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An investigation of mucus/polymer rheological synergism using synthesised and characterised poly(acrylic acid)s

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Abstract

A range of poly(acrylic acid)s with different average degrees of polymerisation and cross-linking densities were synthesised using a solution polymerisation process. The rheological characteristics of aqueous dispersions of these materials and those of mixtures with homogenised pigs gastric mucus were investigated using dynamic oscillatory rheology, and compared to the known mucoadhesive Carbopol 934P. From the storage moduli, the rheological synergy and relative rheological synergy were calculated, and the effects of concentration and pH on this considered. Generally, the larger the molecular weight (and degree of cross-linking), the greater the rheological synergy, with Carbopol 934P giving the most pronounced effect. Rheological synergy was seen to be concentration-dependent, and a maximum concentration to produce an optimum effect was evident. Acid pHs were seen to favour synergy, although in marked contrast to previous literature reports, the optimum mucus–polymer interaction was not observed at the half ionised value ($pH = pK_a$) but at pH regimes that were unique to each polymer type. This could be influenced by the structural constrains imposed on potential hydrogen bonded interactions. It was concluded that synthesising poly(acrylic acid)s with better defined physicochemical properties than commercially available polymers will advance the study of the phenomenon of rheological synergy.

Keywords: Bioadhesion; Homogenised mucus; Mucoadhesion; Poly(acrylic acid); Rheological synergy

1. Introduction

Mucus, a weak viscoelastic gel whose major structural component is glycoproteinic in nature, is known to be capable of associating with certain polymers in the aqueous phase. Interpenetration, entanglement, and interactions at the functional

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group level often result in the formation of mixtures capable of exhibiting rheological synergy (Peppas and Buri, 1985; Caramella et al., 1999) that is, to demonstrate gel-like properties when mixed, greatly in excess of when the mucus and polymer gels are examined separately. Several authors have suggested that the study of the rheological profile of polymer–mucus mixtures provides an acceptable in-vitro model representative of the in-vivo behaviour of a mucoadhesive polymer. As a result, the rheology of many polymer–mucus systems has been investigated (Craig et al., 1994; Mortazavi et al., 1992; Rossi et al., 1995; Fuongfuchat et al., 1996; Madsen et al., 1998). However, it has been suggested that commercial freeze-dried mucins cannot be considered representative of natural mucus, and the use of homogenised mucus for such experiments has been recommended (e.g. Madsen et al., 1996; Zahm et al., 1989).

Polyacrylic acids are comparatively simple polymers largely consisting of a hydrocarbon backbone bearing a carboxyl group on alternate carbons, and can also be cross-linked (Riley et al., 2001). Several workers have investigated commercially available poly(acrylic acid)s using rheological techniques (e.g. Madsen et al., 1998; Mortazavi et al., 1992, 1993). The gel-strengthening effect has been found to be highly pH-dependent with an optimum polymer–mucus interaction occurring at the pK_a of the applied polymer. By examining the rheology of poly(acrylic acid)–mucus mixtures in which the extent of hydrogen bonding had been manipulated through the use of urea and potassium thiocyanate, Mortazavi et al. (1993) have suggested the role of such bonding in the development of rheological synergism.

As previous work has concentrated on often poorly characterised commercially available poly(acrylic acid)s, the aim of this study is to evaluate rheological synergism using polymers synthesised to allow a better definition of their properties. The effect of pH and concentration will also be considered, along with the mechanism of rheological synergism.

2. Experimental

2.1. Materials

Carbopol™934P was obtained as a gift from B.F. Goodrich (Cleveland, OH). Analytical grade monomers and solvents were obtained from Aldrich Chemical Company (Dorset, UK) and, unless otherwise stated, used as supplied.

Acrylic acid was passed through a molecular sieve column to remove the stabiliser (4-methoxyphenol, 0.02% v/v), and stored below 5°C as a solid (m.p. 13°C). The initiators were purified by recrystallisation, and solvents were degassed (by vacuum freezing, or boiling under nitrogen). All polymerisation reactions were performed under nitrogen.

2.2. Synthesis of poly(acrylic acid)s

Six polymers with differing average molecular-weight distribution profiles were synthesised.

2.2.1. Polymer 1

A solution of acrylic acid (10.00 g) in tetrahydrofuran (THF) (35 ml), contained in a round-bottomed flask fitted with a double surface condenser, was mixed with freshly recrystallised benzoyl peroxide (25 mg) and heated under reflux for 5 h (nitrogen atmosphere). The reaction mixture was condensed to half its volume by the removal of THF in the rotary evaporator. Subsequent addition of acetone (175 ml) resulted in the formation of an oil that settled out overnight. The acetone was decanted off, and this procedure was repeated twice. Residual solvent was removed by placing the highly viscous oil in the vacuum oven (50°C, overnight). The poly(acrylic acid) was isolated as a white foam (7.96 g).

2.2.2. Polymer 2

Freshly recrystallised benzoyl peroxide (25 mg) was added to a solution of acrylic acid (10.00 g) in degassed toluene (35 ml; nitrogen atmosphere). At 110°C, a violent reaction took place, resulting in the formation of a white solid. The crude product was recovered by filtration and dried in the vacuum oven. This solid was dissolved in the mini-

mum volume of hot ethanol. On addition of petroleum ether (40–60°C), an oil separated, which was dried to a foam (5.40 g) in the vacuum oven.

2.2.3. Polymer 3

To a stirred solution of acrylic acid (20.00 g) in degassed, distilled water (160 ml), maintained at 90°C under a nitrogen atmosphere, potassium persulphate (400 mg; freshly recrystallised from water) was added. Heating was discontinued after 2 h. The viscous gel that formed was cooled by placing the container in liquid nitrogen and finally, the solvent was removed by freeze-drying. The solid thus isolated was washed with ether (3×100 ml) and dried under vacuum, at 50°C, to yield the product as a white powder (16.89 g).

2.2.4. Polymer 4

Benzoyl peroxide (50 mg), freshly recrystallised from cold chloroform: methanol (1:2 v/v), was added to acrylic acid (10.00 g) contained in a two-neck round bottom flask fitted with a condenser and a nitrogen inlet tube. The reaction mixture was maintained at 100°C. A ''popcorn''-type reaction and the formation of a white solid signalled the end of the reaction. The cooled mixture was purified by repeated washings with acetone (3×100 ml) and dried under vacuum (50°C). The product (6.95 g) was obtained as a white solid.

2.2.5. Polymer 5

To a stirred solution of acrylic acid (50.00 g) in degassed, distilled water (50 ml), maintained at 60°C under a nitrogen atmosphere, potassium persulphate (50 mg; freshly recrystallised from water) was added. Heating was discontinued after 80 min. The viscous gel that formed was quenched in liquid nitrogen, and finally, the solvent was removed by freeze-drying. The solid thus isolated was washed with ether (3×100 ml) and dried under vacuum, at 50°C, to yield the product as a white powder (47.10 g).

2.2.6. Polymer 6

To a solution of acrylic acid (10.00 g) in degassed toluene (70 ml, nitrogen atmosphere), hydroxy-terminated ethylene glycol bis[pentakis-(glycidal allyl ether)] ether (20 μl, 0.01% w/v) was added, followed by benzoyl peroxide [0.200 g; freshly recrystallised from cold chloroform:methanol (1:2 v/v)]. At 110°C, a violent reaction, which resulted in the formation of a white solid, was observed. This product was recovered by filtration, washed with sodium-dried diethyl ether (3×100 ml), dried in a vacuum oven (50°C, 14 h) and isolated as a white powder (7.02 g).

2.3. Derivatisation of poly(acrylic acid)s for GPC work

To a round-bottomed flask containing poly(-acrylic acid) (1.00 g) in absolute ethanol (200 ml), concentrated aqueous H_2SO_4 (0.25ml) was added, and the resulting solution was heated under reflux for 14 h, after which time, the reaction mixture was condensed to 20 ml (rotary evaporator). Dropwise addition to water (300 ml) resulted in the formation of a white precipitate, which was isolated by filtration, washed with THF (3×5 ml) and dried in a vacuum oven at 70°C

2.4. Characterisation

Solution ^{1}H-NMR and ^{13}C-NMR spectra were recorded in D_2O using a Jeol GSX spectrometer operating at 270.05 and 67.80 MHz, respectively. Infra-red spectra (thin film; NaCl plates) were obtained using a Perkin Elmer Paragon 1000 FT-IR spectrophotometer. UV/Vis spectra were obtained employing a CECIL CE-1020-S series scanning spectrophotometer.

Molecular-weight distribution studies of the polymers were performed by gel-permeation chromatography in tetrahydrofuran (THF) solution using a Waters instrument equipped with a PHEONGEL 5 linear column (30 cm × 0.78 cm) and a differential refractometric detector. Measurements were carried out at 35°C with a flow rate of 1 ml/min. A calibration graph was obtained using a series of polystyrene standards (687 to 1.44×10^6 average molecular weight).

2.5. Preparation of samples for rheological studies

2.5.1. Preparation of the homogenised mucus

A batch of crude mucus was obtained by scraping 20 porcine stomachs (1 h after slaughter). Care was taken to avoid contamination from bile and excess food content. An isotonic solution containing sodium chloride (0.9% w/v), the bacteriostatic agent sodium azide (0.02% w/v), a chelating agent (ethylenediaminetetraacetic acid, 0.186% w/v) and protease inhibitor (phenylmethylsulphonyl fluoride, 0.0175% w/v, initially dissolved in 1 ml of absolute alcohol) was prepared and stirred for 12 h. Equal amounts of crude mucus and the isotonic solution were mixed and stirred for 4 min. The resultant mixture was centrifuged at $37\,500 \times g$ for 1 h at 4°C. The supernatant was discarded and the gel layers removed, pooled and exhaustively dialysed (12–14 kDa cut-off) against distilled water for 24 h at 4°C. The dialysed gel was centrifuged at $10\,000 \times g$ for 1 h at 4°C. The gel layers were collected, pooled and mixed for 5 min. Batches (1.6 g) were stored at -20°C for further use. Dry-weight analysis gave $8.14 \pm 0.3\%$ (w/w) solids ($n = 3$). Prior to each experiment, a sample of the homogenised mucus was examined to confirm that it behaved as a viscoelastic gel (Bell et al., 1982; Sellers et al., 1987).

2.5.2. Preparation of stock polymer gels

Solutions and gels were prepared by allowing a mixture of a carefully weighed amount of the powdered polymer in distilled water (60 ml) to stand overnight. After this time, the concentration of the mixture was adjusted to the predetermined value by the addition of appropriate volumes of distilled water. In order to ensure that the polymer was completely hydrated, samples were hermetically sealed and stored at 4°C for at least 7 days prior to each use. Samples that were more than 10 days old were discarded.

2.5.3. Preparation of polymer gels for rheology

The stock polymer gel (1.50 g) was mixed with water (1.50 g), adjusted to the required pH with aqueous sodium hydroxide solution (0.1–1 M) and the resulting sample diluted with water to 4.50 g.

2.5.4. Preparation of mucus for rheology

Homogenised mucus (1.50 g) was mixed with water (1.50 g), adjusted to the required pH with aqueous sodium hydroxide solution (0.1–1 M) and finally diluted with water to 4.50 g.

2.5.5. Preparation of polymer–mucus mixture for rheology

Homogenised mucus (1.50 g) was mixed with an equal quantity of the polymer gel (1.50 g). The pH was adjusted with aqueous sodium hydroxide solution (0.1–1 M) and allowed to equilibrate before addition of distilled water to a total weight of 4.50 g.

2.5.6. Preparation of a low concentration mucus gel for rheology

Homogenised mucus (0.15 g) in water (2.85 g), was adjusted to the required pH with aqueous sodium hydroxide solution (0.1–1 M) and made up to 4.50 g by the addition of further water to give a concentration one-tenth that of the gel used in the rest of this study.

2.5.7. Preparation of 10% mucus–polymer mixture for rheology

Homogenised mucus (0.15 g) in water (1.35 g) was mixed with stock polymer gel (1.50 g). The pH was adjusted with aqueous sodium hydroxide solution (0.1–1 M) and allowed to equilibrate before addition of water to a final weight of 4.50 g.

2.6. Rheological examination

Dynamic oscillatory rheometry was measured on a Carri-Med CSL 100 Rheometer coupled to a PC running Carri-med50 software. All rheological profiles were determined in triplicate, at 15°C, on a 4 cm diameter stainless-steel parallel plate with a 500 μm gap setting, as described by Mortazavi et al. (1993) and Madsen et al. (1998). Polymer dispersions, homogenised mucus and the polymer–mucus mixtures were prepared from the appropriate stock mixture, left at 4°C overnight, equilibrated at 15°C and used within 24 h. Freshly distilled water was used for all these experiments.

Initial experiments were carried out at pH 6.2, the approximate pK_a value of the polymers. All rheological studies were conducted in triplicate. Following sample preparation, each mixture was sealed and allowed to stand overnight at 4°C. Prior to each experiment, samples (1.0–1.5 g) were loaded onto the rheometer platform and left to equilibrate at 15°C for 10 min. This temperature was chosen to minimise edge effects (Ferry, 1970) and sample dehydration (Mortazavi et al., 1993; Madsen et al., 1998).

The first set of measurements involved a torque sweep at 1 Hz to determine the linear viscoelastic region of the sample. The desired torque value and corresponding displacement were selected from the middle of the linear viscoelastic region. A fresh sample was loaded onto the rheometer and a frequency sweep (0.1–10 Hz) was performed at the pre-selected torque and displacement values. The storage modulus, G' (a measure of the resistance to elastic deformation), loss modulus, G'' (a measure of the resistance to viscous flow), and tan δ values (a measure of the relative viscous to elastic properties, G''/G') of 20 data points across the frequency range were then determined.

All data were analysed using the 'Minitab13' package, by a one-way analysis of variance, followed by a Tukey Multiple Comparison Test for individual data sets.

3. Results and discussion

3.1. Chemical synthesis

Two general methods have been employed for the preparation of the poly(acrylic acid)s under consideration, namely: bulk and solution polymerisations. By controlling the reaction conditions, i.e. temperature, amount of initiator, solvent (type and concentration) and presence/absence of a cross-linking agent, a series of polymers with varying degrees of polymerisation and cross-linking densities were prepared.

3.1.1. Synthesis of poly(acrylic acid) in the bulk

The bulk polymerisation was initiated by benzoyl peroxide. The polymer was obtained as a white solid and could be readily freed from unreacted monomer by repeated washings with acetone. The structure of poly(acrylic acid) was confirmed by FTIR and NMR experiments. The infrared spectra of the polymers, as compared to that of the monomer, were characterised by a general broadening of all bands and the disappearance of the $\nu(C=C)$ absorption (1625 cm^{-1}) present in the monomer spectrum; the ($=CH_2$) stretch of the monomer, expected at 3060 cm^{-1}, was masked by the broad carboxyl ν(OH) absorption. In accord with expectations, the main change accompanying the ^{1}H-NMR spectra on polymerisation was the disappearance of the vinylic resonances at 6.2 and 6.4 ppm and their replacement by two broad signals at 1.8 and 2.5 ppm, respectively. A common feature, shared by all polymer samples, was the presence of two multiplets (at 2.8 and 4.5 ppm), attributed to dimer formation (Riley et al., 2001).

3.1.2. Synthesis of poly(acrylic acid) in toluene solution

This reaction resulted in the formation of the polymer as a white solid. Trace amounts of aromatic protons could be seen by ^{1}H-NMR (even after repeated precipitations from acetone), indicating that toluene may be entrapped within the polymer matrix.

3.1.3. Synthesis of poly(acrylic acid) in tetrahydrofuran

This method resulted in the formation of polymers that were highly soluble in the reaction medium.

3.1.4. Synthesis of poly(acrylic acid) in aqueous solution

The polymerisation of acrylic acid in aqueous solution was found to be a highly efficient method for the preparation of these polymers. Careful control of the reaction conditions yielded polymers with varying degrees of polymerisation and minimal dimer content. The polymer could be readily recovered by precipitation from acetone or

ethanol. An attempt to scale up the reaction (fivefold) was also successful, with this polymer exhibiting a molecular-weight distribution profile that was almost identical to that of the product from the small-scale reaction; the amount of dimer present was also similar.

3.1.5. Polymerisation in the presence of a cross-linking agent

The main criterion for this synthesis was the need to produce an ultra-high-molecular-weight material comparable to commercial, cross-linked polymers. Initial investigations were carried out in toluene or aqueous solution using ethylene glycol divinyl ether as the cross-linking agent, but the use of this compound did not prove effective as it required long reaction times and often yielded polymeric products exhibiting brown colouration. Ethylene glycol bis[pentakis (glycidal allyl ether)] ether (hydroxy terminated), a compound that possesses 10 possible cross-linking sites, provided a much better alternative. Reaction in toluene [1% (w/v) cross-linker to monomer] gave a fine white powder that was insoluble in water at the 3% (w/w) level. Reduction of the amount of cross-linker employed for the synthesis to 0.01% (w/v) gave a white powder that, in water at 3% (w/w), exhibited similar dissolution properties to commercial carbomers (i.e. a cloudy gel that turns colourless on addition of aqueous sodium hydroxide solution). Both the polyacid and esterified polymer were insoluble in THF and, hence, could not be subjected to GPC analysis; rheological profiling was used to estimate the average molecular weight of these materials (Riley et al., 2001).

3.2. Rheological profiles

The mechanical spectra obtained with these mixes showed G' and G'' values that were similar throughout the 0.1–10 Hz frequency range (e.g. Fig. 1); which is characteristic of a cross-linked, rather than entangled, system (Mortazavi et al., 1993). This frequency range was selected in view of its likely biological relevance, and because it allowed the experiment to be completed relatively quickly, thus further reducing the possibility of degredation during the procedure. The mean G', G'', and tan δ values at 5 Hz, averaged over three samples, were extracted from the resultant graph to allow comparisons between different materials. It is worth noting, however, that this approach does not describe the complete viscoelastic characteristics of each sample.

The widely utilised, and commercially available, mucoadhesive polymer Carbopol 934P was selected as the benchmark material. Rheological experiments were carried out at two Carbopol 934P-mucus concentration levels: 0.5 and 1% (w/w), respectively (Fig. 2).

The twofold increase in concentration of the polymer gel produces a 35-fold increase in the storage modulus and a 19-fold increase in the loss modulus. However, polymer–mucus mix measurements of G' and G'' only increase fourfold and threefold, respectively. Mixes were more gel-like than the aqueous preparations of the polymer or mucus alone, as indicated by the much lower tan δ and higher G' values at both concentration regimes considered. At the lower concentration of Carbopol 934, there may be insufficient polymer present to allow gelation to occur, hence the tan δ greater than 1.

A set of six of the synthesised poly(acrylic acid)s (Table 1; the reported GPC average molecular-weight values are those of the ester derivatives of these materials Riley et al., 2001) were selected for rheological studies. All the polymers produced synergistic effects when mixed with the homogenised mucus, with both G' and G'' increasing [Fig. 3(a)]. All polymers have tan δ values of

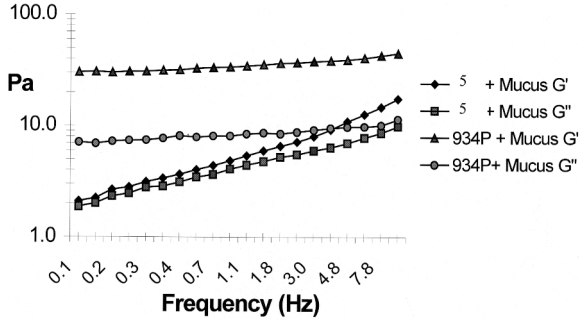

Fig. 1. Examples of 'typical' mechanical spectra for mucus–polymer (polymer 5 and Carbopol 934P) mixes (15°C, 0.1–10 Hz, pH 6.2).

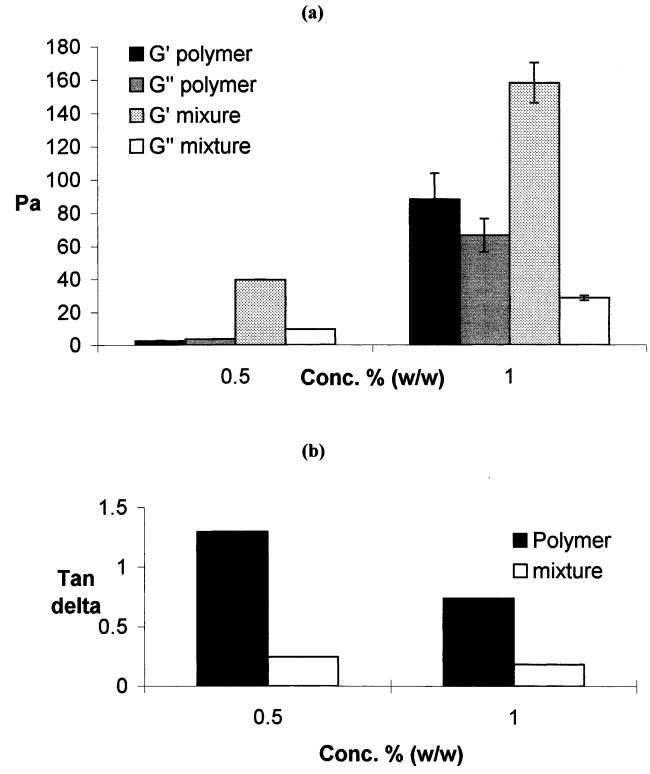

Fig. 2. Effect of concentration, on (a) the storage modulus (G') and loss modulus (G'') and (b) tan δ, for Carbopol 934 alone and when mixed with mucus at initial concentrations of 0.5 and 1% (pH 6.2, 5 Hz, $n = 3$, S.D. bars).

< 1, in accordance with their elastic nature [Fig. 3(b)]. Carbopol 934P, when mixed with mucus, exhibited a significant decrease ($P < 0.05$ Tukey's multiple comparison test) in the tan δ value; representative of an increase in the solid-like nature of the polymer–mucus interaction. In contrast, on mixing with mucus, polymers 1, 2, and 4 show small but insignificant ($P > 0.05$ Tukey's multiple comparison test) increases in tan δ values, perhaps reflective of a loss of the elastic structure.

The polymer–mucus G' and G'' values are most prominent in samples of Carbopol 934P and polymer 3. Interestingly, these are structurally different polymers: Carbopol 934P is an ultra-high-molecular-weight, permanently cross-linked, three-dimensional structure, whereas polymer 3 is a linear, low-average-molecular-weight (140 000) polymer with a polydispersity index of 1.6. To render the comparison of such polymers meaningful, the initial effect of the polymer gel strength has to be taken into account. An expression that allows for the determination of synergistic differences, in terms of G' and G'', between the mixture and the individual components of that mixture is given by Eq. (1) (Barnes et al., 1989; Madsen et al., 1998).

$$\text{Relative } G' = \frac{G'_{\text{Mix}} - (G'_{\text{Poly}} + G'_{\text{Mucus}})}{G'_{\text{Poly}} + G'_{\text{Mucus}}} \quad (1)$$

where: G'_{Mix} = mucus–polymer mixtures, G'_{Poly} = polymer, and G'_{Mucus} = mucus.

A similar equation can be used to calculate Relative G''.

Carbopol 934P (1% w/w), which exhibits the largest gel strength before mixing, can be compared to the synthesised polymers when the corresponding relative G' values are considered (Fig. 4). The largest increase in synergistic interactions with the mucus is that observed with Carbopol 934P at the 0.5% concentration level. Previous

Table 1
Poly(acrylic acid)s used for rheological assessment (from Riley et al., 2001)[a]

Polymer	Polymerisation solvent	Average molecular weight	Interaction with water
1	THF	12 300	Solution
2	Toluene	77 000	Solution
3	Aqueous	140 000	Solution
4	Bulk	154 000	Viscous solution/gel
5	Aqueous	2.9×10^6	Viscous gel
6	Toluene (cross-linked)	$10^6 < Mw < 10^9$	Viscous gel
Carbopol 934P	Commercial	3.5×10^9	Viscous gel

[a] Mw: estimated molecular weight from rheological measurements.

work (Madsen et al., 1998) has shown that an optimum polymer concentration for rheological synergy is evident, above which any synergy is masked by the rheological properties of the polymer alone. This would explain why the lower concentration of polymer showed the greatest synergy, while having smaller loss and storage moduli. By contrast, freshly mixed samples of polymer 3 and mucus exhibited small synergistic interactions; after mixing and overnight storage, such samples showed some phase separation. All other polymers, with the exception of polymer 5, showed no significant rheological synergy at this concentration ($P > 0.05$, Tukey's multiple comparison test). Since polymer 5 exhibited a good affinity for mucus, forming homogeneous samples, this material was selected to study the effects of concentration.

With the high-molecular-weight polymer 5, an increase in G' and G'' values with increasing concentration of polymer was observed [Fig. 5(a)]. When considering relative G' and G'' versus concentration, in agreement with the work of Madsen et al. (1998), there appears to be an optimum concentration for rheological synergy (at pH 6.2), in the range 3–5% (w/w) [Fig. 5(b)]. However, the large standard errors meant that most of these points were not significantly different at the 5% level (Tukey's Multiple comparisons test). The corresponding tan δ plot shows an erratic profile, but there appears to be a plateau above the 3 (w/w) concentration level [Fig. 5(c)].

Four poly(acrylic acid)s were selected for the investigation of the effects of the average molecular weight:

1. low molecular weight, linear polymer 3;
2. high molecular weight, linear polymer 5;
3. cross-linked, ultra-high molecular weight polymer 6; and
4. commercial, cross-linked polymer, Carbopol 934P.

The three synthesised polymers, at 3% (w/w) concentration, and Carbopol 934P, at 0.5% (w/w),

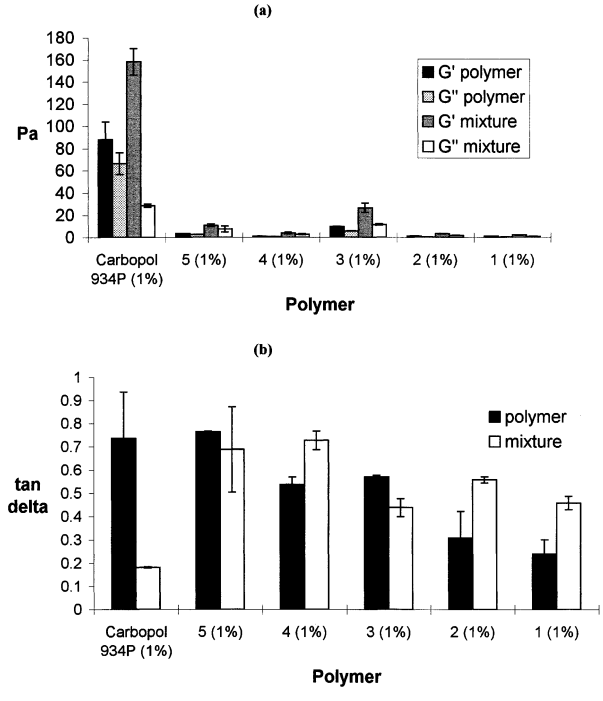

Fig. 3. (a) Rheological properties of selected commercial and synthesised polymers and their corresponding mucus-polymer mixtures (pH 6.2; 5 Hz; $n = 3$, S.D. bars); (b) corresponding tan δ values.

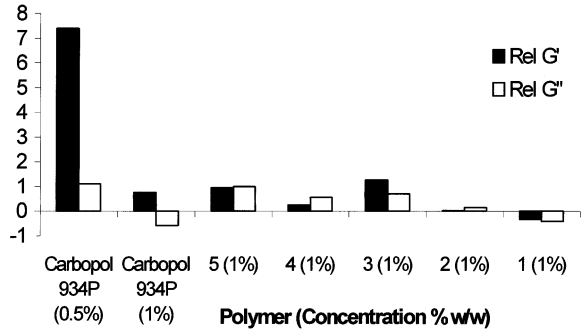

Fig. 4. Relative G' and G'' of selected commercial and synthesised polymers.

were studied over the pH range 3.3–7.3. Results are presented in terms of polymer, mucus, and polymer–mucus mixture rheological profiles.

When the linear low-molecular-weight polymer 3 was mixed with mucus at pH 3.3, a degree of heterogeneity was observed. Both G' and G'' exhibited statistically significant maxima at pH 4.3 ($P < 0.05$ Tukey's Multiple Comparison test), relative to the other pH measurements, consistent with strong viscoelastic interactions (Fig. 6). The polymer–mucus mixture at this pH exhibits significantly larger G' and G'' values ($P < 0.05$ Tukey's multiple comparison test) than that expected from the simple summation of the two components.

It is envisaged that at a low pH, carboxylic acid groups present are able to hydrogen bond resulting in labile intermolecular cross-links. As the pH is increased, the cross-linking hydrogen bonds are replaced by ionic interactions, resulting in a destruction of the hydrogen-bonded network. Thus, mucus–polymer mixtures have less structure and progressively adopt a more viscous behaviour.

High-molecular-weight polymer 5 (Fig. 7) yielded a degree of heterogeneity on mixing with mucus at pH 3.3, but at higher pH values, the samples became homogeneous. Decreases in both G' and G'' were observed with increasing pH. In the low-pH region, the observed G' and G'' of the polymer–mucus mixture are significantly greater than at higher pH values ($P < 0.05$, Tukey's multiple comparison test) and are consistent with large synergistic interactions.

When this material is in a low pH environment, the hydrogen bonding interactions would be predicted to be optimum due to the presence of mainly uncharged carboxyl groups. The behaviour is therefore elastic, although precipitation of the mucus–polymer complex may also occur. Conformation changes (coiling up of the molecule

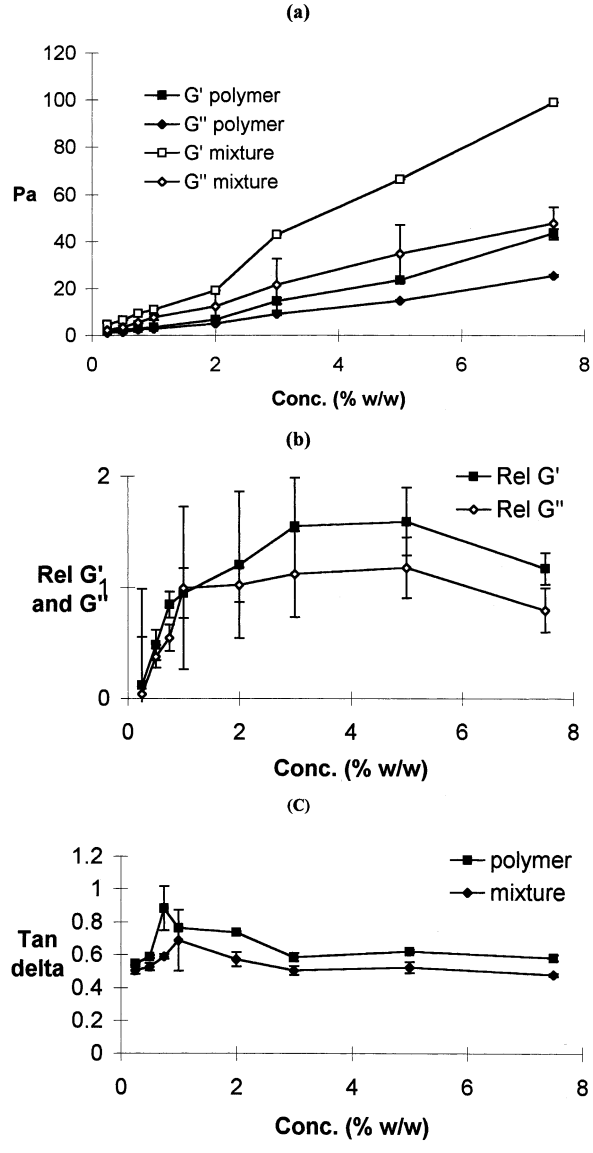

Fig. 5. (a) G' and G'' of polymer 5 gels and mucus–polymer 5 mixtures as a function of concentration (pH 6.2; 5 Hz; $n = 3$, S.D. bars); (b) corresponding values for Relative G' and G''; (c) corresponding values for tan δ.

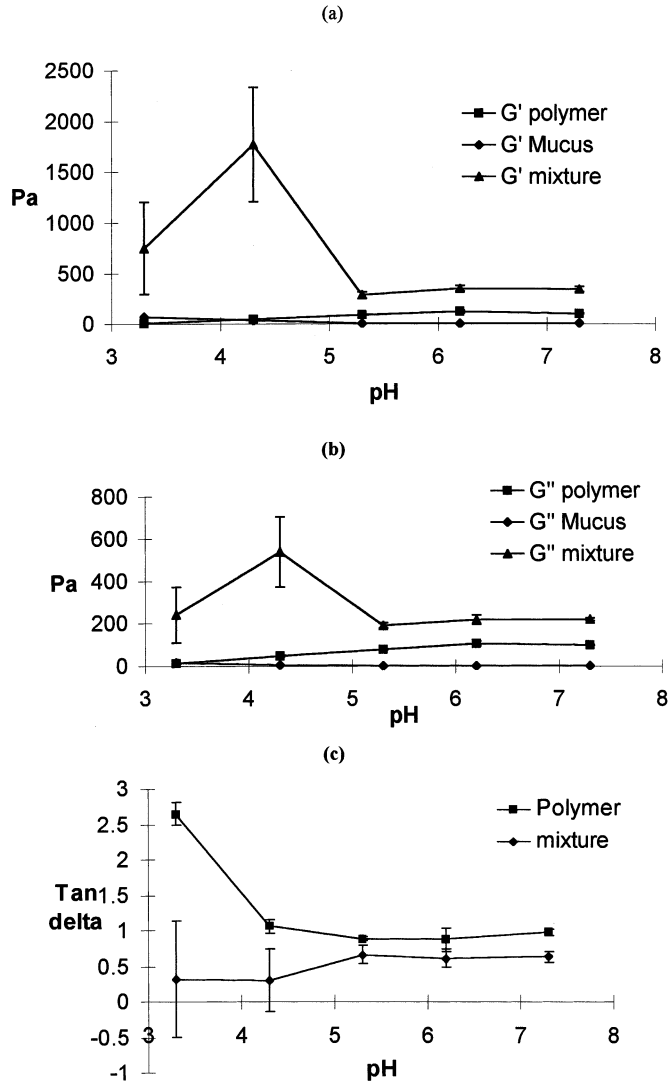

Fig. 6. (a) G' as a function pH for: polymer 3, mucus, and mucus–polymer 3 mixture ($n = 3$; 5 Hz); (b) G'' as a function pH for: polymer 3, mucus alone and mucus–polymer 3 mixtures (5 Hz, $n = 3$, S.D. bars); (c) corresponding tan δ values.

with low ionic charges) might also have an effect. At higher pH values, molecular ionisation increases, resulting in the reduction of hydrogen bonding sites and hence the degree of elasticity. However, the tan δ values do not show a clear trend throughout the pH range.

Ultra-high-molecular-weight polymer 6 (Fig. 8) shows a similar profile to that of polymer 5. However, at a low pH, the larger values of G' and G'' indicate stronger interactions with mucus. As the pH increases to 6.2, the synergistic effect decreases to a minimum, and the mixture rheology approaches that of the pure polymer.

Because of its permanently cross-linked structure, this polymer exhibits a good hydrogen bonding capability at a low pH, as reflected by the high relative G' and G'' values. At a higher pH, the system ionises, and hydrogen bonding is lost. However, the tan δ values do not differ significantly throughout the pH range ($P > 0.05$, one way analysis of variance)

When Carbopol 934P was used, both the G' and G'' plots show decreased values with increasing pH of the mix. The low values of G' and G'' reflect the concentration level of the polymer (0.5% w/w) (Fig. 9), and this may explain the somewhat unexpected tan δ values (showing a maximum value at pH 5.8) for Carbopol 934P in water.

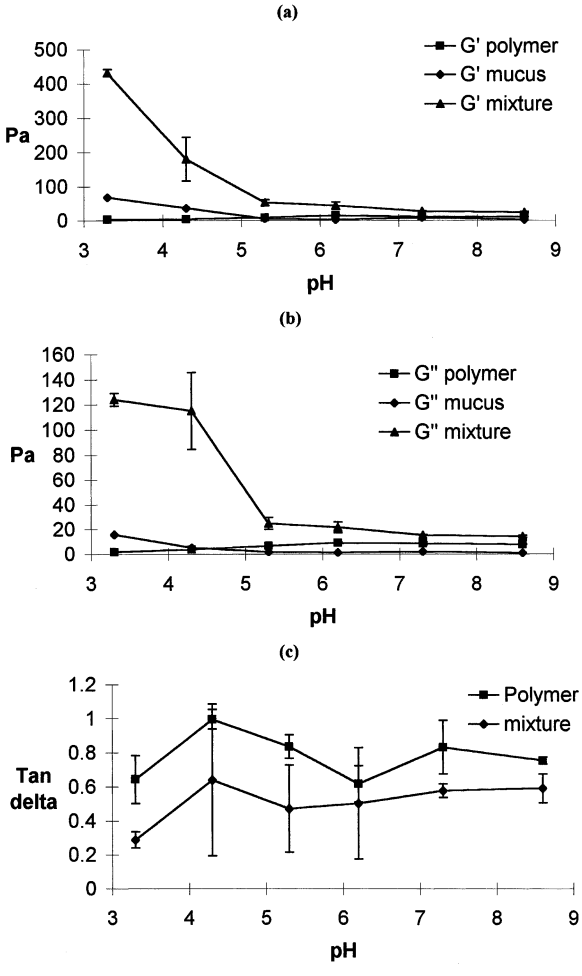

Fig. 7. (a) G' as a function pH for: polymer 5, mucus, and mucus–polymer 3 mixture (5 Hz $n = 3$, S.D. bars); (b) G'' as a function pH for: polymer 5, mucus alone and mucus–polymer 5 mixtures (5 Hz $n = 3$, S.D. bars); (c) corresponding tan δ values.

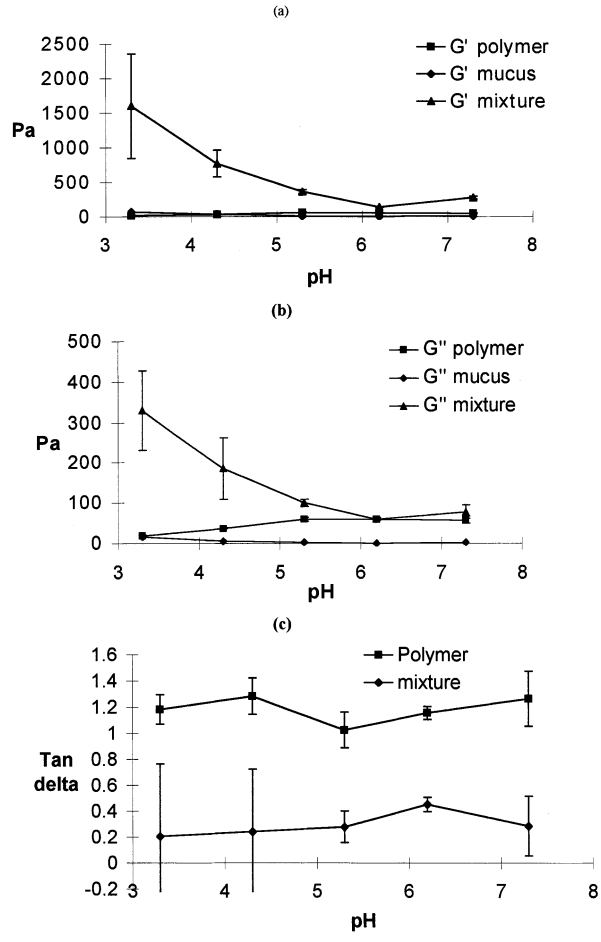

Fig. 8. (a) G' as a function pH for: polymer 6, mucus, and mucus–polymer 6 mixture (5 Hz $n = 3$, S.D. bars); (b) G'' as a function pH for: polymer 6, mucus alone and mucus–polymer 6 mixtures (5 Hz $n = 3$, S.D. bars); (c) corresponding tan δ values.

3.2.1. Comparison of rheological profile in terms of relative G′ and G″ results

Comparison of the rheological profiles (Fig. 10) using the relative values of G' and G'' reveals marked differences in profiles between the polymers. At a low pH, polymer 3 exhibits a relatively large elastic response that may be due to the heterogeneous nature of the mixture. Above pH 5.8, the same polymer shows a significant reduction ($P < 0.05$ Tukey's Multiple Comparison Test) in relative G', indicating weaker interactions with the mucus. Polymer 5 shows a fairly flat elastic

response throughout the pH range; the viscous nature of this polymer is most apparent at low pH. Finally, polymer 6 gives a viscoelastic response that is observed to abate with increasing pH above 4.2 ($P < 0.05$ Tukey's Multiple Comparison Test).

As the gastric mucus gel layer at ulcerated sites is believed to be depleted (Allen et al., 1997) an experiment was performed for the purpose of simulating the interactions between a 90% dilution of the homogenised mucus gel with polymer 5. A mix containing Carbopol 934P was also considered for comparison.

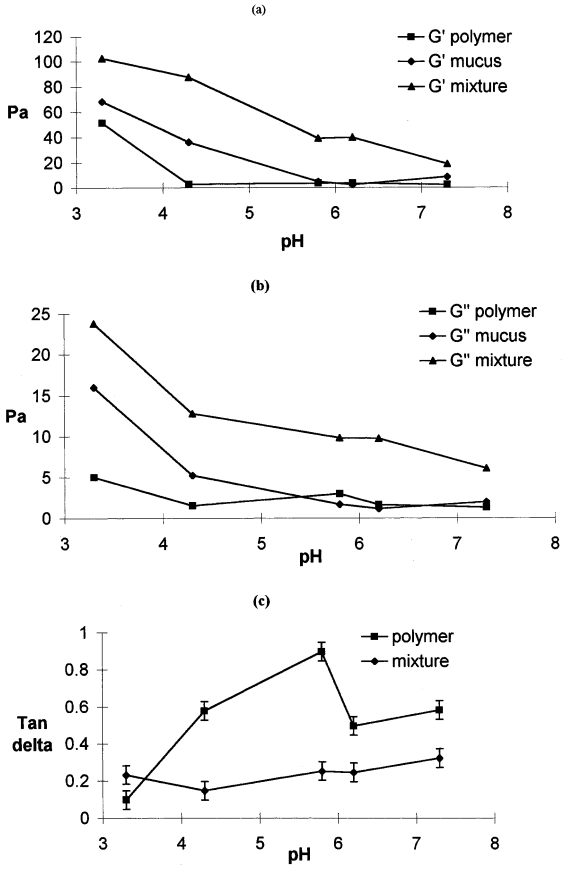

Fig. 9. (a) G' as a function pH for: Carbopol 934P, mucus, and mucus–Carbopol 934P mixture (5 Hz $n = 3$, S.D. bars); (b) G'' as a function pH for: Carbopol 934P, mucus alone and mucus–Carbopol 934P mixtures ($n = 3$; 5 Hz); (c) corresponding tan δ values.

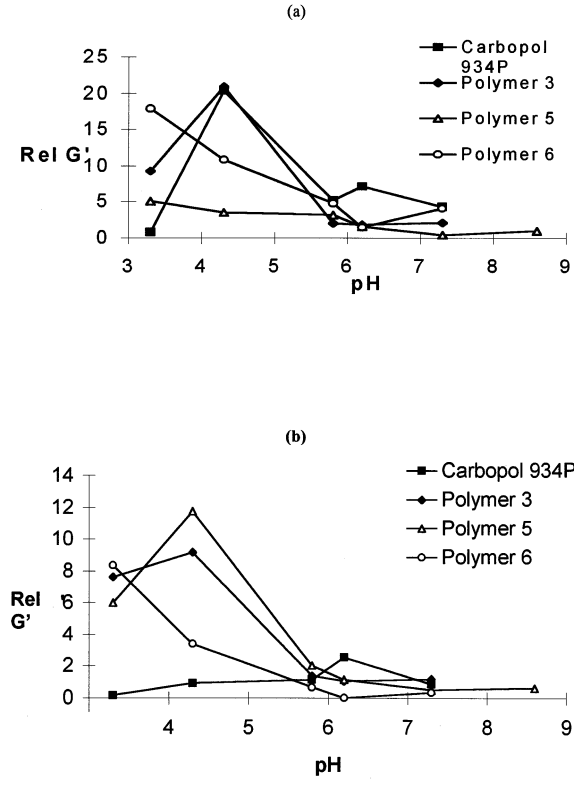

Fig. 10. (a) Relative G' values of polymer–mucus mixtures between pH 3 and pH 9 ($n = 3$, 5 Hz); (b) corresponding relative G'' values.

Mucus gel, diluted with water to 10% of its original concentration, resulted in a small but insignificant ($P > 0.05$ Tukey's multiple comparison test) decrease in G' values relative to the polymer–undiluted mucus mixture for Carbopol 934P and polymer 5 (Fig. 11). The tan δ results indicate that Carbopol 934P changes to a more elastic structure on interaction with both the diluted and undiluted mucus, whereas the polymer 5–mucus mixture shows no significant differences ($P > 0.05$, Tukey's multiple comparison test), in terms of tan δ, when compared to the polymer alone. The results provide some evidence of the potential of such polymers to interact with mucus even in areas with a reduced mucus layer.

4. Conclusions

A range of poly(acrylic acid)s with different average degrees of polymerisation and cross-linking densities have been synthesised for the purpose of examining the effects of these structural features on the rheological characteristics of aqueous dispersions of these materials, as well as those of mixtures with homogenised mucus. The higher-molecular-weight polymers exhibited a rheological synergy when mixed with mucus, but not to the same extent as Carbopol 934P. In marked contrast to previous literature reports (e.g. Madsen et al., 1998), an optimum mucus–polymer interaction was not observed only at the half ionised value [where the pH is numerically equal to the pK_a, 6 ± 0.5 for these polymers (Riley, 1999)] but at pH regimes unique to each polymer type. These would be predicted to be influenced by the structural constrains imposed on potential hydrogen bonded interactions. It was concluded that synthesising poly(acrylic acid)s with better defined physicochemical properties than commercially available polymers will advance the study of

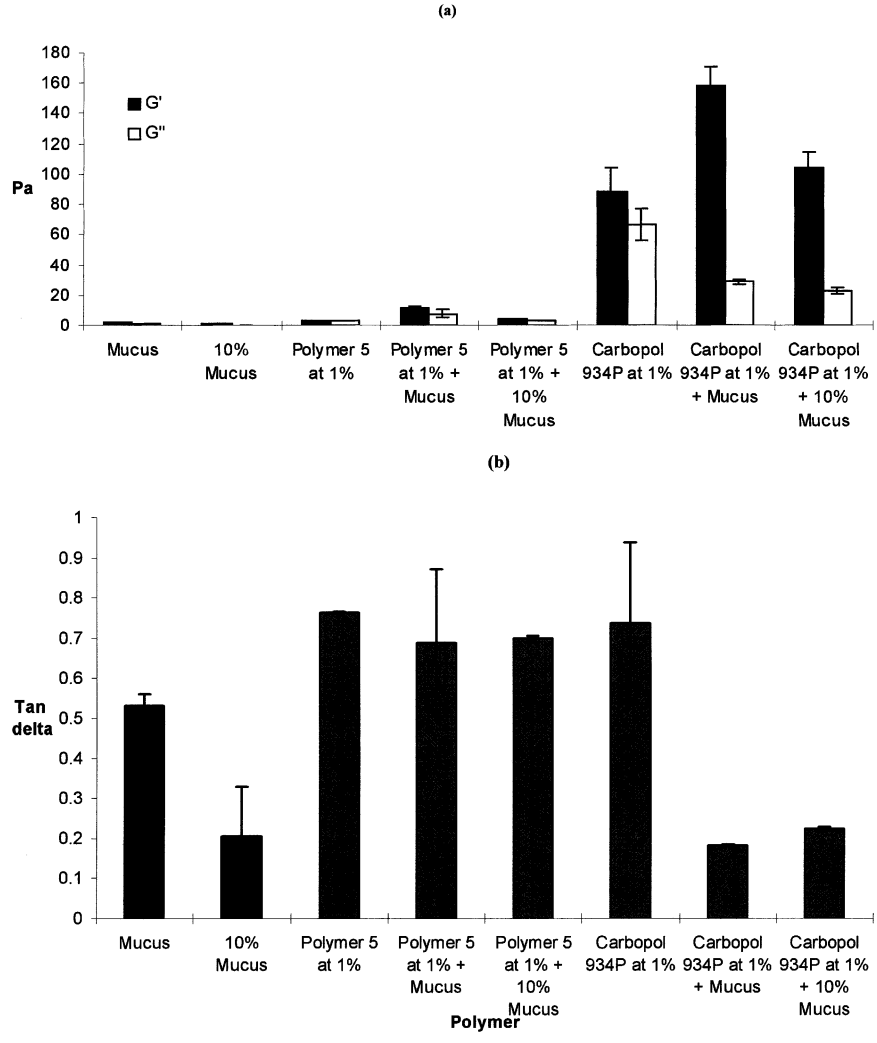

Fig. 11. (a) Effect of mucus dilution (10% of original concentration) on the G' and G'' of polymer–mucus mixtures (pH 6.2, 5 Hz, $n = 3$, S.D. bars); (b) corresponding effect on the tan δ values.

the phenomenon of rheological synergy, and will allow its relevance to the bioadhesion process to be evaluated further.

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References

Allen, A., Newton, J., Oliver, L., Jordan, N., Strugala, V., Pearson, J.P., Dettmar, P.W., 1997. Mucus and *H. pylori*. J. Physiol. Pharmacol. 48, 297–305.

Barnes, H.A., Hutton, J.F., Walters, K., 1989. An Introduction to Rheology. Elsevier Science, Amsterdam.

Bell, A.E., Allen, A., Morris, E., Rees, D.A., 1982. Rheological studies on native pig gastric mucus gel. In: Chantler, E.N., Elder, J.B., Elstein, M. (Eds.), Mucus in Health and Disease II, vol. 144. Plenum Press, New York.

Caramella, C.M., Rossi, S., Bonferoni, M.C., 1999. A rheological approach to explain the mucoadhesive behaviour of polymer hydrogels. In: Mathiowitz, E., Chickering, D.E., Lehr, C.M. (Eds.), Bioadhesive Drug Delivery Systems, Fundamentals, Novel Approaches and Development. Marcel Dekker, New York, pp. 25–66.

Craig, D.Q.M., Tamburic, S., Buckton, G., Michael-Newton, J., 1994. An investigation into the structure and properties of Carbopol 934P gels using dielectric spectroscopy and oscillatory rheometry. J. Contr. Release. 30, 213–223.

Ferry, J.D., 1970. Viscoelastic Properties of Polymers, second ed. John Wiley, New York.

Fuongfuchat, A., Jamieson, A.M., Blackwell, J., Gerken, T.A., 1996. Rheological studies of the interaction of mucins with alginate and polyacrylate. Carbohydr. Res. 284, 85–99.

Madsen, F., Eberth, K., Smart, J.D., 1996. A rheological evaluation of various mucus gels for use in in-vitro mucoadhesion testing. Pharm. Sci. 2, 563–566.

Madsen, F., Eberth, K., Smart, J.D., 1998. A rheological examination of the mucoadhesive/mucus interaction: the effect of mucoadhesive type and concentration. J. Contr. Release 50, 167–178.

Mortazavi, S.A., Carpenter, B.G., Smart, J.D., 1992. An investigation of the rheological behaviour of the mucoadhesive/mucosal interface. Int. J. Pharm. 83, 221–225.

Mortazavi, S.A., Carpenter, B.G., Smart, J.D., 1993. A comparative study on the role played by mucus glycoproteins in the rheological behaviour of the mucoadhesive/mucosal interface. Int. J. Pharm. 94, 195–201.

Peppas, N.A., Buri, P.A., 1985. Surface interfacial and molecular aspects of polymer bioadhesion on soft tissues. J. Contr. Release. 2, 257–275.

Riley, R.G., 1999. Sythesis and characterisation of poly(carboxylic acid)s and their evaluation as gastrointestinal mucoadhesive agents. Ph.D. thesis, University of Portsmouth.

Riley, R.G., Smart, J.D., Tsibouklis, J., Dettmar, P.W., Davis, J.A., Wilber, W.R., 2001. The synthesis of radiolabelled analogues of the carbomers: 14C-labelled poly(acrylic acid)s. J. Biomed. Mater. Res. (Appl. Biomater.) 58, 102–107.

Rossi, S., Bonferoni, M.C., Lippoli, G., Bertoni, M., Ferrari, F., Caramella, C., Conte, U., 1995. Influence of mucin type on polymer–mucin rheological interactions. Biomaterials 16, 1073–1079.

Sellers, L.A., Allen, A., Morris, E.R., Ross-Murphy, S.B., 1987. Mechanical characterisation and properties of gastrointestinal mucus gel. Biorheology 24, 616–623.

Zahm, J.M., Pierrot, D., Fuchey, C., Leverier, J., Duval, D., Lloyd, K.G., Puchelle, E., 1989. Comparative rheological profile of rat gastric and duodenal gel mucus. Biorheology 26, 813–822.