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Factors Influencing Gel-strengthening at the Mucoadhesive-mucus Interface

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Abstract—Mechanical spectroscopy was used to examine some of the factors that may affect mucus gel strengthening: the effect of adding various concentrations of sodium chloride; mucoadhesive polymer molecular weight and its concentration; and the introduction of anionic, cationic and neutral polymers. A reduction in the storage modulus of the mucus/mucoadhesive mixture was observed with the introduction of sodium chloride. A poly(acrylic acid) with a molecular weight of 750 kDa gave the optimum mucus gel strengthening effect relative to other molecular weights. An anionic polymer was found to strengthen the mucus gel much more than a neutral or cationic polymer. It was proposed that the gel strengthening effect could be due to the formation of hydrogen bonded intermolecular complexes between the mucoadhesive and the mucus molecules. Furthermore, the complex formed is influenced by the ionic strength of the environment, and the molecular weight, nature and concentration of the mucoadhesive. In all cases the changes in the rheological properties of the mixes could be correlated directly to the strength of mucoadhesion reported in previous studies.

Bioadhesion is defined as the attachment of synthetic or biological macromolecules to a biological tissue (Peppas & Buri 1985). When applied to a mucosal epithelium, bioadhesive interactions occur primarily with the mucus layer and this phenomenon is referred to as mucoadhesion (Gu et al 1988). Mucoadhesive materials have been identified as being hydrophilic macromolecules possessing numerous hydrogen bond-forming groups (Chen & Cyr 1970; Gu et al 1988; Smart et al 1984).

Formation of a satisfactory adhesive bond between a bioadhesive polymer and the mucus gel can be examined in terms of the contribution of three regions, the surface of the bioadhesive polymer, the interfacial layer between the bioadhesive and mucosa, and the mucosal surface (Peppas & Buri 1985). The weakest component in the adhesive joint would be predicted to be the interfacial layer consisting (at least initially) of mucus. Mucus is a weak viscoelastic gel that lines the epithelium of the gastrointestinal, respiratory and genito-urinary tracts. The basic component of all mucus is the mucin glycoprotein which has an estimated mol. wt of 2–14×10^6 Da (Marriott & Gregory 1990). These glycoprotein molecules associate with each other by non-covalent interactions to form the gel matrix which is responsible for the rheological properties of mucus. It is inconceivable that strong mucoadhesion can occur without a considerable change in the rheological properties of this layer.

Duchene et al (1988) proposed the following stages in mucoadhesion. Initially, an intimate contact (wetting) between the mucus gel and the swelling bioadhesive polymer is required. This is followed by the penetration of the bioadhesive polymer into the mucus gel network, and finally the formation of secondary chemical bonds between the mucus and the mucoadhesive polymer. The importance of the surface free energy thermodynamics of mucus and the hydrated mucoadhesive polymers have been considered in other investigations (Lehr 1991; Lehr et al 1992).

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Previous work by Allen et al (1986) has shown a synergistic increase in the viscosity of gastric mucus glycoprotein by the addition of Carbopol 934P. An increase in viscosity of hog gastric glycoprotein following interaction with Carbopol 934P was also reported by Kellaway (1990) and found to be pH dependent with a maximum effect at pH 6·0. Kerr et al (1990) used mechanical spectroscopy to investigate the effect of pH and polymer chain length on the interaction between glycoprotein gels and poly(acrylic acid). In a previous study (Mortazavi et al 1992), significant mucus gel strengthening was observed on incorporating the mucoadhesive Carbopol 934P. This gel-strengthening phenomenon is thought to result in the formation of a stable mucoadhesive joint and this could explain the large forces required to detach the mucoadhesive dosage form from the mucosal surface. In addition, it was found that this gel-strengthening effect resulted from the formation of both chain entanglement and secondary chemical bonds between the mucoadhesive poly(acrylic acid) and the mucus glycoprotein (Mortazavi et al 1993). This investigation examines some of the factors affecting this mucus gel-strengthening phenomenon, namely the mol. wt, concentration and charge of various mucoadhesive polymers and the effect of introducing an electrolyte (sodium chloride).

Materials and Methods

Materials

Carbopol 934P (C934) (mol. wt 3000 kDa), Carbopol 910 (C910) (mol. wt 750 kDa) and Carbopol 907 (C907) (mol. wt 450 kDa) were obtained as a gift from BF Goodrich (Hounslow, UK). Other poly(acrylic acids) (mol. wts 5 kDa and 90 kDa), hydroxypropyl cellulose (HPC) (average mol. wt 1000 kDa) and polybrene were purchased from Aldrich Chemical Co. Ltd (Gillingham, UK), polyvinyl alcohol (PVA) (mol. wt 100 kDa) was obtained from Fluka Chemicals Ltd (Derbyshire, UK), sodium azide, sodium chloride (NaCl), sodium hydroxide (NaOH) and sodium edetate (disodium salt) were purchased from BDH Chemicals

(Poole, UK) and phenylmethylsulphonylfluoride (PMSF) was obtained from Sigma Chemical Co. Ltd (Poole, UK).

Preparation of homogenized mucus gels

Batches of homogenized mucus were prepared using the method of Mortazavi et al (1992). Crude mucus obtained by scraping 10–20 hog stomachs was homogenized by blending for 4 min with an equal quantity of an isotonic solution containing PMSF (0·0175%, w/v), sodium azide (0·02%, w/v), sodium edetate (0·186%, w/v) and NaCl (0·9%, w/v). The resulting mixture was centrifuged at 2500 *g* for 1 h at 1°C. The gel layers were removed from each centrifuge tube, pooled, exhaustively dialysed for 24 h at 4°C and finally homogenized by blending.

The percentage dry weight was determined for each batch by leaving a small portion (0·5 g) in a weighed open glass vial at 50°C for 48 h. If necessary, the percentage w/w of solids in the homogenized gel was adjusted with purified water to give a gel concentration of 3% w/w. An initial study indicated some batch-to-batch variation in mucus rheology, so the same batch was used for each set of comparative experiments.

Methods

Preparation of test samples. Ionic strength. Samples (100 mL) of 0·1, 0·5, 1·0, 5·0, 10 and 20% w/v NaCl solutions were prepared using freshly boiled and cooled purified water. Samples of homogenized mucus (1·5 g) were manually blended with an equal quantity of a 5 mg g^{-1} C934 (prepared in purified water) until a uniform mix was obtained. One-gram quantities of a NaCl solution were added to the mucus/C934 gel mixture and the pH adjusted to 6·2 using 0·1 M NaOH. The final weight of the sample was then adjusted to 4·5 g using purified water. A control sample was also prepared as before, by replacing the NaCl solution with purified water.

Mucoadhesive mol. wt and concentration. Fifty-gram samples of C934 (5 mg g^{-1}), C910 (0·75, 1·5, 2 and 5 mg g^{-1}), C907 (5, 30, 50 and 75 mg g^{-1}), poly(acrylic acids) (mol. wt 90 kDa, 5 mg g^{-1}; mol. wt 5 kDa, 5 mg g^{-1}) were prepared in purified water. Portions of homogenized mucus (1·5 g) were individually mixed with equal quantities of each mucoadhesive sample and the pH adjusted to 6·20 using 0·1 M NaOH. The final weight of each sample was then adjusted to 4·5 g using purified water.

As controls, mixtures containing 1·5 g of either mucus or each mucoadhesive sample were mixed with an equal quantity of purified water, adjusted to pH 6·20 and diluted to 4·5 g with purified water.

Mucoadhesive polymer comparison. Fifty-gram samples of C934 (5 mg g^{-1}), HPC (5 and 20 mg g^{-1}), PVA (5 and 100 mg g^{-1}) and polybrene (5 and 60 mg g^{-1}) were prepared in purified water. Portions of homogenized mucus (1·5 g) were individually mixed with equal quantities of each mucoadhesive sample and the pH adjusted to 6·20 using 0·1 M NaOH. The final weight of the mix was then adjusted to 4·5 g using purified water.

As controls, 1·5 g of either mucus or each mucoadhesive sample was mixed with an equal quantity of purified water. The pH was adjusted to 6·20 with 0·1 M NaOH and the final weight adjusted to 4·5 g using purified water.

Rheological studies. All samples were allowed to equilibrate at 4°C overnight and then at 15°C for 5 min before testing on a Carri-Med CSL 100 Rheometer using a 4-cm parallel plate with a 0·5 mm gap. The torque values corresponding to the linear viscoelastic region of each sample were determined at 1 Hz, and used in all further studies. The rheological behaviour was then evaluated using a frequency sweep of 10–0·1 Hz that had been previously determined to be representative of the mechanical spectrum of these mixes. The mean G′ ('the storage modulus', a measure of the resistance to elastic deformation which is a reflection of the extent of structuring within the sample) and G″ ('the loss modulus' which is a measure of the resistance to liquid flow, i.e. the viscous nature of the test sample) were then calculated.

Results

Visible breakdown of the mucus/C934 gel mixture was observed when incorporating 20% w/v NaCl concentrations. With 5 and 10% w/v NaCl concentrations, signs of breakdown were visible; nevertheless, samples could still be analysed. No visible breakdown in gel structure was observed with the other salt concentrations employed. A gradual decrease in G′ was observed with increasing salt

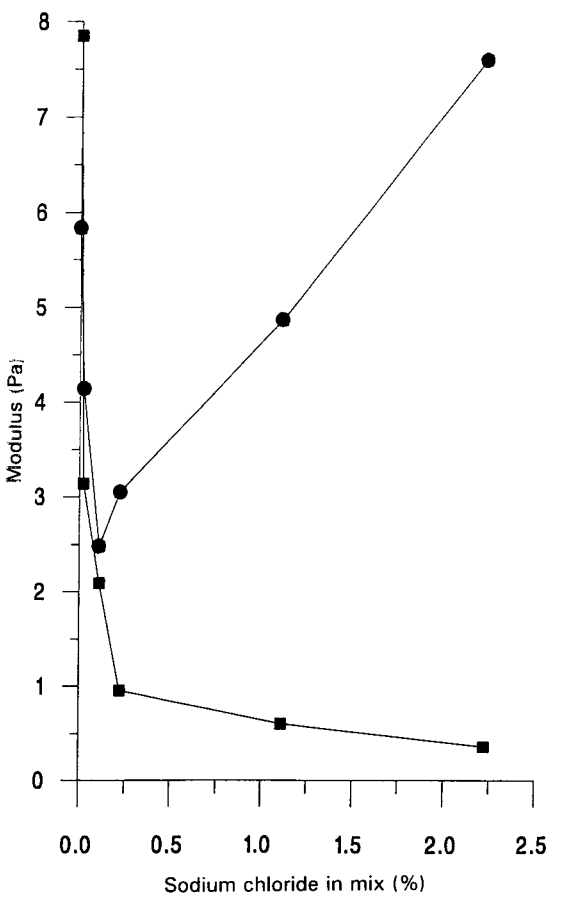

FIG. 1. The effect of NaCl concentration on the G′ (■) and G″ (●) values of mucus/C934 (5 mg g^{-1}) gel mixtures at pH 6·2

Table 1. A comparative rheological assessment of mucus/poly(acrylic acid) and water/poly(acrylic acid) controls using various mol. wts of polymer at a concentration of 5 mg g^{-1} and pH 6·20.

Sample	Mol. wt (kDa)	G′ (Pa)	G″ (Pa)
Mucus/C934	3000	30·77	7·59
Water/C934		2·18	4·39
Mucus/C910	750	35·14	9·45
Water/C910		28·68	13·08
Mucus/C907	450	1·47	2·01
Water/C907		0·46	1·16
Mucus/poly(acrylic acid)	90	0·50	0·53
Water/poly(acrylic acid)		0·30	0·37
Mucus/poly(acrylic acid)	5	0·50	0·35
Water/poly(acrylic acid)		0·38	0·23
Mucus/water		0·95	0·92

Table 2. A comparative rheological examination of different concentrations of C910 in mucus and water at pH 6·2.

Sample	C910 concn (mg g^{-1})	G′ (Pa)	G″ (Pa)
Mucus/C910	5	35·14	9·45
Water/C910		28·68	13·08
Mucus/C910	2	11·28	3·40
Water/C910		1·38	1·77
Mucus/C910	1·5	6·54	2·43
Water/C910		1·27	1·64
Mucus/C910	0·75	1·58	0·96
Water/C910		0·87	1·13
Mucus/water	0 (control)	0·95	0·92

Table 3. A comparative rheological examination of different concentrations of C907 in mucus and water at pH 6·2.

Sample	C907 concn (mg g^{-1})	G′ (Pa)	G″ (Pa)
Mucus/C907	75	11·91	17·51
Water/C907	75	5·12	11·27
Mucus/C907	50	6·61	10·66
Water/C907	50	2·67	6·26
Mucus/C907	30	3·43	5·90
Water/C907	30	1·37	3·47
Mucus/C907	5	1·47	2·01
Water/C907	5	0·46	1·16
Mucus/water	0 (control)	0·95	0·92

concentration (Fig. 1). Increasing the concentration of NaCl resulted in larger G″ values than the G′, suggesting the loss of gel properties and the formation of a more viscous liquid-like mucus/mucoadhesive mixture.

Similar G′ and G″ values to those obtained in previous work (Mortazavi et al 1992) were seen with C934 (Table 1). C910 gave a larger G′ value than C934, while lower mol. wt poly(acrylic acids) showed little effect at this concentration.

The mix containing C910 was found to give the greatest G′ value at a 5 mg g^{-1} gel concentration (Table 2), although the G′ value of C910 alone was close to that of the mix. At a concentration of 2 mg g^{-1}, a substantial increase in the G′ value of the C910/mucus mix was observed in relation to the C910/water control while at lower concentrations little effect was obtained.

Table 4. A comparative rheological assessment of C934, HPC, PVA and polybrene mixes with mucus and water at pH 6·2.

Sample	G′ (Pa)	G″ (Pa)
Mucus/C934 (5 mg g^{-1})	23·89	10·39
Water/C934 (5 mg g^{-1})	2·18	4·39
Mucus/HPC (5 mg g^{-1})	1·04	1·29
Water/HPC (5 mg g^{-1})	0·24	2·43
Mucus/HPC (20 mg g^{-1})	9·20	9·11
Water/HPC (20 mg g^{-1})	8·75	9·23
Mucus/PVA (5 mg g^{-1})	0·39	0·39
Water/PVA (5 mg g^{-1})	0·31	0·23
Mucus/PVA (100 mg g^{-1})	0·91	3·69
Water/PVA (100 mg g^{-1})	0·28	1·69
Mucus/polybrene (5 mg g^{-1})	0·58	0·50
Water/polybrene (5 mg g^{-1})	0·32	0·21
Mucus/polybrene (60 mg g^{-1})	0·21	0·29
Water/polybrene (60 mg g^{-1})	0·27	0·28
Mucus/water	0·43	0·42

A relatively small increase in G′ was observed with low C907 concentrations, but was much larger at higher concentrations (Table 3). All the C907/mucus samples were found to have higher G″ values than G′ and the formation of viscous mixtures rather than gels was observed in all cases.

The anionic C934 gave the greatest G′ value on mixing with mucus (Table 4). At the same concentration of 5 mg g^{-1} as C934, the neutral polymers, HPC and PVA, gave smaller G′ values. A 20 mg g^{-1} HPC gel produced a mucus/gel mixture almost half as strong as that of C934/mucus, but only a small increase in G′ was observed compared with the HPC/water control. No notable increase in G′ was observed even with relatively high PVA concentrations. Both the neutral polymers, HPC and PVA, gave substantial G″ values at higher concentrations which were found to be either equal to or greater than the G′ values. With the cationic polymer, polybrene, visible signs of gel breakdown were observed.

Discussion

In this study, as with previous work (Mortazavi et al 1992), mixing C934 with homogenized pig gastric mucus produces a strengthened gel network as indicated by the G′ values which are substantially greater than the sum of the G′ values obtained when mucus and C934 are evaluated separately at the same concentration. These findings could be extended to the in-vivo conditions, where the formation of a stable and strengthened mucus gel network would be critical in keeping the mucoadhesive dosage form in place. Previous work suggested that the gel network formed incorporates both mucus glycoprotein and the mucoadhesive polymer, and is held together by more than just physical entanglement of the macromolecules (Mortazavi et al 1993). It is known that macromolecules are capable of aggregating with each other in solution, resulting in the formation of intermolecular complexes which are observed as phase-separation pheno-

mena, such as precipitation, coacervation, emulsification, crystallization and gelation (Tsuchida & Abe 1982). These intermolecular complexes are formed as a result of secondary binding forces between the molecules which include electrostatic interactions (Coulombic forces), hydrogen bonds, van der Waal's forces, hydrophobic interactions and charge transfer complexes. Poly(acrylic acids) contain numerous proton-donating carboxyl groups with pK_a values between 5·35 and 7·2 (Park & Robinson 1987) and in the un-ionized form will form hydrogen bonds with proton-accepting groups (Tsuchida & Abe 1982; Bednar et al 1984). It would seem to be reasonable to assume that the rheological changes observed in these studies result from intermolecular complexes formed between the glycoprotein and C934 molecules as a result of hydrogen bonding, with parts of the glycoprotein molecule acting as the proton-accepting group. As both the glycoprotein and C934 (which has a pK_a of 5·79, determined by potentiometric titration) carry a negative charge at the pH values used in this study, the complex formed is unlikely to be due to electrostatic interactions. The stability of poly(acrylic acid) and poly(oxyethylene) complexes are reduced at higher pH values where most of the poly(acrylic acid) carboxyl groups are ionized (Bednar et al 1984). A similar effect would explain the reduction in the G′ value of the C934/mucus mixes at higher pH values (Mortazavi et al 1992).

The addition of NaCl to C934/mucus mixes substantially reduced the G′ value, particularly at higher concentrations. This may be due to the shielding of the negative charges arising from the presence of charged sugar residues, such as sialic acid or sulphated sugars, on the mucus glycoproteins (Allen et al 1984) resulting in a coiled and less expanded molecular conformation. The same explanation could be employed for the anionic C934 with ionizable carboxyl groups (Glavis 1962). Coiling of the macromolecules would be expected to reduce the stability and number of interactions by disrupting hydrogen bonding and limiting molecular entanglement. The increase in G″ at higher NaCl concentrations cannot, however, be explained by this, but probably results from the precipitation observed in these samples which adversely affects the rheological measurements. Increasing the ionic strength of the environment has been reported to reduce the strength of the mucoadhesive joint (Gu et al 1988; Lejoyeux et al 1989a).

C910, which is a cross-linked poly(acrylic acid) with an average mol. wt of 750 kDa, gave the largest G′ value when using concentrations of 5 mg g^{-1}. However, a 2 mg g^{-1} C910 gel resulted in a far larger G′ for the C910/mucus mixture compared with the C910/water control, in contrast to the other C910 gel concentrations studied. This suggests, as would be predicted, that a minimum polymer concentration is required for the formation of a gel (Ross-Murphy & McEvoy 1986). With higher C910 concentrations the G′ values for the mucus/C910 mix were larger but closer to that of the C910/water control. This implies that at higher concentrations, the C910 component has the major effect on the rheology of the mix, minimizing the contributions from mucus/C910 interactions. With C934, which is also cross-linked and has a larger mol. wt, a smaller G′ was observed for the mucus/polymer mix compared with C910. This finding may be explained in terms of the larger mol. wt of C934, resulting in the formation of interchain physical entanglements and hydrogen bonding between the C934 carboxyl groups. This would lead to the adoption of a coiled and entangled conformation which could hinder polymer diffusion into the mucus network as well as screening the potential bonding groups of C934. As would be expected, the shorter chain length poly(acrylic acids) with inherently poor gel-forming properties were found to be less effective in promoting gel strengthening even at high concentrations. A minimum critical chain length is also required for the formation of stable intermolecular complexes, which can be explained in terms of the co-operative nature of the interactions, since there is only a small difference in the stability of the macromolecule/solvent hydrogen bonds relative to the macromolecule/macromolecule hydrogen bonds, a larger number of inter-macromolecular interactions must form to produce a stable complex (Tsuchida & Abe 1982). The increase in G′ of the mucoadhesive/mucus mixes corresponds with the previous in-vivo and in-vitro work on mucosal-adhesion in which an increase in the adhesive strength has been obtained by increasing the mol. wt of the polymer above 100 kDa (Chen & Cyr 1970). Smart et al (1984), using an in-vitro test system, found with sodium carmellose that the optimum adhesive force occurred with mol. wts of approximately 78·6 kDa or greater. In addition, an in-vitro study by Lejoyeux et al (1989b) showed that the optimum detachment force and adhesion work were obtained with C910 and decreased progressively with rising poly(acrylic acid) mol. wt. Similar trends and interpretations have been observed with other polymers in bioadhesion studies (Gurny et al 1984).

Weaker gels were obtained with mixes containing the non-ionic polymers, HPC and PVA, at the same concentration of 5 mg g^{-1}. Increasing the concentration of HPC resulted in some increases in G′ but an almost insignificant effect was obtained with the lower mol. wt PVA. This suggests that these molecules are less able to form the interactions necessary for gelation. These materials differ from C934 in that they do not contain proton-donating carboxyl groups, therefore their ability to form hydrogen bonds is greatly reduced. The rank order of G′ values obtained with the mixes also correspond to the rank order of adhesiveness of these materials (Smart et al 1984; Longer & Robinson 1986) and could explain the lower mucoadhesive strength of the non-ionic polymers compared with the anionic polymers. The cationic polymer, polybrene, was found to produce little mucus gel strengthening, even at a high concentration. However, signs of mucus gel breakdown were observed, suggesting precipitation of an intermolecular complex resulting from electrostatic interactions (Tsuchida & Abe 1982).

It may be concluded that the incorporation of a mucoadhesive material into mucus produces a strengthened gel, mainly as a result of molecular entanglement and interaction in the form of hydrogen bonding. The literature describing intermolecular complex formation seems to be appropriate to explain the nature of these interactions. Furthermore, the same factors such as the ionic strength of the medium and mol. wt and charge of the mucoadhesive polymer, which affect the rheological properties of the mucus/mucoadhesive mixes, have also been shown in other studies to play an important role in mucoadhesion. This work provides further

evidence that rheological methods can be used to evaluate the nature of the interactions between a mucoadhesive macromolecule and a mucus gel, and suggests that molecular interpenetration may be an important factor in mucoadhesion by strengthening the mucus in the mucoadhesive/mucosal interfacial layer.

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