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Interpretation of mucoadhesive properties of polymer gel preparations using a tensile strength method

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Abstract

We have developed a new tensile strength method for assessing mucoadhesive properties of polymer gels utilising freshly excised porcine nasal mucosa and a texture analyser. In conjunction with this, we propose a method for interpreting the mucoadhesive properties that is based on reasoning about the locus of the failure of a mucoadhesive joint. This involves measuring the cohesiveness of the gel and the mucus layer, respectively, and comparing these results with those obtained from a mucoadhesion measurement. Linear polymers (sodium carboxymethylcellulose, poly(acrylic acid) and sodium hyaluronate) and a cross-linked polymer (poly(acrylic acid)) were used as model polymers in this study. It was shown that the withdrawal speed of the probe should be low, about 0.1 mm s^{-1}, and that a contact time of 2 min was sufficient. In the mucoadhesion measurements there was no dependence of the results on the contact time in the interval 2–20 min. The tensile work appeared to be more applicable than the fracture strength for interpreting mucoadhesive properties. Furthermore, it was concluded that the interpretation procedure offers a good basis by which to assess whether the measured tensile work reflects a cohesive failure of the gel or a true interaction of the gel with the mucus layer.

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Introduction

Mucoadhesive dosage forms have gained, and are still gaining, considerable interest as a means of providing intimate contact and prolonging the residence time of a dosage form intended for nasal and ocular administration, for example (Peppas & Buri 1985; Gu et al 1988; Dondeti et al 1996; Lee et al 2000). Many in-vitro methods for measuring mucoadhesion have been reported during the last 20 years, most of them based on tensile or shear strength measurements (Duchene et al 1988; Peppas & Sahlin 1996). The tensile strength methods have been used extensively to study mucoadhesion of solid formulations such as tablets, compacts and microspheres. However, the inconsistencies between apparatuses and instrumental parameters have been pointed out, especially by Tobyn et al (1995), and could provide an explanation for the wide variation in results and conclusions found in the literature.

Several theories have been forwarded to explain the mucoadhesion process (Duchene et al 1988; Gandhi & Robinson 1994; Peppas & Sahlin 1996), but it is likely that different mechanisms are important for dry dosage forms and for fully hydrated systems such as gels (Lehr et al 1992). For gels in particular, it is important to consider the possible regions where the failure of the mucoadhesive joint can take place, and this has been thoroughly discussed by Smart (1999). Which region is the

weakest when the dosage form is in contact with a mucous membrane – the dosage form, the mucus layer or the interface? A strengthening of the mucus layer is necessary for strong mucoadhesion, and such strengthening is generally believed to occur by interpenetration of the polymer chains forming entanglements and secondary chemical bonds with the mucin molecules, and or by dehydration of mucus caused by water movement. The latter is important mainly when dry or partially hydrated dosage forms are concerned (Mortazavi & Smart 1993).

For polymer gels, a mucoadhesion method based on rheological measurements (Hassan & Gallo 1990) has been widely used (e.g. Caramella et al 1994, 1999; Mortazavi 1995; Madsen et al 1998; Hägerström et al 2000). However, evaluation of the method has shown that the results obtained are difficult to interpret and can vary considerably, depending on, for example, the concentration and the ion-sensitivity of the polymer, the quantity of ions present, the mucin type and instrumental factors (Hägerström et al 2000). Another major drawback with the rheological mucoadhesion method is that it can not give information about the weakest region of the mucoadhesive joint since only the interpenetration layer is simulated with this method. With a tensile strength method, however, the different regions of the mucoadhesive joint can be assessed. In our opinion, the interpretation of the results from the measurements should be the subject of greater consideration, to assess whether the mucoadhesion measurement reflects a genuine interaction between the dosage form and the mucus layer, or just a cohesive failure of the dosage form.

In this work we present a tensile strength method suitable for studying the mucoadhesive properties of polymer gels, using freshly excised nasal porcine mucosa and a texture analyser. Previously, a few similar tensile strength methods have been used for polymer gels (e.g. Caramella et al 1994; Jones et al 1997; Tamburic & Craig 1997), but with very different experimental setups and mainly utilising compressed mucin discs or mucin solutions. In this study we investigate the influence of experimental parameters and suggest appropriate settings. As model polymers in developing the tensile strength method we used linear polymers, which were sodium carboxymethylcellulose, sodium hyaluronate and linear poly(acrylic acid), and a cross-linked polymer, poly(acrylic acid). Furthermore, we have put the emphasis on developing a method for interpreting the mucoadhesive properties of the gel formulations, based on the above reasoning about the locus of the failure of the mucoadhesive joint.

Materials and Methods

Materials

The linear polymers used in this study were Carbopol 907 (C907, linear poly(acrylic acid), BF Goodrich, Brecksville, OH), Blanose 7LF and 7HF (B7LF and B7HF, sodium carboxymethylcellulose of low- and high-viscosity grade, Hercules/Aqualon, Alizay, France) and sodium hyaluronate (SH), obtained as Healon 5 (a 23 mg mL^{-1} solution of sodium hyaluronate, MW approx. 4×10^6, Pharmacia Corp., Uppsala, Sweden). The cross-linked polymer used was Carbopol 934P (C934P, cross-linked poly(acrylic acid), BF Goodrich, Brecksville, OH). All the polymers were the kind gifts of the manufacturers. All other chemicals used were purchased from Sigma (St Louis, MO) and were of analytical or 'ultra' quality. Ultra-pure water was used throughout the experiments. Fresh porcine nasal mucosa from Pigham pigs (aged 6 months) was obtained from the local slaughterhouse (Swedish Meats AB, Uppsala, Sweden).

Preparation of gel samples

Carbopol samples were prepared by dispersing the required amount of polymer in 0.9% NaCl, using a magnetic stirrer for about 1 h. The pH was then adjusted to approximately 6.5–7 using 4.5 M NaOH and the sample was equilibrated at 4°C overnight. Next day the pH was finely adjusted to physiological pH (7.4) and 0.9% NaCl was added to obtain the exact polymer concentration required. Blanose samples were prepared by stirring the required amount of polymer and 0.9% NaCl until complete dissolution had taken place (about 15–20 h). Sodium hyaluronate samples were obtained from the manufacturer as solutions containing 23 mg mL^{-1} sodium hyaluronate, which were diluted to exact concentration using a physiological phosphate buffer pH 7.0–7.5 (Pharmacia Corp., Uppsala, Sweden). Careful mixing was carried out to ensure the samples were homogeneous.

Three replicates were made of all gel samples and were stored at 4°C until measurements were performed within 10 days.

The choice of polymer concentration was to some extent based on the rheological behaviour of the samples. For the poly(acrylic acid) polymers (C907 and C934P) two concentrations were chosen: one mutual (2% w/w) and one where the samples had the same elastic modulus (G′) at 1 Hz (approximately 10 Pa) (i.e., 7.4% w/w and 0.75% w/w, respectively). For the linear Blanose polymers the concentrations chosen (2% w/w

B7HF, 7.4% w/w B7LF) matched those of the linear C907. Two concentrations of SH were chosen, 1.5 and 0.5% w/w, the lower one giving an elastic modulus of approximately 10 Pa.

Rheological characterization of gel samples

To characterise the preparations rheological measurements were carried out at 37°C using a Bohlin VOR rheometer (Bohlin Reologi, Lund, Sweden), a controlled rate instrument of the couette type. The measuring system used was a concentric cylinder (C14). After being loaded in the measuring geometry, the samples, previously brought to 37°C, were lightly centrifuged for 1 min at 209 *g* to remove entrapped air. The surface of the sample was covered with silicon oil to avoid dehydration during measurement, and the sample was allowed to equilibrate for at least 5 min. Strain sweep measurements were made to determine the maximum strain amplitude for each of the gel samples, and further measurements of the viscoelastic properties were performed within the linear region (i.e., below the maximum strain amplitude).

Oscillation measurements were performed over the frequency range 0.01–5 Hz. The elastic (storage) modulus (G′), the viscous (loss) modulus (G″) and the phase angle were used as measures of rheological behaviour. In rheological terms a gel has been defined as having a frequency-independent G′ which is considerably higher than G″ in a large frequency range (Ross-Murphy & McEvoy 1986; Almdal et al 1993) resulting in a low phase angle δ ($\tan \delta = G''/G'$). For a concentrated polymer solution, on the other hand, G′, G″ and the phase angle are frequency dependent (Ross-Murphy & McEvoy 1986). A phase angle lower than 45° ($\tan \delta < 1$) indicates a mainly elastic response whereas a phase angle higher than 45° ($\tan \delta > 1$) reflects a more liquid-like, viscous behaviour. Depending on which definition of gels that is used, entangled polymer solutions can be called gels, even though their rheological behaviour does not fall within the rheological definition. Furthermore, the zero-shear viscosity (η_0) of the linear polymer preparations was determined using the rotational viscometry mode of the rheometer. The zero-shear viscosity was estimated from the plateau of the Newtonian region, which was observed at very low shear rates.

Osmolality measurements

The osmolalities of all gel samples and of the solutions used (0.9% NaCl and Tris-buffered sucrose solution) were measured with a Wescor 5500 vapour pressure osmometer.

Preparation and handling of tissue

The time that elapsed from slaughter of the pig to removal of the nose was approximately 2 min. A longitudinal incision was made through the septum wall and the nose was kept in ice until the mucosa was removed. After exposing the nasal cavity on each side of the septum, the cavity mucosa (i.e., the mucous membrane covering the turbinates) was carefully removed using forceps and dissecting scissors, similar to the procedure described by Wadell et al (1999). The removal of the mucosa was completed within 2 h of the slaughter. Three or four small circular pieces of mucosa (diameter 14 mm) were cut out from the central parts of each cavity mucosa, avoiding the regions that had been in contact with the forceps. The pieces were then kept in ice-cold Tris-buffered sucrose solution (Tobyn et al 1995) until use, for a maximum of 5 h. In the mucoadhesion measurements each piece of mucosa was used only once and for each gel preparation the pieces used were always prepared from different pigs, to avoid systematic errors.

To validate the procedure for the preparation of mucosa from the nasal cavity, the uniformity of the mucus layer was assessed qualitatively by staining with Alcian blue 8GX solution (1 mg mL^{-1}). The staining procedure was partly adopted from Corne et al (1974).

Mucoadhesion measurements

A texture analyser, TA.HDi (Stable Micro Systems, Haslemere, UK), equipped with a 5-kg load cell, was used for all tensile strength measurements. The set-up had a force measurement accuracy of 1 mN and a distance resolution of 1 μm. The gel was brought to 37°C and then placed in a specially designed cylindrical plexiglass container (diameter 50 mm), holding approximately 70 mL gel. With sodium hyaluronate gels, however, a smaller container and sample volume (diameter 40 mm, 15 mL) were used because of a limited amount of polymer available. The gel container was then lightly centrifuged for 1 min at 209 *g* to remove entrapped air and ensure an even gel surface and then placed on the stationary surface of the instrument. Using cyanoacrylate adhesive, the piece of mucosa was attached to the upper movable stainless steel cylinder probe (diameter 14 mm) of the instrument, parallel to the stationary surface. The mucosa was lowered towards the gel surface at a constant speed. Having made contact with the gel surface (detected by a triggering force of 2 mN)

it was allowed to penetrate into the gel to a predetermined depth. Note that no force was applied during the contact phase, which is in contrast to most measurements performed with solid dosage forms. After a definite time in contact, the mucosa was slowly withdrawn upwards at a constant speed until a failure occurred between the surfaces. During the entire measurement a force–distance curve was recorded from which the tensile work (i.e., the area under the force–distance curve during the withdrawal phase), the fracture strength (peak force divided by contact area, i.e., 1.54 cm^2) and the deformation to failure were determined using the computer software Texture Expert Exceed (Stable Micro Systems, Haslemere, UK). These three parameters were suggested to be direct predictors of bioadhesive potential in a study by Chickering & Mathiowitz (1995), where the fracture theory of adhesion (Kammer 1983) was applied to analyse tensile strength measurements on bioadhesive microspheres.

The data acquisition rate of the measurements was chosen to give 100 data points/mm. Repeated measurements were performed with each replicate of the gel preparations, but before each measurement a fresh, smooth gel surface was created.

The effect of experimental and instrumental factors

The penetration depth was varied (0.5–2.0 mm) to determine a fixed value for further measurements. The influence of the withdrawal speed on the results was investigated, first with the stainless steel probe alone against the gel (0.1–2.0 mm s^{-1}) to establish the valid range of speeds, and then with mucosa attached to the probe (0.1–0.5 mm s^{-1}). Furthermore, the effect of varying the contact time (2, 8 and 20 min) was investigated for some of the gel preparations.

Measurements for the interpretation of mucoadhesive properties

To assist with the interpretation of the mucoadhesion measurements, we investigated the cohesiveness of the gel and of the mucus layer.

The cohesiveness of the gel was investigated by lowering and withdrawing the stainless steel probe alone against the gel (i.e., without mucosa) with the same experimental settings as used in the mucoadhesion measurements. The failure observed was within the gel for all preparations studied. This was detected by observing that a small amount of gel always remained on the surface of the probe after the measurement.

The cohesiveness of mucus was in preliminary measurements estimated by using the stainless steel probe against the mucosa, which was attached to a stationary plexiglass support. However, the rigid probe surface caused a very large deformation of the thin tissue. With a view to minimizing this problem, we used two other slightly different procedures in which mucosa was attached to both the stationary support and to the probe. In the first one, hereafter called the controlled-depth method, the penetration depth used was 0.6 mm, which ensured complete contact between the two mucous surfaces. With the second procedure, hereafter called the controlled-force method, the penetration depth was not predetermined but instead the force arising from the contact between the mucous surfaces was kept constant at 10 mN during the entire contact phase (2 min). To make the mucous surface more uniform and to minimize the deformation of the tissue, small amounts of mucus were placed on the surface of the stationary piece of mucosa before the measurement. This resulted in a penetration depth that varied from approximately 0.2 mm to 0.6 mm, depending on the resistance exhibited by the mucosa and on the thickness of the tissue. For both of the methods 25 measurements were performed, and the mucosa used was obtained from at least 6 different pigs.

Statistical analysis

For the data obtained from the measurements, the mean values, the standard deviations and the 95 % confidence intervals of the means were calculated. The influence of contact time was evaluated using a one-way analysis of variance. In the interpretation section, the data acquired in a mucoadhesion measurement were compared with those of the cohesiveness of the gel and the cohesiveness of mucus, respectively, using an unpaired, two-tailed *t*-test assuming unequal variances.

Results and Discussion

Properties of the gel preparations

The rheological parameters measured for each gel preparation are presented in Table 1. For the oscillatory data, values are presented for two different frequencies (0.05 and 1 Hz) to indicate the frequency dependence of each gel. The cross-linked gels of C934P were the only preparations exhibiting frequency independence and very low phase angles, and thus have the rheological behaviour of a gel. All other samples showed frequency

Table 1 The measured elastic modulus (G′), viscous modulus (G″) and phase angle (δ) for all preparations. The zero-shear viscosity (η_0) is given for the linear polymer preparations.

Preparation	Frequency (Hz)	G′ (Pa)	G″ (Pa)	δ (°)	tan δ	η_0 (Pa s)
2% C907	0.05	0.0054 (±0.0017)	0.0353 (±0.0048)	81.1 (±3.8)	7.3 (±3.3)	0.126 (±0.0071)
	1.00	0.103 (±0.014)	0.696 (±0.0089)	81.3 (±1.1)	6.63 (±0.87)	
7.4% C907	0.05	0.273 (±0.096)	2.35 (±0.070)	83.3 (±2.5)	9.5 (±4.4)	6.97 (±0.36)
	1.00	9.93 (±0.32)	22.1 (±0.67)	65.3 (±0.11)	2.18 (±0.012)	
7.4% B7LF	0.05	0.419 (±0.090)	1.44 (±0.082)	73.9 (±2.4)	3.51 (±0.56)	7.2 (±1.8)
	1.00	5.36 (±0.34)	14.0 (±0.26)	69.1 (±0.97)	2.62 (±0.13)	
2% B7HF	0.05	4.33 (±0.53)	6.43 (±0.72)	56.1 (±0.52)	1.32 (±0.029)	124 (±18)
	1.00	28.1 (±3.7)	31.4 (±2.9)	48.2 (±1.3)	1.11 (±0.053)	
0.5% SH	0.05	0.719 (±0.053)	1.77 (±0.10)	68.0 (±1.6)	2.48 (±0.20)	8.45 (±0.89)
	1.00	8.53 (±0.64)	6.97 (±0.43)	39.3 (±1.1)	0.818 (±0.032)	
1.5% SH	0.05	53.8 (±1.3)	54.1 (±0.76)	45.1 (±0.30)	1.00 (±0.011)	760 (±12)
	1.00	203 (±2.6)	81.0 (±0.21)	21.8 (±0.27)	0.400 (±0.0054)	
0.75% C934P	0.05	9.5 (±2.2)	0.614 (±0.091)	3.63 (±0.93)	0.064 (±0.016)	a
	1.00	10.1 (±2.4)	1.70 (±0.67)	9.1 (±2.6)	0.161 (±0.046)	
2% C934P	0.05	495 (±15)	17.2 (±2.5)	1.70 (±0.27)	0.0297 (±0.0046)	
	1.00	532 (±17)	24.8 (±0.36)	2.23 (±0.15)	0.0390 (±0.0027)	a

Mean values (±s.d.), n = 3. a, η_0 is not relevant since this is a cross-linked gel.

Table 2 Osmolalities of solutions and polymer preparations.

Sample	Osmolality (mOsmol kg^{-1})
0.9% NaCl	275 (±2.6)
Tris-buffered sucrose	456 (±1.2)
2% C907	362 (±14)
7.4% C907	669 (±52)
7.4% B7LF	520 (±15)
2% B7HF	330 (±6.6)
0.5% SH	306 (±7.1)
1.5% SH	341 (±24)
0.75% C934P	350 (±12)
2% C934P	397 (±43)

Mean values (±s.d.), n = 3–12.

dependence and phase angles greater than 45° (tan $\delta > 1$), reflecting the typical characteristics of entangled polymer solutions. Because of the high molecular weight of sodium hyaluronate, the viscous (liquid-like) behaviour is dominant at low frequencies, whereas a highly elastic behaviour is observed at high frequencies.

The osmolalities of the solutions and the gel preparations are shown in Table 2. The highest osmolalities were exhibited by the preparations with the highest polymer concentration (7.4% C907 and 7.4% B7LF). However, possible osmotic effects did not seem to be reflected in the mucoadhesion measurements. For these preparations the weakest region of the mucoadhesive joint was either the mucus layer or the gel itself, which is further discussed in the section on Interpretation of data.

Handling and staining of tissue

Staining pieces of freshly prepared mucosa with Alcian blue showed that the mucus layer was thick and uniform, thus the preparation and handling of tissue did not destroy the mucus layer. However, it can be noted that a much thinner and more uneven mucus layer was observed with tissue that had been frozen (−20°C) directly after preparation, stored for 24–48 h and thawed in the Tris-buffered sucrose solution before staining. Because of this, only fresh mucosa was used in the mucoadhesion measurements, within 5 h of removal from the nasal cavity.

Experimental and instrumental factors influencing the measured mucoadhesion

In this study we chose to use a large volume of gel, in comparison with some previously described methods

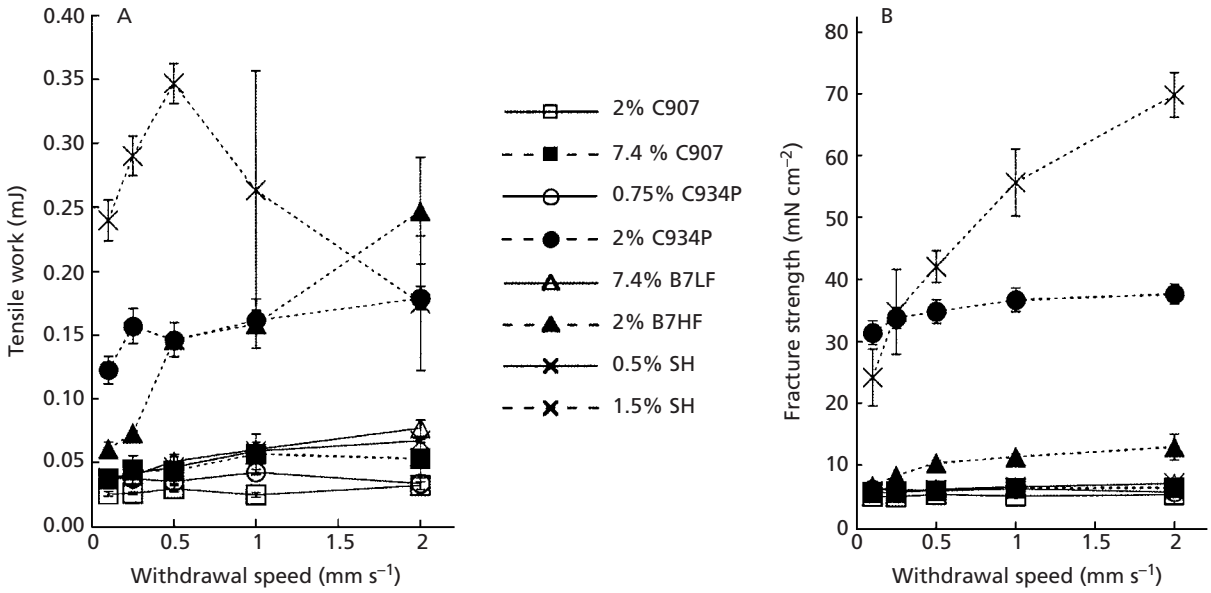

Figure 1 Influence of the withdrawal speed of the probe on the measured tensile work (A) and the fracture strength (B) of the gel preparations. The measurements were performed without mucosa (i.e., with the probe against the gel). Mean values ± 95% CI, n = 3–6.

for gels, where very small volumes have been used (e.g. Caramella et al 1994; Rossi et al 1996; Geraghty et al 1997). With respect to the in-vivo situation, it is certainly most appropriate to use a small gel volume in relation to a large area of mucosa. But our aim was to minimize the risk of creating stress in the gel sample when it was being touched by the mucous surface. If a measurement was performed with gels for which the relaxation was insufficient, systematic errors could be introduced. In a preliminary experiment the probe was allowed to penetrate 1 mm into the gel samples and the relaxation was followed. The force acting on the probe gradually declined and for all preparations based on the linear polymers the force was approximately zero within 2 min. This was interpreted as the preparations having relaxed almost completely. Cross-linked C934P gels, however, showed only a small decrease in the force after 2 min. This was not unexpected because, with its rigid polymer network, this gel would have a very long relaxation time. The risk of dehydration of the gel was evident if the measurements were performed at very long contact times. Therefore, 2 min was the initial contact time chosen.

The initial withdrawal speed of the probe was 0.1 mm s^{-1}, which is a value that had been suggested by other groups for corresponding mucoadhesion measurements on solid formulations (Ponchel et al 1987; Tobyn et al 1995) and with polymer gels (Caramella et al 1994; Tamburic & Craig 1997). These initial parameters were used as a base when investigating the influence of other instrumental settings.

When determining the most appropriate penetration depth we used the probe alone against the linear C907 (2% and 7.4%) and the cross-linked C934P (0.75% and 2%). For each of the gel samples the tensile work and the fracture strength showed consistent values independent of the penetration depth in the range 0.5–2.0 mm. With mucosa attached to the probe the contact between the surfaces was complete with a penetration depth of 1 mm, and the effects of the edges of the probe were negligible. Consequently, we chose this depth for all subsequent mucoadhesion measurements.

The influence of the withdrawal speed on the tensile work and fracture strength is shown in Figures 1 and 2. For highly entangled polymer solutions (i.e., preparations based on linear polymers with a high molecular weight or a high concentration), a dependence on the withdrawal speed was observed in the region 0.1–0.5 mm s^{-1} (Figure 1). Yet, because of the limited precision at higher speed, this was the region we chose to investigate further using mucosa attached to the probe (Figure 2). The tensile work showed less dependence of the withdrawal speed when using mucosa, and the precision for both the work and the fracture strength was best at 0.1 mm s^{-1}. The effect of withdrawal speed was most pronounced for the sodium hyaluronate preparations, particularly the deformation to failure decreased with increasing withdrawal speed (data not

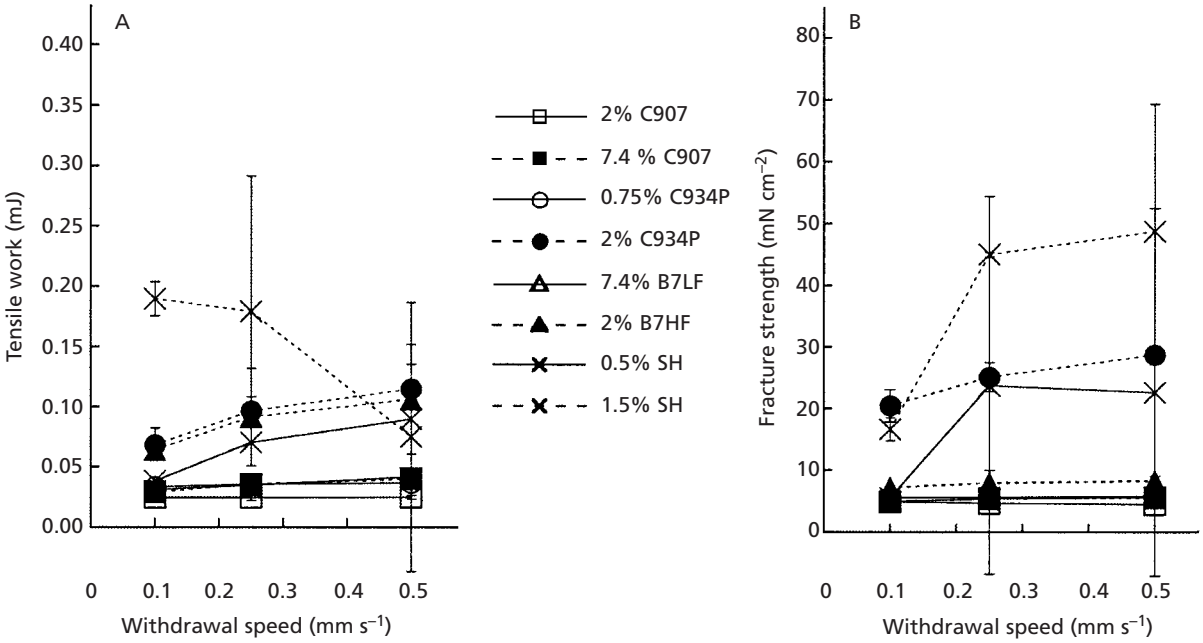

Figure 2 Influence of the withdrawal speed of the probe on the measured tensile work (A) and the fracture strength (B) of the gel preparations. The measurements were performed with mucosa attached to the probe. Mean values ± 95% CI, n = 3–9.

Table 3 The influence of contact time on the mucoadhesion measurements.

	Contact time (min)		
	2	8	20
Preparation	Tensile work (mJ)		
2% C907	0.0244 (±0.0015)	0.0269 (±0.0028)	0.0259 (±0.0011)
7.4% C907	0.0290 (±0.0030)	0.0305 (±0.0018)	0.0313 (±0.0021)
2% C934P	0.079 (±0.017)	0.104 (±0.017)	0.076 (±0.020)
1.5% SH	0.175 (±0.022)	0.1937 (±0.0097)	0.200 (±0.014)
Preparation	Fracture strength (mN cm^{-2})		
2% C907	4.85 (±0.42)	5.06 (±0.38)	5.03 (±0.22)
7.4% C907	4.86 (±0.46)	5.19 (±0.38)	5.08 (±0.25)
2% C934P	20.5 (±2.6)	23.8 (±2.2)	19.7 (±2.9)
1.5% SH	20.3 (±6.4)	17.6 (±2.0)	16.2 (±1.3)

Mean values (±95% CI), n = 3–5.

shown). This should be attributable to the viscoelastic behaviour of the preparations; at high speed mainly the elastic properties are reflected, resulting in a relatively small deformation and a rapid, 'brittle' failure. At 0.1 mm s^{-1} the deformation to failure was larger and more reproducible.

Other authors (Ponchel et al 1987; Caramella et al 1994; Tobyn et al 1995) have suggested a speed of 0.1 mm s^{-1} and our results agreed with this. Moreover, in view of conceivable in-vivo situations, a low withdrawal speed should be preferred, since vertical forces and movements are rare in-vivo.

The influence of contact time was investigated for preparations based on linear polymers with low molecular weight (C907, 2% and 7.4%) and high molecular weight (1.5% SH), and also with the cross-linked polymer (2% C934P), to include gels with different physicochemical and rheological properties. With contact times

of 2, 8 and 20 min, none of the gels studied exhibited any significant differences ($P > 0.1$) in the measured values of the tensile work and the fracture strength (Table 3). This indicates that any influence from the formation of molecular entanglements and secondary chemical bonds (e.g. hydrogen bonds) is rapid and occurs within 2 min.

For polymer gels it is generally accepted that surface and diffusion phenomena, interpenetration and the formation of molecular entanglements and secondary chemical bonds are of great importance in the mucoadhesion process. For dry dosage forms, on the other hand, where water movement and dehydration of mucus would be an important mechanism in the mucoadhesion process, the prehydration time and contact time have been shown to have a considerable influence on mucoadhesion (Ponchel et al 1987; Woolfson et al 1992; Tobyn et al 1995). Osmotic effects may also be of some importance in the case of a highly concentrated gel with limited amounts of water present. In this study, though, the osmotic effects – if there were any at all – were probably too small to be reflected in the mucoadhesion measurements. The systems studied are sufficiently relaxed gels for which a relatively high mobility of the polymer chains would be expected, thus enabling rapid interactions with the mucus.

Interpretation of mucoadhesive behaviour of the polymer gels

To distinguish between the measured parameters, the tensile work is in this section called the mucoadhesion work when obtained from the mucoadhesion measurements, and it is called the cohesive work of the gel and of the mucus, respectively, when acquired in the cohesiveness measurements.

Measurement of the cohesiveness of mucus

In the assessment of the cohesiveness of the mucus layer the choice of procedure proved to be important for the results obtained. With the controlled-depth method, the cohesive work was 0.0361 ± 0.0049 mJ and the fracture strength 33.1 ± 5.9 mN cm^{-2} (mean $\pm$ 95% CI). When the depth was varied initially, we observed a large variation in the data, particularly evident for the fracture strength. This was probably caused by differences in the degree of deformation of the tissue, which was dependent on the thickness of the tissue and on the penetration depth chosen. The influence of the thickness of the tissue has been reported previously by Jacques & Buri (1992) during mucoadhesion measurements on tablets. The controlled-force method seemed to be a better approach, since the deformation of the tissue was less variable and hence the thickness not so important. The cohesive work was 0.0265 ± 0.0018 mJ, and the fracture strength was 17.0 ± 3.6 mN cm^{-2}. The difference in data between the two methods was less considerable for the cohesive work than for the fracture strength. Thus, the cohesive work seems to be less sensitive to experimental factors and may be more applicable for interpreting mucoadhesive properties. However, the influence of the method on the measured cohesiveness of the mucus should be looked into further and is the subject of ongoing studies.

Interpretation of data

During the withdrawal of the mucosa from the gel, a failure will occur in the weakest of the three regions of the mucoadhesive complex – in the gel, in the mucus or in the interpenetration layer (the interface layer between the gel and the mucus where possible interactions strengthen the mucus layer). Consequently, the force–distance curve recorded in the measurement gives a measure of the strength of the bonds in the weakest region. We propose that to interpret the results and to determine in which region the failure occurs (i.e., which bonds that are reflected in the acquired data), the cohesiveness of the single components of the complex (the gel and the mucus) should also be measured. A comparison of the cohesiveness of these components with the results from the mucoadhesion measurement would give a picture of which region is the weakest of the system.

For the first five preparations in Figure 3A, the mucoadhesion work did not differ significantly ($P > 0.1$) from the cohesive works of the gel and the mucus. The weakest region of the mucoadhesive joint appears to be either the mucus layer or the gel itself. The preparations exhibiting this behaviour were those with weak elastic properties and liquid-like (viscous) features (i.e., those gels prepared from linear polymers with low molecular weight and/or those with low concentration).

For the other three preparations the mucoadhesion work was significantly higher ($P < 0.005$) than the cohesive work of the mucus, thus a strengthening of the mucus layer must have occurred. The strengthening might arise from the entanglement of the polymer chains and the mucus glycoproteins, the formation of chemical bonds and/or from dehydration of the mucus layer, as with dry dosage forms. For the two preparations exhibiting the highest elastic properties, 2% C934P and 1.5% SH, the mucoadhesion work was not only higher than the cohesive work of the mucus, but also significantly lower ($P < 0.0005$) than the cohesive work of

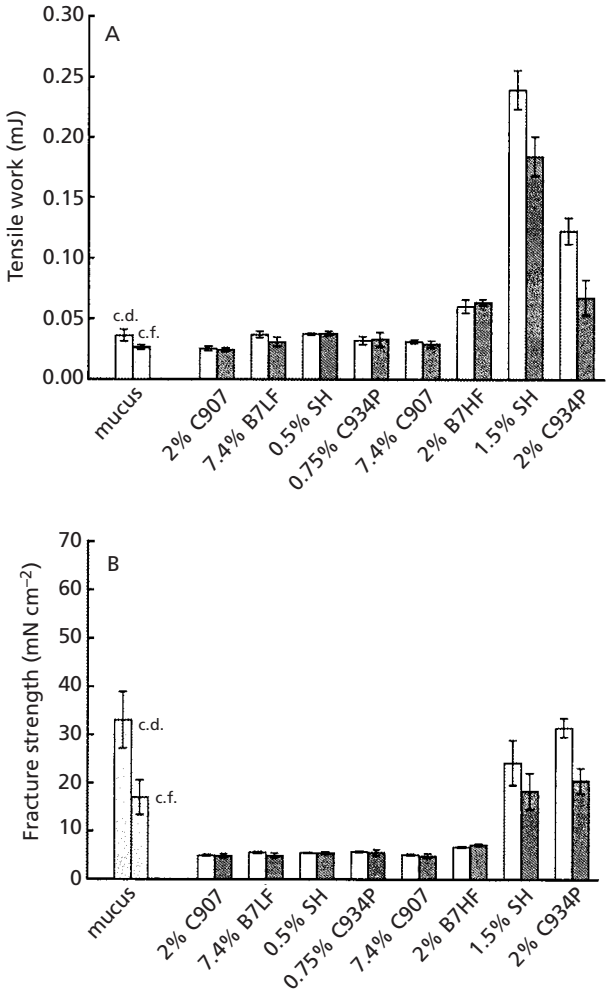

Figure 3 Measurements for the interpretation of mucoadhesive properties of the gel preparations. The measured tensile work (A) and the fracture strength (B) from mucoadhesion measurements (dark grey, n = 3–9) and from measurements of the cohesiveness of the gel preparations (white, n = 6–9) and the mucus layer (light grey; c.d.: controlled-depth method, n = 25; c.f.: controlled-force method, n = 25). Mean values ± 95% CI.

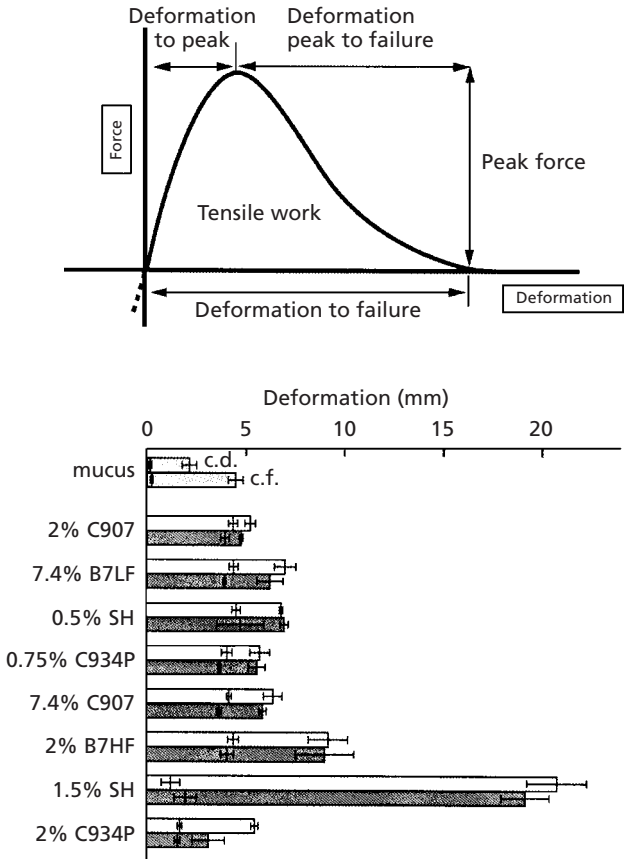

Figure 4 Measurements for the interpretation of mucoadhesive properties of the gel preparations. The upper part shows the definitions of the deformation parameters. The deformation to peak (left part of the bars) and the deformation to failure (the full length of the bars) from mucoadhesion measurements (dark grey, n = 3–9) and from measurements of the cohesiveness of the gel preparations (white, n = 6–9) and the mucus layer (light grey; c.d.: controlled-depth method, n = 25; c.f.: controlled-force method, n = 25). Mean values ± 95% CI.

the gel. In this case, the failure in a mucoadhesion measurement should occur in the strengthened mucus layer. For both of the preparations this was seen irrespective of the contact time.

Similar observations were made for the fracture strength with respect to the mucoadhesion and gel cohesiveness measurements, respectively (Figure 3B). However, the dependence of the cohesiveness of the mucus layer on the method used made it a bit more difficult to interpret any eventual strengthening of the mucus layer, if the fracture strength alone were to be considered.

The shape of the force–deformation curve obtained for polymeric microspheres has been discussed in detail by Chickering & Mathiowitz (1995) and several parallels can be drawn to this study. However, polymer gels can, because of their flexible network structure, be deformed to a larger extent than microspheres. Hence the force–deformation curve would not only reflect the deformation of the tissue and the mucoadhesive bonds but also the deformation and the rheological behaviour of the gel. Dyvik & Graffner (1992) discussed this in terms of high or low viscosity of the samples. In this study, similar observations were made: the viscous and elastic properties of the gel influenced the force–deformation curve, mainly by affecting the deformation to failure. When measuring the cohesiveness of the gels (see part of

Figure 4), the pronounced viscoelastic preparations, 1.5% SH and 2% B7HF for example, were extended during the withdrawal phase to form an elongated string that eventually broke. This was reflected in the data as a large deformation to failure, giving a relatively large tensile work despite the fairly low fracture strength (Figure 3). Not unexpectedly, the deformation to failure was much smaller for the cross-linked highly elastic 2% C934P, leading to a relatively low tensile work though the fracture strength was fairly high (Figure 3).

General discussion

In most mucoadhesion studies published previously, only the mucoadhesion measurements were performed without estimating the cohesiveness of the different regions. For solid dosage forms this could be adequate provided that the dosage form itself is much stronger than the mucus layer. Thus the work and the force measured would reflect a strengthening of the mucus, and the ranking of different dosage forms would probably reflect the in-vivo performance. This could, presumably, also be satisfactory for gel preparations if the aim is to find the dosage form with the most potential to give a prolonged contact with the mucous membrane. But the work and the force from such a measurement of a gel would reflect the failure of the weakest layer, which in many cases may be the dosage form itself, and therefore it would not say anything about true mucoadhesion (i.e., the interactions between the mucus and the gel). The rheological method that has been used for polymer gels (Hassan & Gallo 1990; Caramella et al 1994; Mortazavi 1995; Madsen et al 1998; Caramella et al 1999; Hägerström et al 2000) may, on the other hand, enable an assessment to be made of the interactions between the mucus and the polymer present in the dosage form. However, this information would not reflect the in-vivo performance of the dosage form since it is based on the assumption that the failure always occurs in the interpenetration layer.

In this study, we propose a method that would be useful for understanding the significance of mucoadhesion compared with the importance of the cohesive properties of the gel, for the residence time of the preparation in-vivo. For preparations that do not have a sufficient level of cohesiveness, no information is gained about possible interactions between the mucus and the polymer, but the question is whether such a weak dosage form really can give a long residence time at the mucous membrane. Further work with a wider range of gels is needed to prove a general applicability of the proposed interpretation method, and is the subject of ongoing studies.

Conclusions

This new tensile strength method seems to offer a robust and sensitive way to measure mucoadhesion of polymer gels. We have suggested appropriate values for the withdrawal speed and penetration depth of the mucosa and discussed the choice of contact time. Furthermore, we have proposed a method for interpreting the mucoadhesive properties of gel preparations. This involves assessing the cohesiveness of the gel and of the mucus layer independently, and comparing these with the failure observed in a mucoadhesion measurement. This procedure and the subsequent interpretation of the results offers a good basis from which to assess whether the measured tensile work reflects a cohesive failure of the gel or a genuine interaction of the gel preparation with the mucus layer.

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