International Journal of Pharmaceutics, 83 (1992) 221–225

IJP 02808

An investigation of the rheological behaviour of the mucoadhesive/mucosal interface

S.A. Mortazavi, B.G. Carpenter and J.D. Smart

Drug Delivery Research Group, School of Pharmacy and Biomedical Sciences, Portsmouth Polytechnic, Portsmouth PO1 2DZ (UK)

(Received 9 October 1991)
(Accepted 11 February 1992)

Key words: Bioadhesion; Mucoadhesion; Mucosal adhesion; Carbopol 934P; Polyacrylic acid; Biorheology

Summary

One proposed mechanism of mucoadhesion involves the interpenetration of the mucus/mucoadhesive molecules, followed by the formation of non-covalent interactions. In this study, the effect of introducing a mucoadhesive macromolecule, the polyacrylic acid Carbopol 934P (paa), on the rheological behaviour of a mucus gel was evaluated using mechanical spectroscopy. It was found that a large increase in G' (the storage modulus) occurred in comparison to the values obtained when the mucus gel and the paa gel were evaluated separately at the same concentration. This gel strengthening was markedly affected by pH (i.e., it was minimal at pH values below 4.5 and above 8), while temperatures up to 45°C did not break down this gel. It was concluded that molecular interpenetration resulting in strengthening of the layer between the mucoadhesive/mucosal surface may offer an explanation for the large forces required to break a mucoadhesive joint.

Introduction

Bioadhesion is defined as the attachment of synthetic or biological macromolecules to a biological tissue (Peppas and Buri, 1985). When applied to a mucosal epithelium, bioadhesive interactions occur primarily with the mucus layer, and this phenomenon is referred to as 'mucoadhesion'. In the last decade, bioadhesive polymers have received considerable attention as platforms for controlled delivery due to their ability to prolong the residence time of dosage forms in the gastrointestinal tract as well as localizing in specific regions to enhance drug bioavailability (Gu et al., 1988).

Formation of an adhesive bond between a polymer and mucus gel can be examined in terms of the contributions of three regions: the surface of the bioadhesive polymer, the interfacial layer between the bioadhesive and mucosa, and the mucosal surface (Peppas and Buri, 1985). The weakest component in the adhesive joint would be predicted to be the interfacial layer consisting (at least initially) predominantly of mucus. Mucus is a weak viscoelastic gel that adheres to the epithelium whose major structure-forming component is glycoprotein (molecular weight of 2–14 $\times 10^6$) (Marriott and Gregory, 1990). These glycoprotein molecules associate with each other by

Correspondence: J.D. Smart, Drug Delivery Research Group, School of Pharmacy and Biomedical Sciences, Portsmouth Polytechnic, Portsmouth PO1 2DZ, U.K.

non-covalent interactions to form the gel matrix which is responsible for the rheological properties of mucus. It is inconceivable that strong mucosal adhesion can occur (i.e., withstanding applied tensile forces of up to 3–5 N cm^{-2} (Smart, 1991)) without a considerable change in the rheological properties of this layer.

The following stages in mucoadhesion have been proposed (Duchene et al., 1988). Initially, an intimate contact is formed by wetting of the mucoadhesive surface by the mucus gel. The second stage is the penetration of the mucoadhesive molecules into the mucus gel network, followed by the formation of secondary chemical bonds between the mucus and the mucoadhesive macromolecules. The importance of the surface energy thermodynamics of mucus and the hydrated mucoadhesive polymers has been considered in other work (Lehr, 1991). In this investigation, the second stage, i.e., the molecular interpenetration of the mucus/mucoadhesive macromolecules, is considered and the effect that this would have on the rheological and cohesive nature of the interfacial layer.

In previous work, Hassan and Gallo (1990) performed a simple viscometric method to quantify mucin-polymer bioadhesive bond strength and used this to calculate the viscosity component of bioadhesion. Kerr et al. (1990) used mechanical spectroscopy to investigate the interaction between glycoprotein gels and polyacrylic acids and the effect of pH and polymer chain length on this. Mucoadhesive materials have been identified in previous work (Smart et al., 1984) and this investigation will consider the effect of introducing a known mucoadhesive polymer (the polyacrylic acid Carbopol 934P) on the rheological properties of mucus, along with some of the factors that may affect this.

Materials and Methods

Materials

Carbopol 934P (paa) was obtained as a gift from B.F. Goodrich (Hounslow, U.K.), sodium azide, sodium chloride and sodium edetate (disodium salt) from BDH Chemicals (Poole, U.K.), and phenylmethylsulphonyl fluoride (PMSF) and anhydrous glucose from Sigma Chemical Co. Ltd (Poole, U.K.).

Preparation and characterisation of the homogenised mucus gels

Batches of crude mucus were obtained by scraping 5–20 porcine stomachs obtained fresh from slaughter. These were homogenised by blending for 4 min with an equal portion of an isotonic solution containing PMSF (0.0175% w/v), sodium azide (0.02% w/v), sodium edetate (0.186% w/v) and sodium chloride (0.9% w/v). The resulting mixture was centrifuged at $2500 \times g$ 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 homogenised by blending (when producing a high concentration gel, the sample was dialysed, centrifuged at $20000 \times g$ for 1 h and the supernatant discarded and recentrifuged at $120000 \times g$ for 1 h and the gel layers taken). The dry weight was determined for each batch by leaving a small portion (0.5 g) in an open glass vial at 50°C for 48 h. If necessary, the content (% w/w) of solids in the homogenised gel was adjusted to give a value between 2.8 and 3% (9% for the concentrated gel).

The gel layer and the supernatant of the first batch were analyzed by SDS-polyacrylamide gel electrophoresis for the presence of glycoprotein and protein fractions using a procedure similar to that described by Laemmli (1970). The samples were prepared for electrophoresis by mixing with a loading solution and heating to 100°C for 2 min in a similar manner to the procedure described by Mantle and Allen (1981). The presence of protein and carbohydrate was detected using a Coomassie brilliant blue stain and a danzyl hydrazine stain, respectively. This confirmed the presence of glycoprotein (Mol. Wt > 300000) and lower molecular weight components in the gel. The staining of the supernatant fraction was similar to that of the gel, thus indicating that the mucus components discarded during the homogenisation procedure were similar to those remaining in the gel. The infrared spectrum of the gel layer was then recorded using a Perkin Elmer 377 spectrophotometer over the range

4000–600 cm^{-1}, and all further batches of homogenised mucus compared with this to ensure that no gross differences in the components of the gel occurred from batch to batch. Each set of comparative experiments was completed using homogenised mucus from the same batch.

Experimental procedure

1.5-g samples of homogenised mucus were mixed with an equal quantity of paa gel (5 mg/ml) and the pH was adjusted to the required value (initially 5.1) using either 0.1 M NaOH or 0.1 M HCl. The final weight of the sample was then adjusted to 4.5 g using purified water.

Further mixtures containing 1.5 g mucus alone and 1.5 g paa alone were adjusted to pH 5.1 and diluted to 4.5 g. 1.5 ml of a 3% glucose solution was also added to 1.5 g of paa 5 mg/ml gel, the pH adjusted to 5.1 and then made to 4.5 g.

Using a similar procedure, the effect of pH was investigated by making mucus/paa mixtures at various pH values between 2 and 8.

Each sample was allowed to equilibrate at 4°C overnight prior to testing at 15°C (to minimise drying and sample degradation) using a Carri-Med CSL 100 Rheometer (Carri-Med Ltd, Dorking, England) fitted with a 4 cm stainless-steel parallel plate and a gap setting of 0.5 mm. Samples were then individually loaded, allowed to equilibrate for a further 5 min, tested using a frequency sweep between 10 and 0.1 Hz and the mean storage modulus (G') and loss modulus (G'') calculated.

This work was repeated using the concentrated (9% w/w) mucus sample which, on mixing with the paa gel and adjusting the pH, gave a final concentration of 3% dry weight of solids, which is closer to the in vivo situation.

TABLE 1

Comparative rheological assessment of mucus/paa, mucus/water and paa/water mixtures at pH 5.10 (n = 3)

Sample	G' (S.D.) (Pa)	G'' (S.D.) (Pa)
paa/mucus	33.44 (4.21)	6.83 (0.31)
paa/water	0.91 (0.49)	5.06 (1.74)
Mucus/water	1.31 (0.07)	5.17 (0.89)

A temperature sweep between 5 and 45°C was then completed on the original 'dilute' homogenized mucus/paa mixture at pH 6.18, at a frequency of 1 Hz. This was completed in order to ensure that the rheological behaviour did not completely change when the temperature was increased.

Results

A gel was seen to form within the vial when paa was mixed with mucus and adjusted to pH 5.10 at room temperature, while the mucus/water and paa/water mixtures alone behaved like low-viscosity liquids. This was confirmed when examined by mechanical spectroscopy (Table 1). A much larger G' (a measure of the resistance to elastic deformation) was found for the mucus/paa mixture in comparison with the mucus and paa separately, confirming the formation of a strong gel network. The G'' (a measure of the resistance to liquid flow) was much smaller and did not alter significantly.

When a 3% glucose solution was added to the paa gel in place of the mucus, little effect was

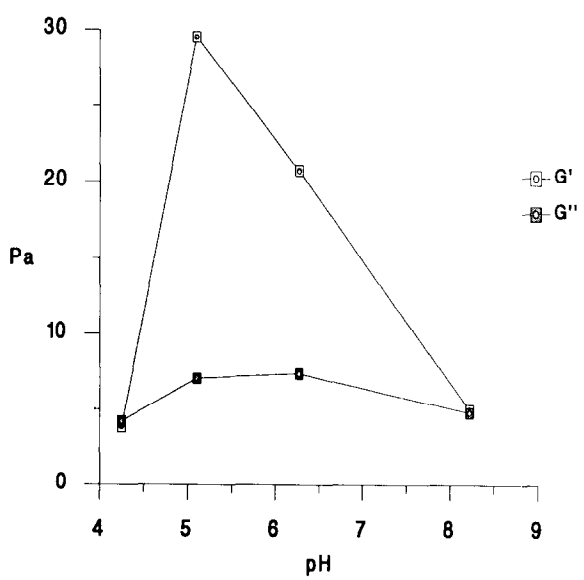

Fig. 1. Effect of pH on the rheological behaviour of paa/mucus mixtures.

TABLE 2

Comparative rheological assessment of concentrated mucus/paa, concentrated mucus/water and paa/water mixtures at pH 6.2

Sample	G' (Pa)	G'' (Pa)
paa/mucus	60.33	13.56
paa/water	3.02	5.89
Mucus/water	2.74	4.15

observed (G' 1.01 Pa, G'' 5.35 Pa) compared to the values obtained for the paa/water mixture in Table 1.

pH was observed to exert a dramatic effect on this gelling phenomenon (Fig. 1). Visible signs of gel breakdown were observed at pH 4.25, which was confirmed by the low G' value.

Similar increases in G' were observed with the concentrated mucus gel at pH 6.2 (Table 2), which was also affected by pH (Fig. 2).

An increase in temperature reduced the G' and G'' values (Fig. 3), but the mixture retained its gel properties throughout this range.

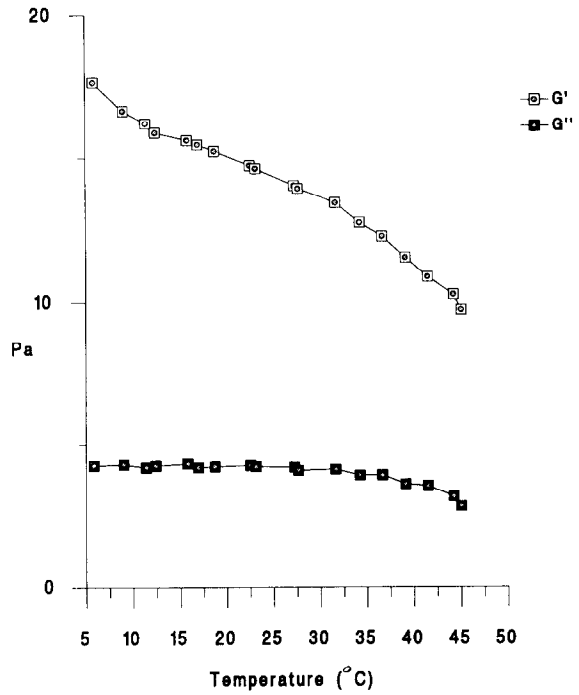

Fig. 3. Effect of temperature on the rheological behaviour of the paa/mucus mixture.

Discussion

In a previous study (Smart et al., 1991) it was proposed that mucus gel dehydration could increase the cohesive and adhesive nature of mucus, thus strengthening the interfacial layer between the bioadhesive and the mucosal surface. This study indicates that interpenetration of the mucus/mucoadhesive molecules will also have a marked effect on the rheological and hence the cohesive nature of this layer. This implies an interaction between the mucosa-adhesive and glycoprotein molecules leading to the formation of the greatly strengthened gel network. If this effect could be explained in terms of a reduction in the available water causing the formation of a paa gel, then the addition of glucose, which would be expected to interact with a similar amount of water, would be predicted to have a similar effect. Although it was necessary to complete this work at a temperature of 15°C to minimise evaporation and instability, the temperature sweep confirmed that this gel structuring was stable at temperatures above that observed physiologically.

The optimum pH for this gel strengthening phenomenon is in the weakly acid to neutral

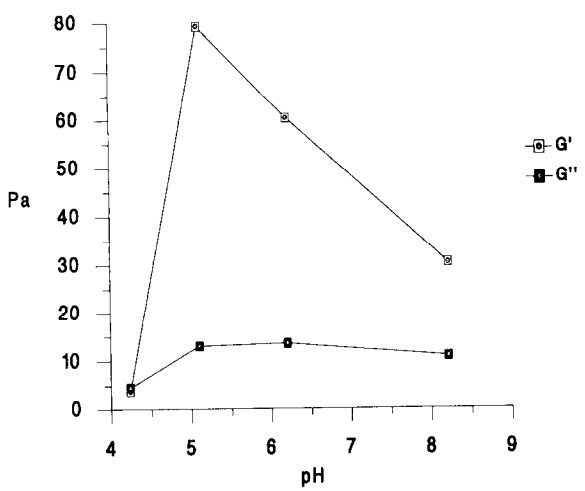

Fig. 2. Effect of pH on the rheological behaviour of paa/concentrated (9% w/w) mucus mixtures.

region, around the pK_a of polyacrylic acid (Park and Robinson, 1987). Kerr et al. (1990), using purified glycoprotein, did not report a similar gel breakdown at lower pH values, implying that this may be caused by other, non-glycoprotein components of the mucus gel. This change in the rheological properties of the mucus not only may strengthen the mucosa-adhesive joint, but also may affect the rate of clearance of mucoadhesive dosage forms (e.g., from the nasal cavity) by the mucociliary transport system or the rate of loss of mucus into the gastrointestinal lumen during normal mucus turnover. It would also confirm the possibility that gel formulations of bioadhesive polymers may interact with the mucus layer and be retained for prolonged periods.

It may be concluded that the process of mucoadhesion is a very complex procedure, that may involve surface wetting, mucus gel dehydration and molecular interpenetration. It is probable that the relative importance of each factor will vary, depending on the contact time, the nature of the mucosal surface, the presence and thickness of the mucus layer (it is possible, for example, that when the mucus layer is comparatively thick, complete interpenetration will be inhibited by the formation of this strengthened gel layer at the interface) and the nature and degree of hydration of the mucoadhesives. The application of one theory for all these circumstances may not therefore be appropriate.

In future work, other factors (e.g., ionic strength and the use of purified glycoprotein fractions) affecting this gel strengthening phenomenon will be considered for polyacrylic acids and other bioadhesive polymers, along with the nature of the interactions between the macromolecules.

Acknowledgement

The authors would like to acknowledge Professor Christopher Marriott for providing the information on which the procedure for preparing the homogenised mucus samples was based.

References

Duchene, D., Touchard, F. and Peppas, N.A., Pharmaceutical and medical aspects of bioadhesive systems for drug administration. *Drug Dev. Ind. Pharm.*, 14 (1988) 283–318.

Gu, J.M., Robinson, J.R. and Leung, S.H.S., Binding of acrylic polymers to mucin/epithelial surfaces: structure-property relationships. *CRC Crit. Rev. Ther. Drug Carrier Systems*, 5 (1988) 21–67.

Hassan, E.E. and Gallo, J.M., A simple rheological method for the in vitro assessment of mucin-polymer bioadhesive bond strength. *Pharm. Res.*, 7 (1990) 491–495.

Kerr, L.J., Kellaway, I.W., Rowlands, C. and Parr, G.D., The influence of poly(acrylic) acids on the rheology of glycoprotein gels. *Proc. Int. Symp. Control. Rel. Bioact. Mater.*, 17 (1990) 122–123.

Laemmli, U.K., Cleavage of structural proteins during the assembly of the head of Bacteriophage T4. *Nature*, 227 (1970) 680–685.

Lehr, C.M., Bioadhesive drug delivery systems for oral applications, Ph.D. Thesis, Leiden University, 1991.

Mantle, M. and Allen, A., Isolation and characterization of the native glycoprotein from pig small intestinal mucus. *Biochem. J.*, 195 (1981) 267–275.

Marriott, C. and Gregory, N.P., Mucus physiology and pathology. In Lenaerts, V, and Gurny, R. (Eds), *Bioadhesive Drug Delivery Systems*, CRC Press, Boca Raton, FL, 1990, pp. 1–23.

Park, H. and Robinson, J.R., Mechanisms of mucoadhesion of poly(acrylic acid) hydrogels. *Pharm. Res.*, 4 (1987) 457–464.

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

Smart, J.D., An in vitro assessment of some mucosa-adhesive dosage forms. *Int. J. Pharm.*, 73 (1991) 69–74.

Smart, J.D., Kellaway, I.W. and Worthington, H.E.C., An in-vitro investigation of mucosa-adhesive materials for use in controlled drug delivery. *J. Pharm. Pharmacol.*, 36 (1984) 295–299.

Smart J.D., Carpenter, B.G. and Mortazavi, S.A., Is mucus dehydration an important factor in mucoadhesion? *Proc. Int. Symp. Control. Rel. Bioact. Mater.*, 18 (1991) 629–630.