* (○), 0.05 *M* (●), 0.15 *M* (□), and 0.5 M (△).

Reactivity Ratios of Copolymers

The density of carboxyl groups in the low-conversion copolymers was measured by potentiometric titration. The density was then converted into the mole fraction F_1 of the acrylic acid moiety in the copolymer and used to calculate reactivity ratios from Eq. (A). The data on f_1 (mole fraction of acrylic acid in the reaction feed) and F_1 (mole fraction of acrylic acid in the copolymer) are listed in Table II. The reactivity ratios were calculated from the data using Eq. (3). The values of r_1 and r_2 were 0.43 and 0.71, respectively. From the known relationship between copolymer structure and r_1r_2 product (7), it is clear that the P(AA-co-AM) copolymers produced here are not block copolymers, since neither r_1 nor r_2 is larger than 1. It also appears that the

Table II. F_1 and f_1 Values for Low-Conversion AA-AM Copolymers (F_1 and f_1 values refer to AA)[a]

Copolymer (AA:AM)	F_1	f_1	$\frac{f_1(1 - 2F_1)}{(1 - f_1)F_1}$	$\frac{f_1^2(F_1 - 1)}{(1 - f_1)^2 F_1}$
1:9	0.1	0.123	0.681	−0.088
3:7	0.3	0.302	0.562	−0.425
5:5	0.5	0.473	0.001	−1.114
6:4	0.6	0.501	−0.006	−2.241
8:2	0.8	0.699	−2.273	−6.870

[a] $[f_1(1 - 2F_1)/(1 - f_1)F_1] = 0.7139 + (0.4296)\,[f_1^2(F_1 - 1)/(1 - f_1)^2 F_1]$.

copolymers are not ideal, since $r_1 r_2$ is not equal to 1. For the case in which both r_1 and r_2 are less than unity, there is an increasing tendency toward alternating copolymerization. These data suggest that the acrylic acid–acrylamide system falls into the category of nonideal azeotropic copolymerization, which shows mixed tendencies between ideal and an alternating character (15). The results indicate that the charged groups in P(AA-co-AM) are probably fairly evenly distributed, although they are not perfectly random. Thus, it is safe to use these copolymers to study the effect of charge density on mucoadhesion. The same results were obtained with full conversion linear and cross-linked copolymers.

Equilibrium Swelling

The swelling ratios (wet weight/dry weight) of polymers were measured at various pH values ranging from 1.2 to 7.0 (Fig. 5). As expected, the swelling ratio was increased as a function of pH. At pH 7.0, swelling was increased linearly as the acrylic acid content increased until 0.6 mole fraction of copolymers. Above this value, the swelling increased more than a hundred times. The swelling of the copolymers at pH 1.2 is shown in Fig. 6. Figure 6 presents more useful information for studying mucoadhesion, since the actual mucoadhesion of the copolymers was measured at a gastric pH. It is noted that minimum swelling occurs when the mole fraction of acrylic acid is about 0.5. The fact that cross-linked polyacrylamide showed higher swelling than cross-linked PAA or copolymers implies that there may be an interaction between acrylic acid and acrylamide at this pH. The swelling ratio of cross-linked PAA is more than twice as large as that for other copolymers. At this point, it is necessary to calculate the actual density of carboxyl groups at the interface, since the same surface area was used to test mucoadhesion. From Fig. 6, the surface area expansion ratios of cross-linked PAA and cross-linked 5:5 P(AA-co-AM) are 4.5 and 2.3, respectively. Thus, the effective carboxyl-group densities of cross-linked PAA and cross-linked 5:5 P(AA-co-AM) in the swollen state should be the same. Therefore, a comparison of mucoadhesive properties of cross-linked PAA and cross-linked 5:5 P(AA-co-AM) should provide useful information on the role of the charge density on the polymer backbone, in addition to the effective charge density in a given volume.

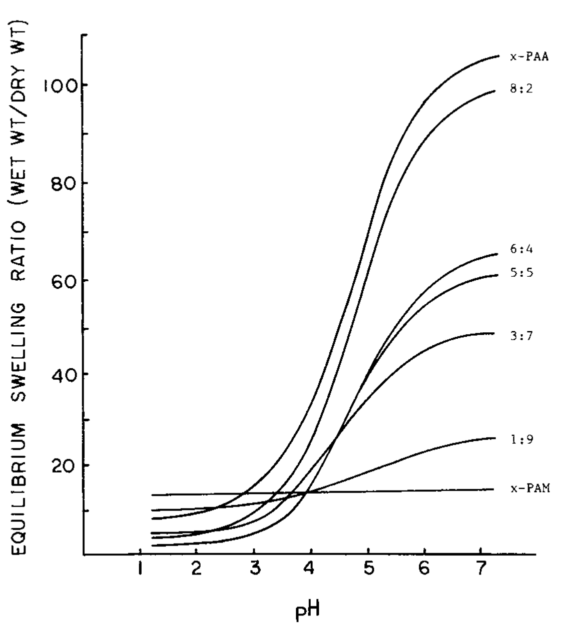

Fig. 5. Equilibrium swelling ratios of cross-linked P(AA-co-AM) at various pH values. The ratio of each curve indicates acrylic acid:acrylamide of the copolymer. The concentration of NaCl was 0.15 M. Equilibrium swelling ratios of cross-linked PAA (x-PAA) and cross-linked PAM (x-PAM) are shown as a comparison.

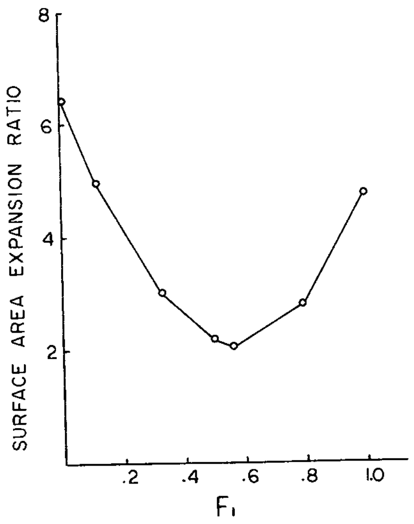

Fig. 6. Surface area expansion ratio of cross-linked P(AA-co-AM) at pH 1.2. The pH was adjusted with HCl and the concentration of NaCl was 0.15 M. The surface expansion ratio represents the surface area of a hydrated state divided by the surface area in a dry state.

To examine the role of chain flexibility in mucoadhesion, PAA was cross-lined with various concentrations of cross-linker. The data on PAA swelling at different cross-linking densities are plotted in Fig. 7. The swelling ratio of cross-linked PAA decreased almost linearly as the cross-linking concentration was increased. As swelling was increased, the effective carboxyl-group density in a given surface area was reduced. Since the same surface area was used to test mucoadhesion, the mucoadhesion results of various PAA with different cross-linking densities should provide information on chain flexibility.

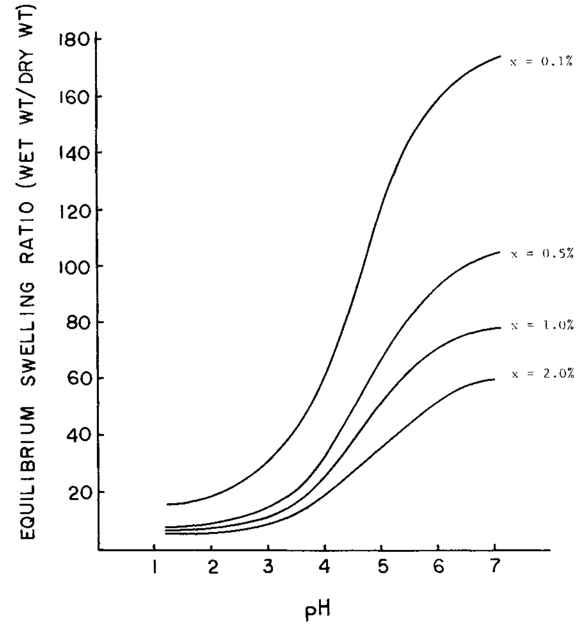

Fig. 7. Equilibrium swelling ratio of cross-linked PAA with different cross-linking densities. The concentration of NaCl was 0.15 M. x represents the weight percent of 2,5-dimethyl-1,5-hexadiene (cross-linking agent) per monomer in the feed.

Effect of pH in the *in Vitro* Mucoadhesion Test

The bioadhesive properties of synthesized cross-linked polymers were tested using rabbit gastric tissue. We have shown that the adhesion force of cross-linked PAA to the gastric tissue is a function of pH and the adhesion force drops sharply at a pH of about 4 (2). These data clearly suggest that mucoadhesion occurs only when carboxyl groups are in an acid form, and the role of adhesive bonding becomes insignificant when charge repulsion dominates at a pH higher than 6. Since mucoadhesion decreased sharply at pH's above 4, the fraction of protonated carboxyl groups at pH 4 was calculated. The α value at pH 4 was approximately 0.2 as obtained from potentiometric titration curves of cross-linked PAA which were measured by a discontinuous titration method. Thus, about 80% of the protonated carboxyl groups are necessary for favorable mucoadhesion. The effective carboxyl-group density is dramatically reduced at pH values greater than 6 because of swelling. As observed in Fig. 5, x-PAA swells more than 100 times above pH 6. Thus, it is also possible to suggest that the total number of PAA chains in a given surface area is lower at pH values above 6, which makes mucoadhesion less favorable, although the intrinsic mucoadhesion remains the same. To examine this possibility, the cross-linking density of cross-linked PAA was varied.

Effect of the Cross-Linking Density

The cross-linking density of cross-linked PAA is expected to influence mucoadhesion by changing the effective number of PAA chains in a given volume (chain density) and the mobility of PAA chains. It is very difficult to change only chain density while maintaining mobility, or vice versa. As a first step to this problem, however, the cross-linking density was changed while the same monomer concentration was maintained. The mucoadhesion data on cross-linked PAA at different cross-linking densities are shown in Fig. 8. It is clear that mucoadhesion decreases as the concentration of cross-linking agent is increased. When the concentration of cross-linking agent was increased from 0.1 to 0.5%, a sharp drop in mucoadhesion was observed. Above 0.5%, the drop in mucoadhesive ability was linear. This marked decrease from a cross-linking concentration of 0.1 to 0.5% deserves further consideration. The hydrogels with 0.1% cross-linking agent are extremely loosely cross-linked and show very flexible and elastic properties. Once this hydrogel adheres to the mucus layer, the adhesion is so tight that removal of the gel from the mucus layer is not possible using the automatic tensiometer, because the maximum force that can be generated by the device was only about 3g. When the gel was removed from the mucus layer by manual separation, the cross-linked gel was elongated and contact at the gel–mucus interface remained intact. Although an exact adhesion strength could not be measured, it was obvious that the strength was much larger than those obtained at a higher cross-linking density. As shown in Fig. 7, the increased cross-linking density resulted in lower swelling. The lower swelling at a higher cross-linking density should have resulted in a higher number of carboxyl groups in a given surface area used to test mucoadhesion. The reduced mucoadhesion, despite the increased number of carboxyl groups, suggests the importance of chain flexibility. Chains with a higher flexibility may create more depth of the interfacial region for contact and subsequently provide a better environment for entanglement between the adhesive polymer and the mucin molecules. It may also be true that increased flexibility increases the chance of interaction, which may require the right configuration between two interacting chains.

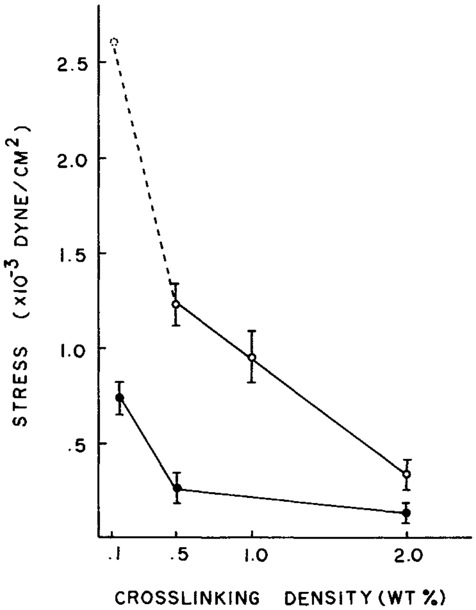

Fig. 8. Effect of the nominal cross-linking density on *in vitro* mucoadhesion of cross-linked PAA (○) and cross-linked P(AA-co-AM) (●). The nominal cross-linking density is presented as the weight percent of monomer in the feed.

Since the absolute number of carboxyl groups does not appear to be important, it may be said that mucoadhesion occurs only when carboxyl groups are in an acid form.

Effect of the Carboxyl-Group Density

After cross-linked P(AA-co-AM) were made, the exact mole fraction of acrylic acid in each copolymer was determined by titration as described earlier. The mucoadhesive properties of various copolymers are shown in Fig. 9. There was only a small decrease when the mole fraction of acrylic acid was reduced to about 0.8 but a dramatic decrease at 0.66. Before $F_1 = 0.66$, no difference in mucoadhesion was observed. As mentioned under Equilibrium Swelling, the effective carboxyl-group densities of cross-linked PAA and cross-linked 5:5 P(AA-co-AM) in the swollen state are the same. Yet the mucoadhesive property of the 5:5 copolymer is much less than that of the homo PAA. Thus, the total number of carboxyl groups in a given volume or surface area did not result in the same mucoadhesive strength. The adhesion strength of the 8:2 copolymer is statistically the same as that of the homo PAA. The steep decrease in adhesion suggests that the density of carboxyl groups on the polymer backbone has to be above a critical value to be mucoadhesive. The actual mole fraction of acrylic acid in the 8:2 copolymer was 0.78. Since mucoadhesion was measured at pH 1.2, practically all the carboxyl groups are present in the protonated form. The presence of 78% of the total functional groups in the protonated form is about the same as the value which was necessary for favorable mucoadhesion. It was calculated earlier that about 80% of the protonated carboxyl groups was necessary for favorable mucoadhesion. Since 80% appeared to be the critical carboxyl-group concentration, the sharp decrease in mucoadhesion observed with other copolymers of lower acrylic acid contents is understandable.

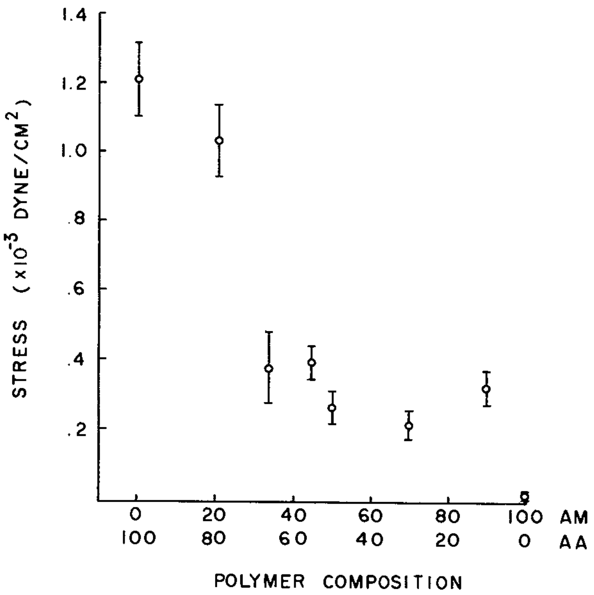

Fig. 9. Effect of the contents of acrylic acid (AA) and acrylamide (AM) on *in vitro* mucoadhesion of cross-linked P(AA-co-AM).

DISCUSSION

Mucoadhesive properties occur mainly because of the hydrogen bonding ability at acidic pH. The hydrogen bonding between PAA and mucin molecules can be directly tested using other spectroscopic techniques such as infrared (IR) spectroscopy (15,16). Although mucin glycoproteins possess a high percentage of carbohydrates, including sialic acid (17), the exact composition of the mucus layer and the structure of mucin molecules are not well understood. Because of this poor understanding of the nature of the mucus layer, it may be difficult to conduct spectroscopic studies. More information on mucin glycoproteins and the mucus layer and the separation of pure mucin glycoproteins is necessary for such studies.

Some polymers, such as poly(methacrylic acid) (11) and maleic acid copolymers with vinyl methyl ether and styrene (18), are reported to show a transition in the titration curve at a critical charge density. Such a transition has been interpreted to result from cohesion of the hydrophobic methyl or phenyl residues that resist expansion of the polymer chain until a critical charge density is attained. A transition in the titration curve can also be observed when a polymer such as poly(glutamic acid) undergoes a helix-coil transition (19). The fact that there is no transition in the titration curves of PAA (Fig. 1) implies that no conformational change of PAA occurs throughout the titration. This implies that there is no hydrophobic interaction, which is observed with polymethacrylic acid (11). Thus, it appears that the hydrophobic interaction between PAA and mucin molecules is not significant.

The concept of mucoadhesives was derived from the need to increase the gastric retention time of oral dosage forms. Although the mucoadhesives are expected to interact and adhere to the mucus layer of the stomach, it is still not clear whether such an adhesion can really occur in the presence of gastric juice, which includes soluble mucins and proteins. Soluble mucins and proteins have the same ability as the mucus layer to interact with the mucoadhesive. Thus, it is possible that the mucoadhesive interacts with soluble mucins or other proteins before it reaches the surface of the mucus layer. An effective mucoadhesive should be able to avoid various interferences which potentially inhibit its adhesion to the mucus layer. It is still not known to what extent soluble mucin molecules will affect adhesion to the mucus layer. This problem can be minimized by changing the shape or size of the mucoadhesive polymer. Even when mucoadhesives are successful in binding tightly to the mucus layer, the residence time of the mucoadhesives may be determined by the turnover time of the mucin molecules which remains unknown. The effect of mucin turnover in humans on the application of mucoadhesives as potential oral drug delivery systems remains to be determined.

REFERENCES

1. K. Park and J. R. Robinson. Int. J. Pharm **19**:107–127 (1984).
2. H. Park and J. R. Robinson. *J. Controlled Release* **2**:47–57 (1985).
3. N. A. Peppas and P. A. Buri. *J. Controlled Release* **2**:257–275 (1985).

4. A. Katchalsky. *J. Polym. Sci.* 7:393–412 (1951).
5. A. Katchalsky and P. Spitnik. *J. Polym. Sci.* 2:432–446 (1947).
6. M. Mandel. *Eur. Polym. J.* 6:807–822 (1970).
7. A. Rudin. *The Elements of Polymer Science and Engineering*, Academic Press, New York, 1982, pp. 266–270.
8. W. R. Carbaness, T. Y. C. Lyn, and C. Parkanyi. *J. Polym. Sci. (A-1)* 9:2155–2170 (1971).
9. G. Hardy and L. Nagy. *J. Polym. Sci. (C)* 16:2667–2673 (1967).
10. G. Smets and A. M. Hesbain. *J. Polym. Sci.* 40:217–226 (1959).
11. R. Arnold. *J. Colloid Sci.* 12:549–556 (1957).
12. A. M. Liquori, G. Barone, V. Crescenzi, F. Quadrifoglio, and V. Vitagliano. *J. Macromol. Chem.* 1:291–305 (1966).
13. H. L. Greenwald and L. S. Luskin. In R. L. Davidson (ed.), *Handbook of Water-Soluble Gums and Resins*, McGraw–Hill, New York, 1980, pp. 17-1–17-19.
14. S. Hattori. Ph.D. thesis, University of Utah, Salt Lake City, 1980, pp 36–49.
15. S. Nishi and T. Kotaka. *Macromolecules* 18:1519–1525 (1985).
16. O. E. Philippova, S. I. Kuchanov, I. N. Topchieva, and V. A. Kabanov. *Macromolecules* 18:1628–1633 (1985).
17. A. Allen. *Br. Med. Bull.* 34:28–33 (1978).
18. J. D. Ferry, D. C. Udy, F. C. Wu, G. E. Heckler, and D. B. Fordyce. *J. Colloid Sci.* 6:429–442 (1951).
19. A. Wada. *Mol. Phys.* 3:409–416 (1960).