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An in-vitro investigation of mucosa-adhesive materials for use in controlled drug delivery

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An in-vitro test system was developed to investigate the adhesiveness of various materials to mucus. The results obtained showed good agreement with the findings of previous in-vivo evaluations of mucosa-adhesives. Further investigations found that these materials become adhesive on hydration. Chain length, and the presence of ionizable groups in the molecule, were found to be determinate factors. The physical nature of the gel, and the location at which the mucoadhesive materials hydrated, were of less importance.

Recent interest has been expressed in the delivery of drugs to, or via mucous membranes by the use of adhesive materials. Several mucosa-adhesive formulations are now available or under development (Chen & Cyr 1970; Simpson 1968; Nagai et al 1979; Abrams 1982).

In most cases, assessment of adhesive performance was determined in-vivo using subjective evaluation. In this work an in-vitro model was developed to investigate mucosa-adhesive materials in terms of strength of adhesive bonding and the mechanism of such interactions.

The surfaces of mucous membranes are covered with a continuous layer of mucus. Penetration of, or adhesion to, this layer would be a requirement for mucosa-adhesion. Therefore, in this model the force required to detach a glass plate, coated with the test material, from an isolated mucus gel was measured.

MATERIALS AND METHODS

Materials

Test materials were: sodium alginate (Alginate Industries Ltd., Girvan, Ayrshire), sodium carboxymethylcellulose (SCMC) and hydroxypropylmethyl cellulose (Hypromellose, British Celanese Ltd., Spondon, Derby), gelatin, pectin and polyvinylpyrrolidone 44000 (PVP) (BDH Chemicals Ltd., Poole, Dorset), acacia (Brome and Schimmer Ltd, Romsey, Hants), Carbopol 934 (BF Goodrich, Heston, Middlesex), polyethylene glycol (PEG) 6000 and tragacanth BP (Macarthys, Romford, Essex), hydroxypropylcellulose (mol. wt 300 000) (Aldrich Chemical Company Inc., Milwaukee, U.S.A.).

Gantrez-AN was kindly donated by GAF (Great Britain) Ltd., Wythenshawe, Manchester.

Polystyrene latex spheres (1·15 μm diameter) were obtained from Coulter Electronics Ltd., Luton, Beds.

'Homogenized' mucus samples were prepared as follows: crude mucus samples, obtained by scraping guinea-pig intestines, were contaminated with heterogeneous cellular material. These samples were bulked, and slowly stirred for 24 h at 4 °C with distilled water sufficient to give twice the initial volume. This mixture was then centrifuged at 32 500*g* for 30 min, the supernatant and sedimented solids discarded and the middle gel layer retained. This was divided into 1 ml aliquots and deep frozen until required. These samples were less contaminated and more homogeneous than the crude scrapings. However, a marked reduction in gel properties was evident due either to molecular damage to the gel forming glycoprotein component during 'solubilization' (Pain 1980) or to some loss of the glycoprotein component during centrifugation.

Rheological and biochemical characterization of the mucus was considered unnecessary as all results were expressed as a percentage of a reference standard, i.e. the force required to pull the clean plate from the same mucus sample. For each set of comparative experiments, samples from the same batch of crude mucus were used.

Model gel samples were prepared by placing 5 ml samples into jacketed water baths and allowing them to equilibrate at 4 °C for 24 h, then 1 h at 20 °C before testing at 20 °C.

Methods

This is based on the Wilhelmy plate method used for surface tension determination, and consists of (Fig. 1) a glass plate, 11 mm wide, suspended from a microforce balance (C.I. Robal, C.I. Electronics, Salisbury, Wilts.). A 5 ml glass vial (20 mm i.d. × 33 mm depth) containing the mucus samples, was

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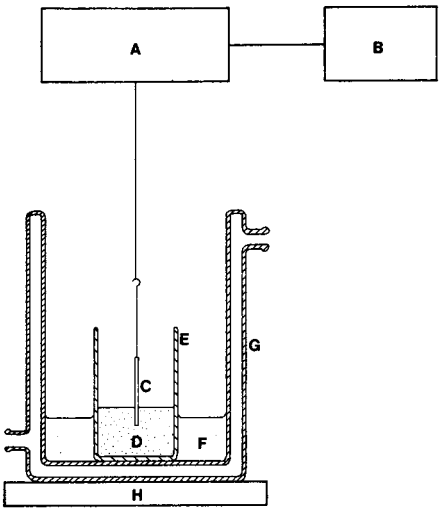

FIG. 1. The in vitro mucoadhesion apparatus. Key: A, Microforce balance; B, Chart recorder; C, Glass plate (side on); D, 1 ml Homogenized mucus; E, Glass vial; F, Water; G, Water jacket at 20 °C; H, platform moving in vertical direction.

placed into the water bath at 20 °C. The model gels were prepared directly in the water bath. This was then placed on a platform that could be mechanically lowered at a rate of 1 mm min^{-1} by the use of a slow infusion apparatus (Scientific and Research Instruments Ltd., Edenbridge, Kent).

The platform was raised until the plate had penetrated the mucus gel or model gel to touch the base of the container. The plate was left in contact with the gel for 7 min, before the platform was lowered at 1 mm min^{-1}. The maximum force recorded by the microforce balance and displayed on a y–t recorder when the plate detaches from the gel, was noted.

Plates were coated by dipping into a 1% solution of the test material, and oven drying at 60 °C to constant weight.

As a standard the clean plate was tested before and after coating with the test material, and the mean of the two forces found. The coated plate force was then expressed as a percentage of the clean plate force. Three plates were tested for each material, and the mean and standard deviation of the percentage forces found.

RESULTS AND DISCUSSION

The reproducibility of this test system is such that when the same clean plate is pulled five times from an homogenized mucus sample, a mean maximum force of 120 mg was recorded, with a standard deviation of 2·24.

A rank order of adhesiveness was obtained for several materials when tested in the mucoadhesion apparatus (see Table 1). The results obtained agree with previous published data on mucosa-adhesion. Chen & Cyr (1970) used a subjective assessment of adhesion of discs to rabbit oral mucosa. They reported that SCMC, tragacanth and sodium alginate were excellent adhesives; gelatin satisfactory; pectin fair and PVP, acacia and PEG poor.

Table 1. Rank order of mucoadhesive force.

Coating material	Mean % adhesive force	Standard deviation
75P SCMC	192·5	12·0
Carbopol 934	185·0	10·3
Tragacanth	154·4	7·5
Gantrez AN	147·7	9·7
Sodium Alginate (H.V.)	126·2	12·0
Hypromellose (M.V.)	125·2	4·8
Gelatin	115·8	5·6
Pectin	100·0	2·4
P.V.P.	97·6	3·9
Acacia	97·6	5·9
PEG 6000	96·0	7·6

Carbopol 934, a polymer of acrylic acid, was reported to have good mucosa-adhesive properties (Nagai et al 1979), as were maleic anhydride co-polymers, e.g. Gantrez. AN (Banker 1980 personal communication). Modified hypromellose is used in the 'Synchron' controlled release formulation that adheres to oral mucosa (Abrams 1982). Generally, those molecules with a large molecular weight and containing ionizable groups were the most adhesive. Park & Robinson (1982a, b) also noted the importance of ionizable groups when they studied the binding of various polymers to the mucin/epithelial cell surface.

Nagai et al (1979) used blends of Carbopol 934 and hydroxypropylcellulose in their mucosa adhesive formulations. Various coating solutions containing these macromolecules were tested (Fig. 2). Carbopol is a highly mucoadhesive macromolecule, but blends with hydroxypropylcellulose resulted in a loss of adhesion. However, greater adhesive forces were recorded for blends than would be predicted from the additive effects of the component polymers.

The mechanism and factors affecting mucoadhesion were investigated using SCMC. The molecular weight of four viscosity grades of SCMC had previously been determined (Kellaway & Najib 1980). The effect of molecular weight on adhesion is shown in Table 2. It was found that optimum adhesive forces were obtained with molecular

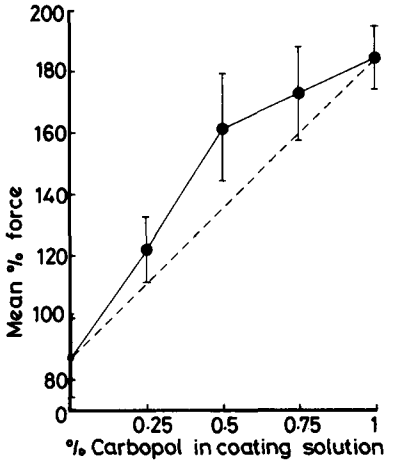

FIG. 2. The influence of blends of Carbopol 934 and hydroxypropylcellulose on the mucoadhesive force. Clean plate force = 100% s.d. bars, n = 3.

weights ≥78 600 daltons; Chen & Cyr (1970) have observed optimal adhesion for fractions exceeding 1×10^5 daltons.

Using the highest and lowest molecular weight samples, the effect of contact time between the coated plate and mucus gel was investigated (Fig. 3). The adhesive force was seen to increase with time. Chen & Cyr (1970) reported that these materials only become adhesive on hydrating to form a 'tacky' film. The increase in adhesive force may be related to the amount of water taken up by the SCMC coat as it hydrates in contact with the mucus gel. However, in this in-vitro test the available water in the mucus sample is limited and this may explain why over-hydration, to form a slippery mucilage, does not apparently occur. In order to minimize biological degeneration of the mucus sample, a 7 min contact time, the shortest to achieve a measure of muco-adhesion, was used in all experiments.

The effect of coat thickness, expressed as coat weight, on adhesion is shown in Fig. 4. When attempting to produce a uniform increase in coat weight, it was found that the standard deviations of the adhesive force increased considerably. However, the adhesive force increased with coat weight.

Table 2. The effect of molecular weight of SCMC on mucoadhesion

Grade SCMC	Mol. wt	Mean % force	Standard deviation
20	60 000	126	6·7
40	78 600	151	7·0
75	94 300	155	7·8
1000	654 000	157	10·3

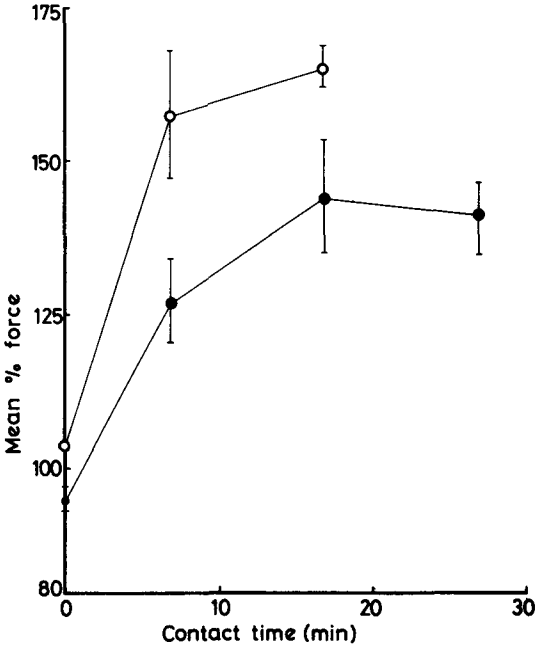

FIG. 3. The effect of contact time on mucoadhesion for SCMC. (○) SCMC P1000, (●) SCMC P20. Clean plate force = 100%, s.d. bars, n = 3.

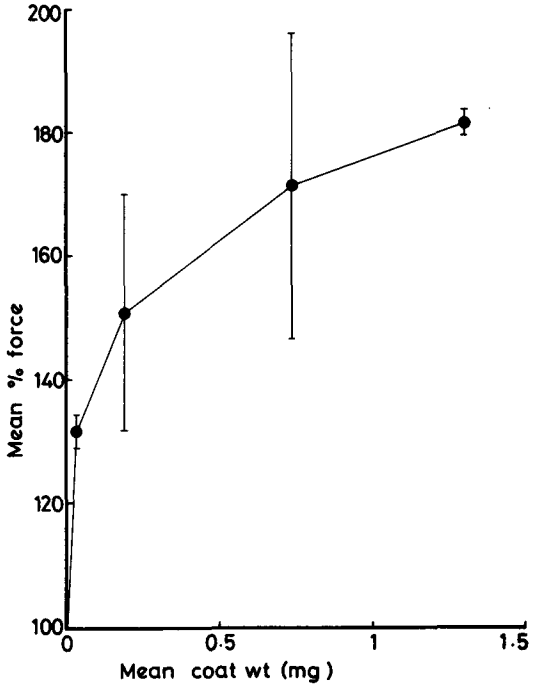

FIG. 4. The effect of coat thickness on mucoadhesion for SCMC P75. Clean plate force = 100%, s.d. bars, n = 3.

The importance of hydration occurring in contact with, or before contact with, the mucus sample was investigated. Prehydration was achieved by placing a drop of water onto either side of a coated plate for 7 min, before placing the plate in the mucus and testing immediately. This method was compared with the previous technique where the coat was allowed to hydrate in contact with the mucus sample. As there is no significant difference in the adhesive forces obtained with the two methods (157·4 ± 6·8, 154·8 ± 7·8%), it is apparently not important for hydration to occur in contact with the mucus.

In the following work, the mucus was replaced with model gels. It was thought that a better understanding of the factors affecting, and the mechanism of, adhesion to, gels could be achieved with these simpler systems.

At 20 °C, 1% w/v gelatin gels have viscoelastic properties (Robinson et al 1975). The effect of contact time on adhesion of P75 SCMC coated plates to this gelatin gel is shown in Fig. 5. The decrease in adhesive force after 3 min may be explained by the greater quantity of water available in this system, allowing overhydration of the SCMC coat. In further work with model gels, the plates were tested immediately after contact with the gel.

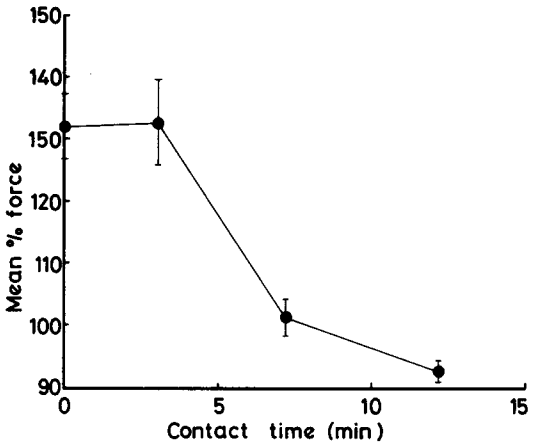

FIG. 5. The effect of contact time with a 1% gelatin gel for SCMC P75. Clean plate force = 100%, s.d. bars, n = 3.

Various materials were tested in this system using 1% w/v gelatin and 1% w/v alginic acid gels. The variation in the rank order of results obtained, shown in Table 3, may be due to pH differences, variations in water availability, or a degree of specificity in the interactions.

Gelatin gels were prepared at various pH's and the adhesive force between these and plates coated with

Table 3. Comparison of a mucus gel and model gel systems.

Polymer coat	Mean % force: Mucus	Gelatin 1% gel	Alginic acid 1% gel
P75 SCMC	195·4	131·5	160·5
Tragacanth	154·4	97·8	145·5
Hypromellose	125·2	92·5	90·3
Gelatin	115·8	111·8	134·7
Pectin	100·0	110·0	—
P.V.P.	97·6	98·4	96·1
P.V.A.	94·8	75·6	85·4

P75 SCMC and tragacanth found (Fig. 6). A low pH favours adhesion. The net charge carried by the SCMC, tragacanth and gelatin molecules was found using the method of Kellaway & Najib (1981), the charge being expressed as the electrophoretic mobility of latex spheres coated with the test polymer at various pH's (Fig. 7).

Adhesion is seen to increase at pH's below the isoelectric point of the gelatin molecule. At these pH's gelatin carries a net positive charge while, in contrast, SCMC and tragacanth carry a net negative charge. Hence an opposite charge favours adhesion.

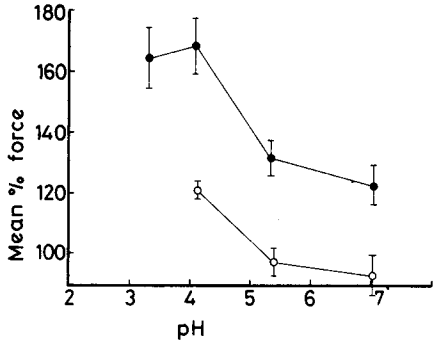

FIG. 6. The effect of pH on adhesion of SCMC P75 and tragacanth to a 1% gelatin gel. (●) SCMC P75, (○) tragacanth. The gelatin isoelectric point = 4·82 and the clean plate force = 100%, s.d. bars, n = 3.

The importance of the presence of a gel network was investigated using a gelatin gel and sol. Sols were prepared by omitting the equilibration for 24 h at 4 °C stage of the gel preparation. From the results obtained in Table 4, it would appear that the presence of a gel network is not a prime requisite. However, the forces in mg required to pull the plates from the sol for both coated and uncoated plates are considerably lower than those required to pull the plates from the gel, and this may have some bearing on the results obtained.

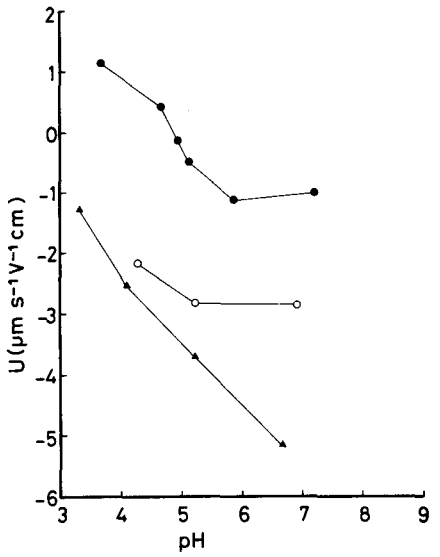

FIG. 7. The effect of pH on the electrophoretic mobility (U) of polystyrene latex coated with gelatin, tragacanth and SCMC P75. (●) gelatin; (○) tragacanth; (▲) SCMC P75. s.d. bars, n = 3.

Table 4. P75 SCMC—adhesion to 1% gelatin gel and sol.

Physical state	Mean max. clean plate force (mg)	Coated plate force (mg)	% Force	Mean force %	Standard deviation
Gel	129	164	127	132	5·2
	138	190	138		
	145	188	130		
Sol	101	149	147	139	7·7
	102	138	136		
	103	137	133		

CONCLUSIONS

From the agreement between this and previous work, it is clear that materials that adhere to isolated mucus will also adhere to mucus membranes. These materials become adhesive on hydration which occurs as water is extracted from the mucus gel. If an excessive quantity of water is available, the hydrated polymers start to form gels and eventually a slippery mucilage. In this state all adhesive properties are lost as the polymers dissolve in the available water. Mucoadhesive materials are macromolecular hydrocolloids with numerous hydrogen bond forming groups. The presence of ionizable groups favours adhesion.

From the work with model gels, the presence of a gel network is not a prime requisite, therefore the role played by physical or mechanical bonding in this form of adhesion may be limited. The mechanism of adhesion is therefore predominantly by primary and secondary molecular interactions, opposite charges being seen to favour adhesion.

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