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Mucoadhesion of both film-forming and non-film-forming polymeric materials as evaluated with the Wilhelmy plate method

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Summary

In contrast to previous studies applying the Wilhelmy plate method, this investigation used native mucus to evaluate the mucoadhesion tendency of polymeric materials. The mucus was obtained by scraping freshly isolated porcine small intestines and was used without further processing or purification. A very strict experimental set-up and procedure ensured reproducible bioadhesion measurements. Polymer coated glass plates were vertically pulled out of mucus samples and extraction forces were continuously measured. Two types of film-forming polymers could be distinguished. After extraction of the polymer coated plate, mucus clots were formed on the surface of the carboxymethylcellulose (CMC), hydroxypropylmethylcellulose (HPMC) and hydroxypropylmethylcellulose phthalate (HPMCP) coated glass plates, whereas no permanent mucus clots were formed on the Eudragit RS 100 coated glass plates. When clots were formed the mucoadhesive force depended linearly on the polymer surface area submerged in the mucus. In order to be able also to evaluate the mucoadhesion tendency of non-film-forming polymers and particulate systems, a double coating technique was applied with Eudragit RS 100 as primer. The obtained rank order for mucoadhesive tendency was: CMC > HPMC K100M > HPMCP > polycarbophil > HPMC K4M > amylopectin > Eudragit RS 100. Inclusion of calcium chloride in the HPMC and CMC coats resulted in higher mucoadhesive forces. Inclusion of the mucolytic *N*-acetylcysteine only marginally increased the mucoadhesive force of these polymers.

Introduction

Several apparatus for the in vitro determination of bioadhesion have been described (Duchêne et al., 1988; Rao and Buri, 1989). The Wilhelmy plate surface tension technique (Weser, 1980), modified by Smart et al. (1984) to measure mucoadhesion, is a relatively inexpensive, rapid and simple in vitro method. With this method a polymer coated glass plate is suspended from a microbalance into a mucus sample. After an equilibration time of 10 min the plate is withdrawn from the mucus. One approach to quantitate bioadhesion is to calculate the fracture energy from the surface under the corresponding force-elongation curves (Ponchel et al., 1987). The more simple approach used in this study is to record the maximum force by the microbalance at the

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moment the plate is detached from the mucus. In their experiments, Smart et al. (1984) applied 'homogenized' guinea-pig mucus, with a marked reduction in gel properties compared to native crude mucus. In order to perform the Wilhelmy plate mucoadhesion testing of polymeric materials under more physiologically valid conditions, in the present study only native mucus possessing its original gel properties was used. Experimental variables considered in this study were the penetration depth and the rate at which the plate is pulled out of the mucus gel. Originally, the test was only used for the evaluation of film-forming polymers. In order to make the test also applicable to non-film-forming and particulate systems, a double coating technique was applied.

Materials and Methods

Materials

Calcium polycarbophil (Lee Lab. Inc., U.S.A.), sodium carboxymethylcellulose (Akzo, The Netherlands), cross-linked amylopectin (AveBe, Veendam, The Netherlands), Eudragit RS 100 (Röhm Pharma, Germany), hydroxypropylmethylcellulose K4M and K100M Premium (Dow Chemical, U.S.A.), hydroxypropylmethylcellulose phthalate (Shinetsu, Japan), *N*-acetylcysteine (OPG, The Netherlands), and calcium chloride and sodium chloride (Baker, The Netherlands) were used as obtained from the manufacturer.

Isolation of mucus

The procedure of isolation of native mucus was essentially that of Allen (1978). Mucus was scraped from the interior of the small intestines, freshly isolated from Yorkshire pigs. Pieces of intestines contaminated with food or faeces were discarded. The scraping was performed in a gentle way such that only the upper layer of the mucosa was gathered. Contamination of the mucus with blood or ruptured cells was avoided, as was checked by light microscopy. The yield of this isolation procedure was approx. 70 ml of mucus for each length of porcine small intestines (approx. 20–25 m). The mucus was stored in small portions at −20 °C and thawed 1 h before the experiments. The pH of the thawed mucus was 6.5.

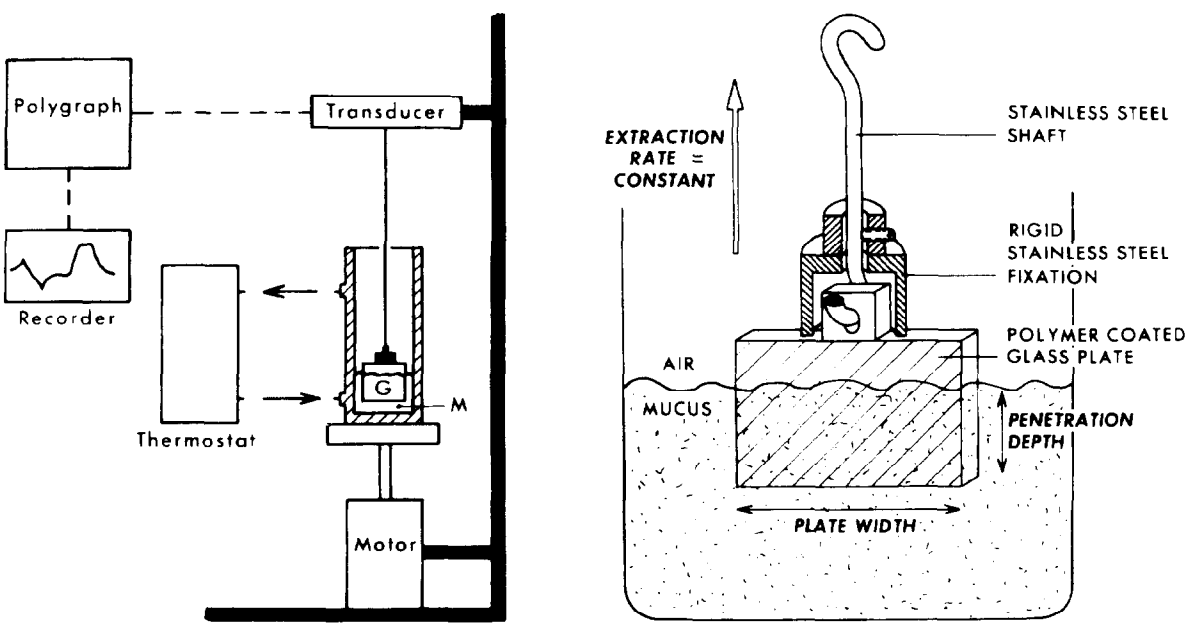

Fig. 1. Schematic representation of the Wilhelmy plate mucoadhesive force measurement apparatus and the device for the rigid attachment of (polymer coated) glass plates. G, glass plate; M, mucus gel.

'Homogenized' mucus was prepared from native mucus essentially according to the method described by Smart et al. (1984). 200 ml of scraped mucus was gently stirred in the presence of 200 ml of distilled water for 24 h at 4 °C and subsequently centrifuged for 30 min at 27500 × *g*. Approx. 200 ml of homogenized mucus was obtained, divided into aliquots and frozen at −20 °C. The dry weight content of the homogenized mucus was 14% (w/w) as was determined by drying the mucus for 18 h at 60 °C in vacuo.

Apparatus

The set-up for bioadhesive force measurement is a modified surface tension apparatus, equipped with a glass Wilhelmy plate, which is suspended in medium (Fig. 1). This modified Wilhelmy plate surface tension apparatus consists of a thermostated glass container and a transducer (FT-03c), which is connected to a polygraph (Grass 79c, Quincy Mass) and a recorder (Kipp en Zonen, type BD 41). The plate is moved into or out of the medium by increasing or lowering the height of the bath with the help of an infusion pump (Precidor 5003). Calibration of the system showed that the responses measured by the transducer as displayed on the recorder were linearly proportional to the applied forces for all sensitivities of transducer and recorder employed.

In the case where clean glass plates are applied, the F_{max} value obtained can be used to calculate the surface free energy (Γ) of the penetrated liquid, by applying the formula: $\Gamma = F_{max}/L_b \cdot \cos \Theta$ (Weser, 1980). In this equation F_{max} represents the maximal force measured by the transducer, L_b the perimeter of the glass plate and Θ the contact angle.

Mucoadhesive force measurements

An uncoated or polymer coated glass plate was submerged at a controlled speed into 15 ml of freshly thawed mucus at 37 °C to a predetermined depth (2–8 mm). After 10 min of equilibration the plate was pulled out of the mucus at the same rate but in the reverse direction. During the equilibration time, the mucus gel attains its original level and the polymer is hydrated by the mucus water. Accurate rates in the range between 1.0 and 10.0 mm/min were achieved by using the synchronous motor of the infusion pump. In order to ensure a reproducible vertical movement of the glass plates into and out of the mucus gel, the glass plates were rigidly connected with a stainless steel shaft directly to the transducer (Fig. 1). This resulted in very reproducible adhesion measurements with only small variances in the evaluated mucoadhesive forces.

Three different series of glass plates were used with nominal widths of 5, 10 and 25 mm. The actual dimensions (width × thickness) of the glass plates were 5.00 × 1.54 mm, 10.02 × 1.55 mm and 24.65 × 1.62 mm. The internal diameter of the container used was 3 cm. The glass plates were coated with polymer by dipping into 10% (w/w) solutions of the polymers, and drying for 1 h at 60 °C. An alternative coating technique employed was precoating the glass plate with Eudragit RS 100 from an ethanolic solution, drying the plate for 3 min at 60 °C, and covering the sticky plate with dry polymeric particles.

Viscosity determinations

The viscosity of both native and homogenized mucus was determined at 37 ± 0.5 °C with a cone and plate viscometer (Rheomat 15). The shear stresses were read from the instrument at a number of fixed settings of the shear rate. Mucus was temperature equilibrated in the viscometer for 10 min before starting the series of measurements. For both increasing and decreasing shear rates, the shear stresses were read after 1 min. Because of the large difference in viscoelastic behaviour between native and homogenized mucus, two different cones (with plate 1/2) and subsequently two different sets of shear rate settings had to be used.

Results and Discussion

Native mucus appeared to differ strongly from homogenized mucus in its rheological properties, as determined by viscometry (Fig. 2). For both the homogenized and native mucus, the standard deviation in the shear stress values is approx. 10% ($n = 3$). To make our results as meaningful

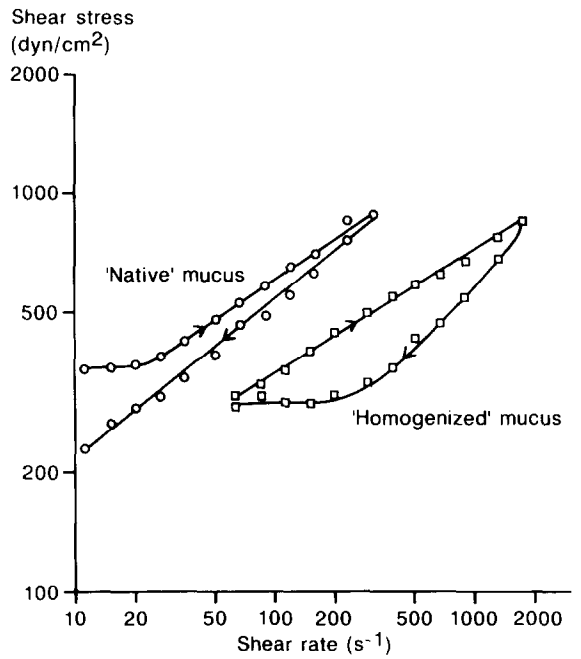

Fig. 2. Rheology of native and homogenized mucus as measured with a cone and plate viscometer at a temperature of 37 °C. The arrows indicate the shear stress determinations at the applied series of increasing and decreasing shear rates.

for the physiological situation as possible, it was therefore decided to use only native mucus for the determination of the mucoadhesion of polymers.

For all mucoadhesion determinations with the Wilhelmy plate method, complete force/time diagrams were recorded. Several stages can be discerned (Fig. 3). The experiment commences with the recording of the weight of the plate in air and zeroing the amplifier (stage 1). Upon raising the bath, the plate penetrates the medium at a constant velocity. As soon as the penetration movement is stopped the most negative value is found (stage 4), relaxing to an equilibrium value (stage 5). When the plate movement is reversed the plate is removed from the medium, reaching a maximum value (stage 7) at a penetration depth of approx. 0 mm. At this point a mucus bridge between polymer coated glass plate and mucus has been formed. Upon further extraction the perimeter of the mucus bridge is reduced and eventually fractures. The force measured after fracture equals the gross weight of the glass plate including adhering mucus (stage 8). The mucoadhesive force F_{max} was therefore defined as the net force difference between the forces measured at stages 7 and 8.

Mucoadhesive measurements with uncoated glass plates

In order to understand more completely the results of the mucoadhesive experiments in which polymer coated glass plates are pulled out of mucus, measurements were also performed with uncoated glass plates.

By pulling glass plates from water and measuring the force of detachment (F_{max}) the surface free energy of water (Γ) can be calculated (Weser, 1980). Assuming a contact angle of 90° between clean glass and water, an average surface free energy of $(69 \pm 5) \times 10^{-3}$ J m^{-2} ($n = 9$) can be calculated (Table 1). This result is close to the literature value of 70.0×10^{-3} J m^{-2} (Weast, 1975). As expected, the values obtained for the

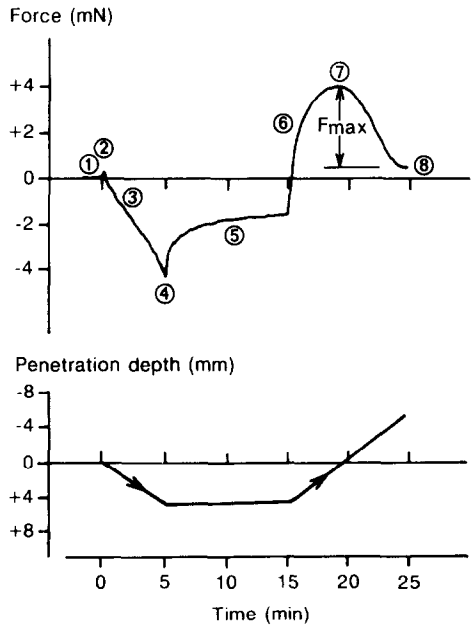

Fig. 3. A representative force/time diagram for the mucoadhesive force (F_{max}) determination for a polymer coated plate with a width of 5 mm, a penetration depth of 5 mm, a penetration rate and extraction rate of 1 mm/min and an equilibration time of 10 min.

TABLE 1

Maximum forces as measured by pulling clean glass plates with nominal widths of 5, 10 and 25 mm at a rate of 1 mm / min out of water or out of mucus at 37 °C (values are given with their standard deviations (n = 3))

Plate		Water		Mucus	
Width (mm)	Perimeter (mm)	F_{max} (mN)	$\Gamma_w \cdot \cos \Theta$ (10^{-3} J m^{-2})	F_{max} (mN)	F_{max} ratio
5	13.08 ± 0.08	0.78 ± 0.10	60 ± 8	1.57 ± 0.10	2.01
10	23.14 ± 0.12	1.57 ± 0.10	68 ± 4	3.13 ± 0.21	1.99
25	52.54 ± 0.10	4.18 ± 0.10	80 ± 2	8.35 ± 0.11	2.00

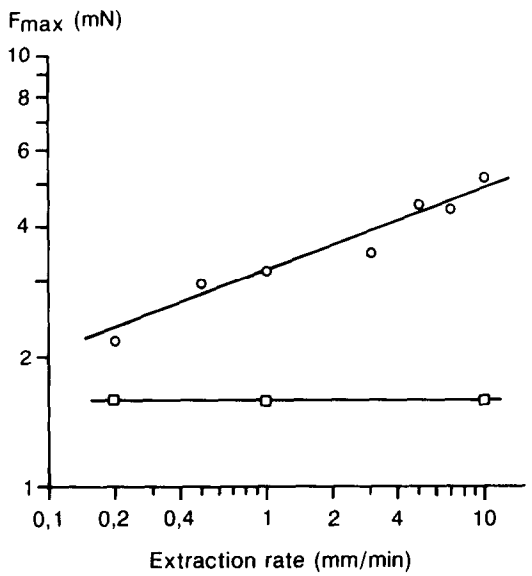

Fig. 4. Effect of plate extraction rate on the mucoadhesive force (F_{max}) for a clean glass plate with a width of 10 mm. Each point is the mean of 2–4 experiments.

surface free energy of water were independent of the velocity at which the glass plate was pulled out of the water (Fig. 4) and of the penetration depth of the glass plate in water (Fig. 5). Also, after immersion in mucus for clean glass plates, no effect of penetration depth was found (Fig. 5). However, for mucus the F_{max} values obtained for uncoated glass plates appeared to be dependent on the rate of plate extraction out of mucus (Fig. 4). Fig. 4 clearly demonstrates that the viscoelastic nature of mucus influences the mucoadhesive measurements and that the degree of bioadhesiveness (F_{max} value) of a certain surface will depend on the applied shear rate. Smart et al. (1984) defined the mucoadhesive force of a polymer as the ratio of the maximum force of detachment measured for the polymer coated glass plates and the corresponding uncoated glass plate. From this study it can be concluded that although the mucoadhesive force is defined as a ratio, it will still depend on the applied plate extraction rate.

Comparison of the F_{max} values obtained for clean glass plates extracted from water and from

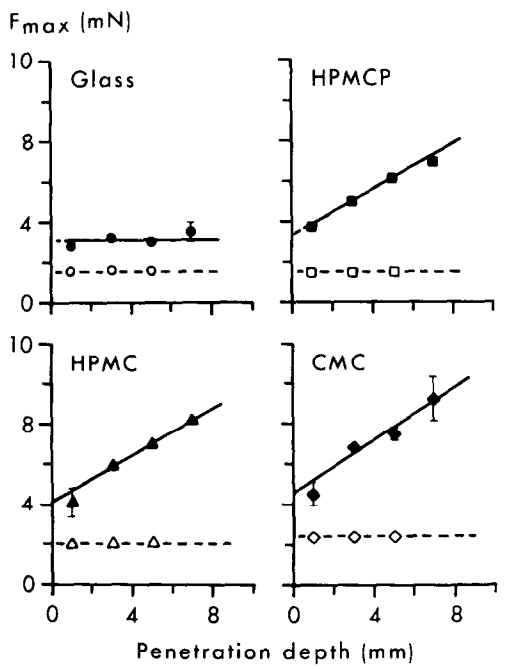

Fig. 5. Effect of penetration depth on the adhesive forces (F_{max}) as measured for clean glass plates and polymer coated glass plates. The open symbols refer to water as extraction medium, the closed symbols to mucus as extraction medium. Plate extraction rates: 1 mm/min.

mucus at a constant extraction rate of 1 mm/min, indicated a constant factor of 2 higher F_{max} value for mucus (Table 1), independent of the plate width and the penetration depth employed (Fig. 5). Extrapolation of the data of Fig. 4 indicates that the F_{max} ratio for clean glass plates becomes 1 at plate extraction rates of approx. $2-3 \times 10^{-2}$ mm/min. It is difficult to give a reliable estimate for the true in vivo shearing rate in the small intestines. However, for a human small intestinal length of 3 m and a small colon transit time of 5 h, an educated guess for the physiological shearing rate is 10 mm/min. For this shearing rate, an F_{max} ratio of 3 is found (Fig. 4). In order to standardize the experimental conditions, all the subsequent experiments with polymer coated glass plates were performed at a relatively low plate extraction rate of 1 mm/min. It should be borne in mind, however, that due to the higher in vivo shear rates the in vivo mucoadhesion forces of these polymers might be slightly higher (approx. 50%) than the in vitro experimentally determined F_{max} values.

Mucoadhesive measurements with polymer coated glass plates

Experiments were started with Eudragit RS 100, since it is a polymer often used in coatings of oral extended-release dosage forms. Eudragit RS 100 is a water-insoluble copolymer of acrylic and methacrylic esters with a low content of quaternary ammonium ions (Table 2). The F_{max} value determined for Eudragit RS 100 coated glass plates was even smaller than for uncoated glass plates (cf. Table 4 with Table 1), indicating that this film-forming polymer has no mucoadhesive tendency. The low value for Eudragit RS 100 can be explained by poor wetting of the polymer ($\cos \Theta \ll 1$), or in terms of the interpenetration theory as extended to mucoadhesion (Peppas and Buri, 1985). Eudragit RS 100 does not dissolve in water but swells only slightly Lehmann and Dreher, 1969), making the interpenetration of Eudragit RS 100 and mucus polymeric chains less likely. No visible mucus permanently adhered to the Eudragit RS 100 coated glass plate after extraction.

The second class of film-forming polymers tested for mucoadhesion was the cellulose derivatives. The polymers studied were all more or less soluble in mucus isolated from porcine small intestines (Table 2). Unlike Eudragit RS 100 coated glass plates, the immersion of HPMCP, HPMC K100M, and CMC coated glass plates in mucus gel resulted in formation of permanent clots of mucus on the polymer surface. Upon extraction the mucus clot did not fracture at the polymer/mucus interface, but the locus of failure was in

TABLE 2

Characteristics of the film-forming polymers used in this study

Film former	Type	Molecular weight indication	Aqueous solubility
Eudragit RS 100	Copolymer of acrylic and methacrylic acid esters with a low content of quaternary ammonium groups	150 000	practically insoluble
HPMCP	Hydroxypropylmethylcellulose phthalate (anionic polymer)	20 000	insoluble in water; soluble in small intestinal fluid
CMC	Sodium carboxymethylcellulose (anionic polymer)	6650 cps [a]	soluble
HPMC K4M	Hydroxypropylmethylcellulose (non-ionic copolymer of hydroxypropyl- and methoxylcellulose)	4×10^3 cps [a]	soluble
HPMC K100M	Hydroxypropylmethylcellulose (non-ionic copolymer of hydroxypropyl- and methoxylcellulose)	108×10^3 cps [a]	soluble

[a] Viscosity of a 2% solution at 20 °C.

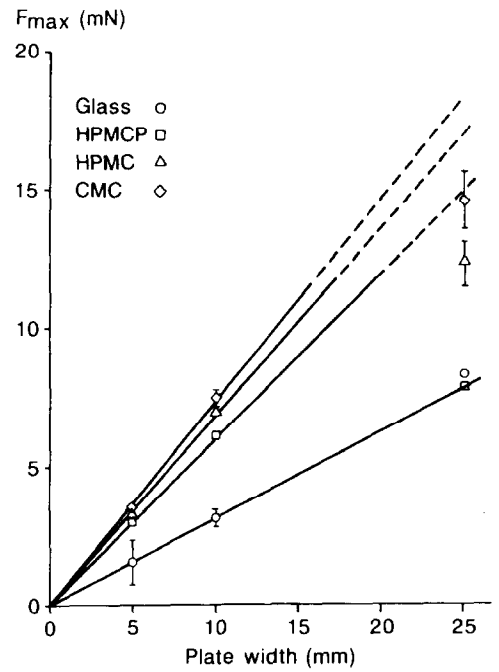

Fig. 6. Effect of plate width on the mucoadhesive force (F_{max}) as measured for clean glass plates and for plates covered with the polymers HPMCP, HPMC K100M and CMC. The extraction rate was 1 mm/min, the penetration depth 5 mm and the equilibration time 10 min.

the mucus gel matrix itself. What is apparently measured in these mucoadhesion experiments is not the mucoadhesion force of the polymer itself, but the mucus/mucus fracture force, modulated by long-distance influences of the type of cellulose polymer. Fracture occurred after the plate had passed through the mucus/air surface, explaining why correction for the adhering mucus had to take place (the mucoadhesive force F_{max} was defined as the force difference between stages 7 and 8 (Fig. 3)). Linearity of the results was found up to plate widths of 10 mm (Fig. 6); for plate widths of 25 mm the container became too small to accommodate the adhering clots. The obtained rank order for the bioadhesive force measured at 37 °C with native mucus was: CMC > HPMC K100M > HPMCP (Fig. 6), but the differences were only small. Although Smart et al. (1984) performed their experiments at 20 °C with homogenized mucus, the rank order of mucoadhesive force for CMC and HPMC is in agreement with their findings. However, for CMC and HPMC, Smart et al. found mean bioadhesive forces of 193 and 125% relative to forces measured for clean glass, whereas in this study values of 217 and 187% were determined for the respective polymers. Because the applied extraction rates (1 mm/min) and penetration depths (5 mm) were the same in both sets of experiments, the quantitative differences must be explained in terms of the differences in physico-chemical properties between native and homogenized mucus. One important difference could be the pH, since CMC can be expected to have a mucoadhesive pH dependency. In a separate series of experiments, it was shown that the F_{max} value obtained for the cellulose derivatives was linearly proportional to the applied penetration depth (Fig. 5). This contrasts with the measurements using clean and Eudragit RS 100 coated glass plates, where for both the extraction media water and mucus, F_{max} values independent of the penetration depths were found, just as in ordinary surface tension measurements (data for Eudragit RS 100 not shown).

An alternative approach to increasing the mucoadhesive force of a polymer is by altering the physical or chemical structure of the mucus at its interface. The mucolytic *N*-acetylcysteine is expected to lower the viscosity of the mucus gel. Direct coating of the glass plates with *N*-acetylcysteine resulted in a doubling of the adhesive force of mucus for glass (Table 3). However, the incorporation of *N*-acetylcysteine in the polymer coat of the glass plate only slightly enhanced the bioadhesive force (Table 3). Possibly longer contact times could lead to larger increases in the values of F_{max}. An agent that has been described as lowering the mucoadhesion of CMC is calcium (Gayot, 1985). The reason given is that calcium lowers the aqueous solubility of this anionic polymer (Gayot, 1985), leading to less polymer/mucus chain interpenetration. Leung and Robinson (1988) explained a decreased mucin-mucin tangential shear on addition of calcium in terms of a reduced expansion of the mucus network. However, in our experimental set-up, incorporation of calcium chloride in the coat layer doubled the

TABLE 3

Muco-adhesive forces measured for three types of film-forming cellulose polymers with and without incorporation of N-acetylcysteine and calcium chloride in the polymeric film (plate extraction rate, 1 mm / min; penetration depth, 5 mm)

Surface	Additive	F_{max} (mN) ± S.D.
Glass	–	3.2 ± 0.1
	N-acetylcysteine	6.5 ± 0.1
HPMCP	–	6.6 ± 0.2
	N-acetylcysteine	7.4 ± 0.2
HPMC K100M	–	7.0 ± 0.1
	N-acetylcysteine	7.4 ± 0.4
	calcium chloride	12.7 ± 0.6
CMC	–	7.5 ± 0.3
	N-acetylcysteine	7.6 ± 0.4
	calcium chloride	11.2 ± 0.4

mucoadhesive forces for both the ionic polymer CMC and the nonionic polymer HPMC (Table 3).

Extension of the Wilhelmy plate method to non-film-forming polymers can be achieved by applying a double coating technique. According to this technique, polymer powders are adhered to partially dried Eudragit RS 100 film-coated glass plates. Since the mucoadhesion of Eudragit RS 100 is equal to that of glass, the results obtained with the double coating technique can be directly compared with those obtained with the film-forming technique. In most cases the increase in plate surface area will be negligible. For CMC no differences were found for the two coating techniques (Table 4), leading to the conclusion that for CMC bioadhesion and surface area are probably not affected by the coating technique. For large particles of HPMCP doubled F_{max} values were determined, when compared to F_{max} values found for small particle coated plates and film-coated plates. Possibly the applied contact time of 10 min is not sufficient for large HPMCP particles to establish an equilibrium hydrated state at the polymer/mucus interface, leading to different viscoelastic properties and consequently to altered F_{max} values. This idea is supported by the differences in F_{max} values observed for two different viscosity grades of HPMC. For the high viscosity grade K100M of HPMC a much higher F_{max} value is obtained than for the corresponding low viscosity grade K4M (Table 3), in agreement with the finding that the mucoadhesive force increases with the molecular weight of the mucoadhesive polymer (Gurny et al., 1984). Application of this double coating technique to amylopectin and polycarbophil particles resulted in relatively low F_{max} values (Table 4), indicating that these two non-film-forming polymers are only slightly mucoadhesive under the conditions tested.

Concluding Remarks

This paper describes an evaluation and modification of the Wilhelmy plate mucoadhesion test. It was demonstrated that native mucus can be used directly without further homogenization to

TABLE 4

Muco-adhesive forces (n = 2) obtained for Eudragit coated glass plates, surface coated with solid particles (plate extraction rate, 1 mm / min; penetration depth, 5 mm; values in parentheses are the corresponding values obtained with film-coated glass plates)

Surface of Wilhelmy plate		F_{max} (mN)	Mass of adherent mucus (mg)
Eudragit film-coated glass plates	without additional coating	2.9	0
	+ HPMCP small particles	6.5 (6.6)	15
	+ HPMCP large particles	13.6 (6.6)	15
	+ CMC particles	7.3 (7.5)	16
	+ HPMC K4M particles	4.7	19
	+ HPMC K100M particles	8.1 (7.0)	10
	+ amylopectin particles	4.2	14
	+ polycarbophil particles	5.7	10

test the mucoadhesion tendency of polymers. Variables such as plate extraction rate, plate width, and penetration depth were examined. By applying particles on the surface of Eudragit RS 100 coated glass plates the method was demonstrated to be suitable for the testing of non-film forming polymers. Under the experimental conditions selected, the mucoadhesive forces of quite distinctive polymers such as CMC, HPMC and HPMCP appeared to be only marginally different. This can be explained by realising that the highest mucoadhesive force measurable with this technique is the cohesion force of mucus for itself. Once mucus is strongly bound to the polymer surface the forces to detach a mucus covered plate from mucus will be measured. This could indicate that it is of no use to synthesize polymers for the coating of peroral dosage forms that form stronger mucoadhesive bonds than the mucin-mucin bonds.

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