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Rheological characterisation of mixed gels of mucin and alginate

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Abstract

Aqueous mixtures of purified mucin and alginate have been shown to form weak viscoelastic gels under appropriate conditions. These mixed gels of mucin and alginate have been studied by both small deformation and large deformation rheology, and the effects of temperature and ionic strength on small deformation rheology have been investigated. The gels were rheologically reversible, flowing as the shear stress was increased and recovering their solid dominant properties as the shear stress decreased, and thermally stable showing little change in small deformation rheology in the range 10–60 °C. The effect of ionic strength on gel rheology was indicative of the presence of electrostatic interactions within the gel matrix. Based on these investigations, we propose that mixed mucin alginate gels are maintained by both heteropolymeric mucin–alginate interactions and homopolymeric mucin–mucin interactions. The ability of alginate to interact with mucin and promote mucin–mucin interactions may also have relevance to understanding the clinical problems associated with pulmonary infection with the alginate secreting bacterium *Pseudomonas aeruginosa* in cystic fibrosis patients.

Keywords: Biopolymer; Mucus

1. Introduction

Gel forming mucins are a family of polymeric glycoproteins that are responsible for the functional rheological properties of epithelial mucous secretions (Allen, 1989). Each mucin subunit is comprised of a protein backbone, containing a large tandem repeat region that is rich in serine and/or threonine and is heavily *O*-glycosylated. Polymerisation of mucin subunits occurs via disulfide bonding in the C and N terminal regions, which are non-glycosylated and rich in cysteine (Desseyn, Aubert, Porchet, & Laine, 2000). Mucins are secreted from the epithelial surfaces of the gastrointestinal, respiratory and genitourinary tracts where they hydrate to form mucus gels. The rheological properties of mucus gels cannot be accounted for by simple topological entanglement but rather include contributions from a wide range of mucin–mucin interactions (Bell, Allen, Morris, & Ross-Murphy, 1984; Taylor, Allen, Dettmar, & Pearson, 2003). Whilst increased knowledge and understanding of the mechanisms by which mucins themselves interact to form the mucus gel is of undoubted interest in terms of understanding many of the body's protection mechanisms, there is also significant interest in investigating the behaviour of mixed polymers systems including mucin. A substantial number of studies have investigated the rheological properties of mucin–polymer mixtures, in particular synergistic increases in viscosity upon mixing, as a method of studying mucoadhesive interactions (Ceulemans, Vinckier, & Ludwig, 2002; Fuongfuchat, Jamieson, Blackwell, & Gerken, 1996; Jones, Lawlor, & Woolfson, 2003; Madsen, Eberth, & Smart, 1998; Patel, Smart, Nevell, Ewen, Eaton, & Tsibouklis, 2003; Riley et al., 2001; Rossi, Ferrari, Bonferoni, & Caramella, 2000). Other studies have considered how the presence of other polymers may alter the functional rheological properties of mucus secretions in vivo (Brownlee, Allen, Dettmar, & Pearson, 2002; Dasgupta, Brown, & King, 1998; King, Dasgupta, Tomkiewicz, & Brown, 1997). In view of

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the similarities in the structure and hydrodynamic properties of the polymeric secreted mucins (Harding, 1995; Perez-Vilar & Hill, 1999) and the fact that the MUC5AC gene product is a major mucin in the stomach and lungs of humans (and pigs) (Gendler & Spicer, 1995), this study is also relevant to the influence bacterial exopolysaccharides may exert on the rheological properties of tracheobronchial mucus (Mrsny, Lazazzera, Daugherty, Schiller, & Patapoff, 1994). These properties are important as they are directly related to the ability of the mucociliary transport system to clear the mucus from the lung in the normal way.

Comparing published data is difficult for several reasons. Firstly, although the majority of the studies investigate rheological synergism, the methods of determining such synergism differ. Some use the increase in viscosity above the additive viscosity of the two polymers whereas others use the increase in viscosity relative to the original viscosity and whilst some compare polymers at a given concentration, others compare at a given viscosity. These differences in choice of conditions limit the information that can be gained from data relating to rheological synergism (Hagerstrom & Edsman, 2003). In addition to this, the method of purification of mucin has significant effects on its viscoelastic properties. Many studies utilise commercial mucin preparations due to the low inter-batch variability allowing better comparisons between polymers, however, it has been shown that such mucins are unable to produce rheological properties comparable to those of the native mucus secretions (KocevarNared, Kristl, & SmidKorbar, 1997). The use of freshly purified mucins introduces greater inter-batch variability but does produce mucins that are more comparable to those in the native secretion. Even so, mucins extracted under highly protein denaturing conditions are not able to fully replicate the rheological behaviour of the native secretion (Raynal, Hardingham, Sheehan, & Thornton, 2003) whilst mechanically extracting mucins with high shear may reduce the length of the polymers extracted.

Despite the variations in experimental conditions there is a significant body of evidence indicating mucins are able to interact with a variety of unlike polymers. Of particular interest is the interaction of mucins with alginates. Alginates are linear polysaccharides produced as a structural component in marine brown algae (*Phaeophyceae*) and also as a capsular component in some bacteria such as *Azotobacter vinelandii* and several species of *Pseudomonas* (Smidsrød & Draget, 1996). They are used extensively in the food and chemical industries as thickening, stabilising and emulsifying agents (Moe, Draget, Skjåk-Bræk, & Smidsrød, 1995) and also have pharmaceutical applications as actives for the treatment of oesophageal reflux, as excipients and also as sustained drug release systems (Skaugrud, 1995). Alginates are composed of two sugar monomers β-D-mannuronate and α-L-guluronate known as M units and G units, respectively (Haug, Larsen, & Smidsrod, 1967; Smidsrød & Draget, 1996). The distribution of monomers within the alginate chain is not random and structure of the alginate chain can be described as consisting of regions of repeating M units (M blocks), regions of repeating G units (G blocks) and regions of alternating structure (MG blocks). The sequence of the alginate chain in terms of M and G and alternating blocks influences the viscosity of the molecule with G blocks being the stiffest and MG blocks being the most flexible. The sequence also determines the ability of the molecule to form gels with divalent cations, as these cations (particularly calcium) are able to bind to G blocks to form intermolecular junction zones (Smidsrød & Draget, 1996). One particularly relevant study has shown that aqueous systems composed of freshly purified porcine or ovine submaxilliary mucins and alginate demonstrated an enhanced elastic response and formation of a viscoelastic gel when the mucin was present in excess indicating a substantial binding interaction between mucin and alginate (Fuongfuchat et al., 1996). Here, we investigate the rheological behaviour of gelled aqueous systems of purified mucin and alginate without the presence of divalent cations, which could induce alginate gelation.

2. Methods

2.1. Purification of mucin

Pig stomachs were obtained immediately after slaughter and were rinsed under gently running cold tap water to remove food debris. The firm adherent mucus gel was removed from the cardia and fundus of the stomach by gentle scraping with a microscope slide. Purified mucins were prepared according to the method of Fogg et al. (1996). Freshly removed mucus gel was briefly homogenised (1 min) in 67 mM pH 6.5 phosphate buffer containing a cocktail of proteolytic inhibitors. (1 mM iodoacetamide, 100 mM aminocaproic acid, 10 mM EDTA, 10 mM *N*-ethylmaleamide, 5 mM benzamidine HC1 and 1 mM phenylmethylsulphonylfluoride) and then centrifuged (9000 rpm, 4 °C, 1 h) to remove insoluble material. This supernatant was purified by equilibrium density gradient centrifugation in CsCl (1.42 g/ml starting density), fractionated (taking mucins banding between 1.45 and 1.49 g/ml), exhaustively dialysed, freeze-dried and stored at −20 °C.

2.2. Preparation of mucin and alginate solutions

Mucins (purified as above) were solubilised in deionised water at 4 °C overnight with gently agitation. Two different alginate samples were used, A—m.w. (weight average) 380,000, F_G (fraction of G residues), 0.68; B—m.w. 350,000, F_G, 0.45. Alginate solutions were prepared by dissolving powdered freeze-dried alginate (FMC Biopolymer) in deionised water under vortex created by a magnetic stirrer for 30 min or until the alginate was all in solution. Alginate solutions were then stored at 4 °C overnight before use to ensure the solution was homogeneous and free of bubbles. Alginates were a gift from FMC Biopolymer (Drammen, Norway). Mucin and alginate solutions were diluted before use to give the required final concentrations and ionic strengths.

2.3. Preparation of mucin alginate gels

Mucin alginate gels were prepared by gentle mixing of the mucin and alginate solutions in varying ratios to reach the desired final concentration. Vigorous agitation of the samples was avoided as gelling occurred spontaneously on mixing and vigorous mixing lead to the introduction of bubbles in the gel.

2.4. Rheological methods

Small deformation measurements were carried out using a Bohlin CVO50 rheometer or a Rheologica StressTech rheometer both fitted with 40 mm diameter serrated parallel plates, set at a gap width of 1 mm and operating in oscillatory mode. An initial amplitude sweep, stress range 0.1–3 Pa, was carried out to determine the shear independent plateau of the elastic modulus G'. Subsequent frequency sweeps were carried out at stress/strain values in the shear independent plateau over the frequency range 0.005–10 Hz. These frequency sweeps were used to determine the complex modulus (G^*) and the phase angle (δ), allowing the storage modulus (G') and the loss modulus (G'') for the gel to be calculated. From this, it was determined whether elastic, solid like behaviour was dominant over viscous, liquid like behaviour. Large deformation measurements involving increase–decrease stress sweeps were carried out using the Bohlin CVO50 rheometer fitted with 25 mm diameter serrated parallel plates to minimise slippage and amplitude sweeps were carried out over the range 0.5–50–0.5 Pa. Monitoring of the storage modulus (G') and the loss modulus (G'') over this range of shear stress allowed gel breakdown to be determined as the point at which $G'' > G'$ (liquid like behaviour dominates in the system).

3. Results and discussion

3.1. Formation conditions

Initial studies were carried out to investigate the concentrations at which mixtures of mucin and alginate would form a viscoelastic gel in deionised water. The mucin concentration was kept at a maximum of 9 mg/ml as at this concentration, the mucin preparation was well below the critical overlap concentration (intrinsic viscosity ≈200 ml/g) and well below the concentration needed to form a elastic dominant gel (≈50 mg/ml).

Mucin conc. (mg/ml)	Alginate A conc. (mg/ml)	Alginate B conc. (mg/ml)	Gelation
7	3		No
7		3	No
8	2		No
8		2	No
9	1		No
9		1	No
9	0.5		Yes
9		0.5	Yes

All mucin alginate mixtures and gels were transparent and non-turbid and no turbidity developed on standing.

Having determined the conditions under which a mucin alginate mixed gel could be formed, subsequent investigations of the rheological behaviour of the gel were carried out using the system containing 9 mg/ml mucin and 0.5 mg/ml alginate B (m.w. 350,000; F_G, 0.45)

3.2. Small deformation rheology

Freshly prepared mucin alginate gels (9 mg/ml mucin, 0.5 mg/ml alginate in deionised water) were subject to an initial amplitude sweep to determine the linear viscoelastic region where the values of the moduli are independent of the applied deformation. Subsequent frequency sweeps were carried out at stress/strain values within the linear region in the frequency range 0.005–10 Hz. The gel showed elastic dominant behaviour across the accessed frequency range, however, the gel was unstable at frequencies above 3 Hz so these measurements were disregarded. Although the elastic response was dominant across the whole frequency range, the gel maintained a significant viscous response with a phase angle of 10–20°. Both the elastic and viscous modulus show weak frequency dependence (Fig. 1). However, the frequency dependence is not to the degree expected from

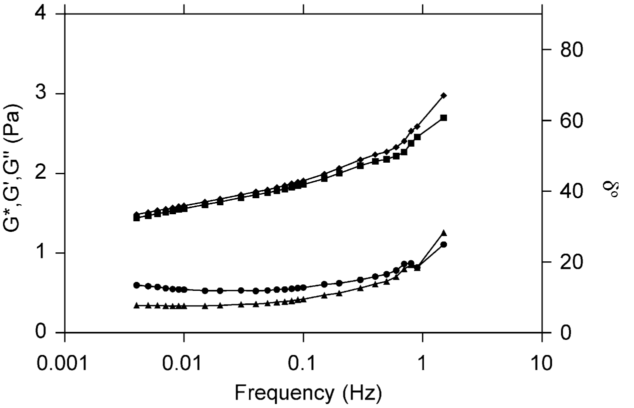

Fig. 1. Frequency sweep of mucin alginate gel showing a solid dominant system with slight frequency dependence of the moduli values G' –■–, G'' –▲–, G^* –◆–, δ –●–.

a purely entangled polymer system maintained solely by topological interactions.

These observations are typical of weak physical viscoelastic gels (Kavanagh & Ross-Murphy, 1998) and show similarities with the behaviour of the native pig gastric mucus gel (Bell et al., 1984; Taylor et al., 2003) although the absolute moduli values are significantly higher for the native mucus gels presumably due at least in part to the higher polymer concentration (pig gastric mucus typically 50 mg/ml mucin). The instability seen at frequencies above 3 Hz may be due to a complex regime of relaxation times at shorter timescales.

3.3. Effect of alginate concentration on gel formation

Mucin alginate mixtures were prepared with 9 mg/ml mucin and a range of alginate concentrations (1, 0.5, 0.25, 0.1 mg/ml) in deionised water. Each mixture was subject to a frequency sweep in the linear viscoelastic region to determine whether the behaviour was elastic dominant.

As noted above, increasing alginate concentration above the ‘gelling ratio’ results in no gel formation. However, gel formation is less sensitive to reducing the alginate concentration. As observed 9 mg/ml mucin 1 mg/ml alginate lies above the gelation threshold with the phase angle of the mixture remaining above 45. At lower concentrations of alginate, the phase angle is independent of alginate concentration over a five fold concentration range (Fig. 2). Below the minimum concentration of 0.1 mg/ml alginate per 9 mg/ml mucin, no gel formation was observed. Additional experiments were conducted using 9 mg/ml mucin and various concentrations of guar gum in place of the alginate. No mucin–guar combination resulted in gel formation. This, and the wide range of alginate concentrations that lead to gelation in mucin alginate mixtures suggest that the gelation does not arise simply from segregative interactions between the two polymers resulting in mucin selfassociation.

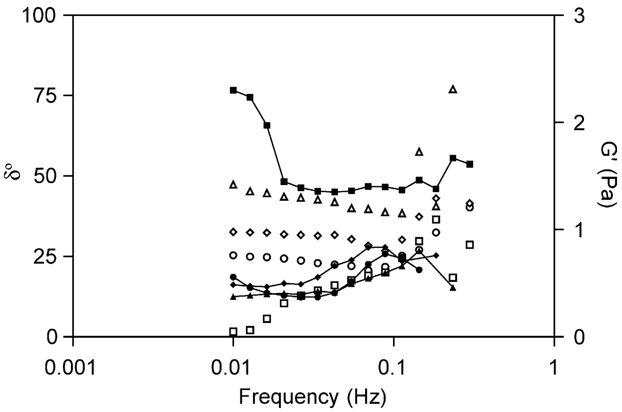

Fig. 2. Effect of lowering alginate content on phase angle and elastic modulus of mucin alginate gel. Systems prepared with 9 mg/ml mucin 1 mg/ml alginate were viscous dominant across the frequency range whereas those prepared with lower concentrations of alginate (0.5, 0.25 and 0.1 mg/ml) showed solid dominant behaviour. Lower levels of alginate produced a system with very low moduli values below the limits of resolution of the instrument. In the range of alginate concentration were solid dominant behaviour occurred, G' increased with increasing alginate content. Closed symbols δ, open symbols G'-1 mg/ml –■–, 0.5 mg/ml –▲–, 0.25 mg/ml –◆–, 0.1 mg/ml –●–.

The observation that addition of excess alginate leads to a non-gelling mixture is in agreement with Fuongfuchat et al. (1996). They propose that the alginate molecule possesses a high number of mucin interaction sites whereas the mucin has relatively few sites for alginate interaction and that these mucin–alginate interactions provide the crosslinks in the mucin alginate gel network. This theory can be developed further to suggest that both mucin–alginate and mucin–mucin interactions contribute to the gel network. In this case, mucin–alginate interactions may introduce entanglements with longer lifetimes which then allow the formation of mucin–mucin interactions resulting in gel formation. Increasing the amount of alginate in the system may then act to inhibit mucin–mucin interactions and thereby interfere with gelling. The presence of mucin–mucin interactions within the gel network may account for the broad range of alginate concentrations that can induce gelation.

3.4. Large deformation rheology

The mucin alginate gel (9 mg/ml mucin, 0.5 mg/ml alginate in deionised water) was subject to an increase–decrease amplitude sweep (0.5–50–0.5 Pa) at a frequency of 1 Hz. The maximum stress in the gel was subjected to lay well outside the linear viscoelastic region to investigate the rheological behaviour of the gel under conditions that cause disruption of the gel network. Serrated lower and upper plates were used to minimise gel slippage at high shear stresses.

The mucin alginate gel was rheologically reversible undergoing a transition from elastic dominant to viscous dominant behaviour as the shear stress was increased and then a transition back to elastic dominant behaviour as the shear stress was decreased again (Fig. 3). The increase–decrease stress sweep was repeated at frequencies between 1 and 0.1 Hz. The shear stress needed to induce a cross-over to a viscous dominant regime ($\delta > 45°$) was frequency-dependent showing a pronounced increase at frequencies above approx. 0.5 Hz (Fig. 4).

This behaviour is again rather similar to the native pig gastric mucus gel, which is also rheologically reversible and shows a comparable frequency dependence of the stress needed to induce a cross-over into a viscous dominant regime (Taylor et al., 2003).

3.5. Temperature effects

Frequency sweeps of mucin alginate gels (9 mg/ml mucin, 0.5 mg/ml alginate in deionised water) were carried out at temperatures of 10, 25, 37, 45 and 60°. The phase angle (δ) and the complex modulus (G^*) were plotted as a function of frequency for each temperature. There were no

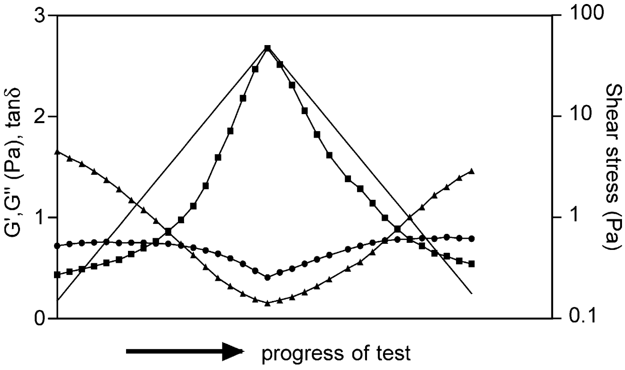

Fig. 3. Breakdown-recovery behaviour of mucin alginate gel at 1 Hz. The mucin alginate gel was subjected to increasing applied stress (from 0.5 to 50 Pa) and then decreasing applied stress (50–0.5 Pa) at a frequency of 1 Hz. As the shear stress is increased above that is necessary for gel breakdown, the gel flows as a viscous liquid with $G'' > G'$. As the shear stress is reduced, the gel regains its solid dominant behaviour $G' > G''$. tan δ –■–, G' –▲– G'' –●–, shear stress —.

significant differences in the gel behaviour over the range of temperatures (Fig. 5).

The gelation in the mucin alginate gel is not thermoreversible, which indicates gelling is not due to a significant conformational change such as the random coil–helix transition in the gelling of gelatine. The small deformation rheology of the gel is relatively independent of temperature. As most types of interaction become either stronger (e.g. hydrophobic interactions) or weaker (e.g. hydrogen bonds) with increasing temperature this stability suggests that the gel is not maintained by a single interaction type, or is maintained by interactions that are reasonably independent of temperature.

3.6. Ionic strength effects

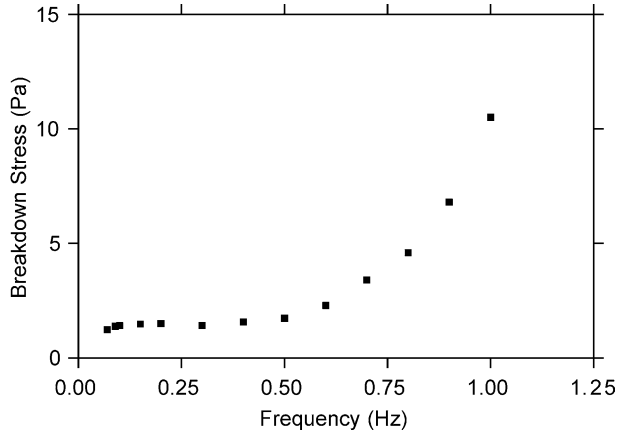

Fig. 4. Effect of frequency on breakdown stress of mucin alginate gel. The breakdown stress defined as the shear stress necessary to induce flow within the system ($G'' > G'$) showed a pronounced frequency dependence particularly above a frequency of 0.5 Hz.

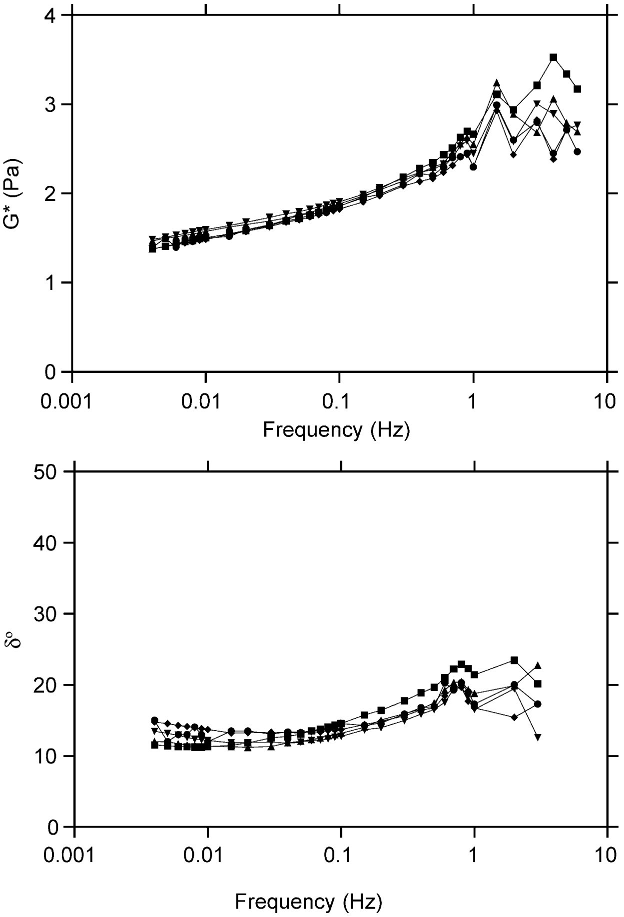

Fig. 5. Effect of temperature on small deformation rheology of mucin alginate gel. Both the phase angle (δ) and the complex modulus (G^*) remained relatively constant when the gel is subjected to testing at temperatures between 10 and 60°. 10° –■–, 25° –▲–, 37° –▼–, 45° –◆–, 60° –●–.

Mucin and alginate solutions were prepared in deionised water and then diluted with NaCl solutions to give a final polymer concentration of 10 mg/ml in deionised water and 0.02, 0.05, 0.1 and 2 M NaCl. These mucin–alginate solutions were mixed in the ratio to give final polymer concentrations of 9 mg/ml mucin, 0.5 mg/ml alginate as for the mucin alginate gels in deionised water.

The solutions prepared in 0.02, 0.05 and 0.1 M NaCl (in addition to those in deionised water) formed gels upon mixing whereas the solutions prepared at 2 M NaCl did not gel. When NaCl was added to a preformed mucin alginate gel in deionised water to a concentration of 2 M, the gel partitioned into a gel phase and a sol phase.

The phase angle and the moduli G' and G'' of the gels were determined at an oscillatory frequency of 1 Hz within the linear viscoelastic regime and plotted as a function of increasing NaCl concentration. At low ionic strength (0.02 M NaCl), the gel had an increased elastic modulus (88% increase) and decreased phase angle (33% decrease) when compared to the deionised water gel. Further increases in the concentration of NaCl lead to a decrease in the elastic modulus and an increase in the phase angle. The gel phase that remained after addition of 2 M NaCl to a deionised

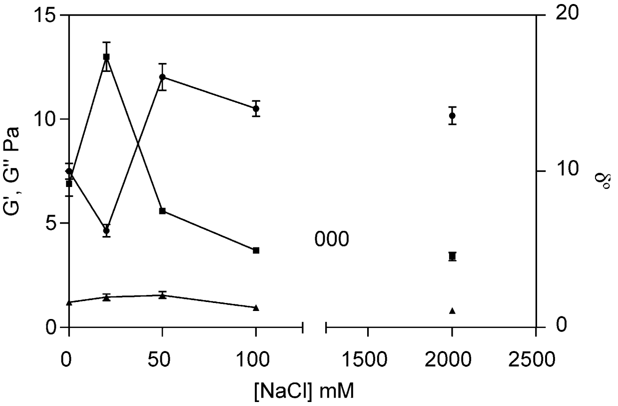

Fig. 6. Effect of ionic strength on small deformation rheology of mucin alginate gels. Mucin alginate gels prepared in 0.02, 0.05 and 0.1 M NaCl and the gel pellet that remained after addition of 2 M NaCl to a preformed mucin alginate gel in deionised water were tested rheologically. The effect of increasing ionic strength on the elastic modulus (G') and the phase angle (δ) is typical of that seen in a system maintained by ionic interactions. G' –■–, G'' –▲–, δ –●–.

water mucin alginate gel showed similar rheology to a mucin alginate gel formed at 0.1 M (Fig. 6).

Considering that alginate is negatively charged and mucin is amphoteric, this behaviour can be explained in terms of electrostatic interactions within the gel matrix (Neiser, Draget, & Smidsrod, 1999). As the ionic strength is increased, it screens off first long-range repulsive forces leading to a strengthening of the gel network. Further increases in ionic strength additionally screen off short-range attractive forces thus weakening the gel network. This strengthening and then the weakening, as the ionic strength is increased, is a fingerprint for the presence of electrostatic interactions within the system. No gel formation occurs when the ionic strength is increased to 2 M NaCl, however, addition of 2 M NaCl to a preformed gel does not completely dissociate the gel. Rather, it leads to partitioning into a gel and sol phase. The remaining gel phase has rheology very similar to that observed in a gel formed in an ionic strength of 0.1 M NaCl. This would indicate that not all interactions within the gel matrix are electrostatic in nature or that the electrostatic interactions show large hysteresis behaviour. The fact that the gel will not form in 2 M NaCl probably reflects the fact that attractive electrostatic interactions are totally screened at this ionic strength preventing association between the mucin and alginate. The lack of gel formation in mixtures of mucin and the uncharged polymer guar gum supports this explanation.

4. Conclusions

If certain conditions are met (regarding relative concentrations), then aqueous mixtures of mucins and alginates are able to form weak viscoelastic gels. These gels are thermostable and rheologically reversible showing significant similarities to the native mucus gels, although with lower moduli values. Indications are a variety of interaction types that maintain the gel network and it is likely that both electrostatic hetropolymeric interactions between mucin and alginate and homopolymeric mucin–mucin interactions contribute. The formation of these mixed gels is likely to be relevant to both to pharmaceutical uses of alginate in ingested dosage forms and the situation in the lung of cystic fibrosis patients infected with alginate secreting *Pseudomonas aeruginosa*. In both cases, the range of mucin: alginate ratios required for gel formation is likely to be met at some point due to concentration gradients in both alginate and mucin; with alginate being most concentrated in the region of dosage form or bacteria and mucin being most concentrated as it is secreted from the epithelium. In the case of *P. aeruginosa* infection in the lungs any enhancement of sputum elasticity through mucin alginate interactions is likely to have significant consequences for mucociliary clearance, even if the conditions for large-scale formation of mucin alginate gels are not completely satisfied.

Acknowledgements

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References

Allen, A. (1989). Gastrointestinal mucus. In J. G. Forte (Ed.), *Handbook of physiology—the gastrointestinal system 111*. Am. Physiol. Soc.

Bell, A. E., Allen, A., Morris, E. R., & Ross-Murphy, S. B. (1984). Functional interactions of gastric mucus glycoprotein. *International Journal of Biological Macromolecules*, *6*, 309–315.

Brownlee, I. A., Allen, A., Dettmar, P. W., & Pearson, J. P. (2002). Mucosal protective properties of dietary fibre may be mediated through an interaction with mucus. *Journal of Gastroenterology and Hepatology*, *17*, A991.

Ceulemans, J., Vinckier, I., & Ludwig, A. (2002). The use of xanthan gum in an ophthalmic liquid dosage form: Rheological characterization of the interaction with mucin. *Journal of Pharmaceutical Sciences*, *91*, 1117–1127.

Dasgupta, B., Brown, N. E., & King, M. (1998). Effects of sputum oscillations and rhDNase in vitro: A combined approach to treat cystic fibrosis lung disease. *Pediatric Pulmonology*, *26*, 250–255.

Desseyn, J. L., Aubert, J. P., Porchet, N., & Laine, A. (2000). Evolution of the large secreted gel-forming mucins. *Molecular Biology and Evolution*, *17*, 1175–1184.

Fogg, F. J. J., Hutton, D. A., Jumel, K., Pearson, J. P., Harding, S. E., & Allen, A. (1996). Characterisation of pig colonic mucins. *Journal of Biochemistry*, *316*, 937–942.

Fuongfuchat, A., Jamieson, A. M., Blackwell, J., & Gerken, T. A. (1996). Rheological studies of the interaction of mucins with alginate and polyacrylate. *Carbohydrate Research*, *284*, 85–99.

Gendler, S. J., & Spicer, A. P. (1995). Epithelial mucin genes. *Annual Review of Physiology*, *57*, 607–634.

Hagerstrom, H., & Edsman, K. (2003). Limitations of the rheological mucoadhesion method: The effect of the choice of conditions and the rheological synergism parameter. *European Journal of Pharmaceutical Sciences*, *18*, 349–357.

Harding, S. E. (1995). On the hydrodynamic analysis of macromolecular conformation. *Biophysical Chemistry*, *55*, 69–93.

Haug, A., Larsen, B., & Smidsrod, O. (1967). Studies on sequence of uronic acid residues in alginic acid. *Acta Chemica Scandinavica*, *21*, 691.

Jones, D. S., Lawlor, M. S., & Woolfson, A. D. (2003). Rheological and mucoadhesive characterization of polymeric systems composed of poly(methylvinylether-co-maleic anhydride) and poly(vinylpyrrolidone), designed as platforms for topical drug delivery. *Journal of Pharmaceutical Sciences*, *92*, 995–1007.

Kavanagh, G. M., & Ross-Murphy, S. B. (1998). Rheological characterisation of polymer gels. *Progress in Polymer Science*, *23*, 533–562.

King, M., Dasgupta, B., Tomkiewicz, R. P., & Brown, N. E. (1997). Rheology of cystic fibrosis sputum after in vitro treatment with hypertonic saline alone and in combination with recombinant human deoxyribonuclease I. *American Journal of Respiratory and Critical Care Medicine*, *156*, 173–177.

KocevarNared, J., Kristl, J., & SmidKorbar, J. (1997). Comparative rheological investigation of crude gastric mucin and natural gastric mucus. *Biomaterials*, *18*, 677–681.

Madsen, F., Eberth, K., & Smart, J. D. (1998). A rheological assessment of the nature of interactions between mucoadhesive polymers and a homogenised mucus gel. *Biomaterials*, *19*, 1083–1092.

Moe, S., Draget, K. I., Skjåk-Bræk, G., & Smidsrød, O. (1995). Alginates. In A. M. Stephen (Ed.), *Food polysaccharides and their applications*. New York: Marcel Dekker.

Mrsny, R. J., Lazazzera, B. A., Daugherty, A. L., Schiller, N. L., & Patapoff, T. W. (1994). Addition of a bacterial alginate lyase to purulent cf sputum in-vitro can result in the disruption of alginate and modification of sputum viscoelasticity. *Pulmonary Pharmacology*, *7*, 357–366.

Neiser, S., Draget, K. I., & Smidsrod, O. (1999). Interactions in bovine serum albumin–calcium alginate gel systems. *Food Hydrocolloids*, *13*, 445–458.

Patel, M. M., Smart, J. D., Nevell, T. G., Ewen, R. J., Eaton, P. J., & Tsibouklis, J. (2003). Mucin/poly(acrylic acid) interactions: A spectroscopic investigation of mucoadhesion. *Biomacromolecules*, *4*, 1184–1190.

Perez-Vilar, J., & Hill, R. L. (1999). The structure and assembly of secreted mucins. *Journal of Biological Chemistry*, *274*, 31751–31754.

Raynal, B. D. E., Hardingham, T. E., Sheehan, J. K., & Thornton, D. J. (2003). Calcium-dependent protein interactions in MUC5B provide reversible cross-links in salivary mucus. *Journal of Biological Chemistry*, *278*, 28703–28710.

Riley, R. G., Smart, J. D., Tsibouklis, J., Dettmar, P. W., Hampson, F., Davis, J. A., et al. (2001). An investigation of mucus/polymer rheological synergism using synthesised and characterised poly(acrylic acid)s. *International Journal of Pharmaceutics*, *217*, 87–100.

Rossi, S., Ferrari, F., Bonferoni, M. C., & Caramella, C. (2000). Characterization of chitosan hydrochloride–mucin interaction by means of viscosimetric and turbidimetric measurements. *European Journal of Pharmaceutical Sciences*, *10*, 251–257.

Skaugrud, Ø. (1995). Drug delivery systems with alginate and chitosan. In D. A. Karsa, & R. A. Stephenson (Eds.), *Excipients and delivery systems for pharmaceutical formulations* (pp. 96–107). Cambridge: Royal Society of Chemistry.

Smidsrød, O., & Draget, K. I. (1996). Chemistry and physical properties of alginates. *Carbohydrates in Europe*, *14*, 7–13.

Taylor, C., Allen, A., Dettmar, P. W., & Pearson, J. P. (2003). The gel matrix of gastric mucus is maintained by a complex interplay of transient and nontransient associations. *Biomacromolecules*, *4*, 922–927.