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In-vitro evaluation of bioadhesion in particulate systems and possible improvement using interactive mixtures

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Abstract

The bioadhesion of tablet components was tested using the fracture method (maximum tensile stress in detaching the sample from mucus membrane) and comparing traditional tablet specimens with powder monolayers. Both nonadhesive excipients and established mucoadhesive materials were investigated. Some nonadhesive materials showed unexpectedly good adhesive properties when tested as tablets but not as powders. Conversely, some bioadhesive materials had unexpectedly low adhesive properties when tested as tablets. Thus, powder specimens of some materials appear to give more realistic results than tablet specimens in this respect. The use of powder specimens seems particularly applicable for testing dispersible tablets intended for transmucosal absorption. Potential for increasing the bioadhesive properties of coarse, nonadhesive carrier particles by coating them with fine particles of bioadhesive materials during dry mixing (forming interactive mixtures) was also studied. The tensile strength of the adhesive bond between the mucosa and the nonadhesive excipients was improved when fine cross-linked carboxymethyl cellulose sodium (Ac-Di-Sol) particles were added. The addition of increased proportions of Ac-Di-Sol initially improved the bioadhesive properties until a plateau was reached. A standardised test of bioadhesive capacity could therefore involve the addition of fine bioadhesive powders to coarse carriers in proportions close to those providing monoparticulate surface coverage. Interactive mixtures such as these may also offer potential as a tool for use in the development of bioadhesive drug formulations.

Introduction

Drug formulations that have bioadhesive properties can prolong the residence time for the drug at the site of absorption, thus potentially improving membrane transport. The ability to increase bioadhesion would be especially important for active compounds that are poorly soluble or permeate the mucosa poorly. One of the difficulties in developing bioadhesive formulations is that the mechanism of bioadhesion is not yet fully understood. The most common theories have been reviewed by Chickering & Mathiowitz (1999). The electronic theory suggests an electronic transfer between the two materials causing a double layer of electrical charge, which results in attraction forces. The adsorption theory suggests that adhesion between the mucosa and the adhesive material is due to van der Waals interactions, hydrogen bonds and related forces. The wetting theory suggests interfacial tensions between the two materials, while penetration of polymer chains into the mucus network and vice versa, causing a mechanical bond, is referred to as the diffusion theory. The importance of water content and movement of water into the bioadhesive material from the mucosa (i.e. dehydration of the mucosa) has also been suggested as a mechanism for adhesion (Duchêne et al 1988; Mortazavi & Smart 1993).

Methods of evaluation of the bioadhesive properties of a compound also pose difficulties. One common in-vitro method is based on the fracture theory (i.e. evaluating the force required to separate the formulation from the mucosa after keeping them in contact under a specified force for a specified time). The tensile stress can then be determined by dividing the maximum force of detachment by the total surface area involved in the adhesive interaction (Chickering & Mathiowitz 1999). This method has been used for evaluating the bioadhesive properties of both pure materials and

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formulations (e.g. Ponchel et al 1987; Tobyn et al 1997). Tablets were most often used in these studies (Ponchel et al 1987; Tobyn et al 1997) but individual microspheres and powders have also been investigated (Chickering & Mathiowitz 1995; Mahrag Tur & Ch'ng 1998). Robert et al (1988) used a method for assessing bioadhesion similar to that used in the study reported here. They suggest that using powders provides a simple, rapid method of measuring the adhesive properties of a material. However, they do not appear to have performed any comparative experiments using other specimen types (such as tablets) to evaluate the influence of specimen type on the bioadhesive results.

Normally, the concept of enhanced bioadhesion is discussed in the preparation of controlled release formulations and, in these cases, it is obviously important to use the actual product for the measurement of bioadhesion. However, such concepts could also be applicable to instant-release formulations, such as tablets for sublingual administration. In these formulations, after initial rapid disintegration, the tablet subunits formed should preferably adhere for a limited period to the sublingual mucosa, so as to avoid swallowing and systemic uptake from the intestine. For such a specific application, a non-disintegrating tablet form will obviously not be a suitable specimen for bioadhesion testing. The use of powder particles would better reflect the adhesion of subunits or particles to the mucosa after tablet disintegration.

The aim of this study was to quantify the bioadhesion of various materials using both powder and tablet specimen forms. In addition, the possibility of increasing the bioadhesive properties of coarse, nonadhesive carrier particles by coating them with fine particulate bioadhesive materials (i.e. forming ordered mixtures, in this paper referred to as interactive mixtures) was studied. The use of common superdisintegrants (i.e. substances which readily absorb water) as a method of improving the bioadhesion of these coarse particles was also evaluated.

Materials and Methods

Materials

Dibasic calcium phosphate dihydrate (DCP) (Emcompress, Edward Mendell Co. Inc., USA) and mannitol (granulated quality, Roquette, France) were used both as nonadhesive materials in the baseline bioadhesion studies and also as carrier materials in the preparation of interactive mixtures. A size fraction of 180–355 μm for each material was obtained by dry sieving (Retsch, Germany). Sodium alginate (viscosity 400–600 mPas for a 1% solution) (Carl Roth GmbH, Germany), cross-linked polyvinylpyrrolidone (Kollidon CL and Kollidon CLM, BASF, Germany) and cross-linked carboxymethyl cellulose sodium (Ac-Di-Sol, FMC, Cork, Ireland) were used as supplied to represent materials with potential bioadhesive properties. Finer particle size fractions of Ac-Di-Sol were obtained by milling in a mortar grinder (Retsch, Germany) followed by air classification (100 MZR, Alpine, Germany).

Primary characterisation of test materials

All powders were stored at 40% r.h. and room temperature, for at least 48 h before characterisation and mixing. The apparent particle density of the materials (n = 3) was assessed using a helium pycnometer (AccuPyc 1330 Pycnometer, Micromeritics, USA) (Table 1). The external surface area of the coarser size fractions (180–355 μm) of mannitol and DCP was determined using Friedrich permeametry (n = 3) (Eriksson et al 1990). Blaine permeametry was used to determine the external surface area of all other powders (Alderborn et al 1985) (Table 1).

Table 1 Primary characteristics of test materials.

Material	Particle size fraction (μm)	Apparent particle density (g cm^{-3})[a]	External specific surface area (cm^2 g^{-1})[b]	Tablet porosity (%)[c]
Sodium alginate	—	1.717 ± 0.001	2000 ± 25	36 ± 0.11
Kollidon CL	—	1.224 ± 0.001	4200 ± 250	33 ± 0.25
Kollidon CLM[d]	—	1.212 ± 0.001	32600 ± 375	—
Ac-Di-Sol				
As supplied	—	1.607 ± 0.001	2600 ± 25	49 ± 0.62
Coarse	>5	1.607 ± 0.001[e]	3200 ± 330	
Medium	—	1.607 ± 0.001[e]	6400 ± 91, 6700 ± 180	
Fine	<5	1.607 ± 0.001[e]	12600 ± 1100	
Mannitol	180–355	1.486 ± 0.000	290 ± 6.5	18 ± 0.63
DCP	180–355	2.884 ± 0.001	440 ± 3.7	42 ± 0.11

Mean values ± s.d. [a]Measured with a helium pycnometer (AccuPyc 1330 Pycnometer, Micromeritics, USA), n = 3. [b]Measured with a Friedrich permeameter (Eriksson et al 1990) or Blaine permeameter (Alderborn et al 1985), n = 3. [c]Porosity of the tablets, compressed at 100 MPa, was calculated from the weight and dimensions of the tablets and the apparent particle density of the material or mixture, n = 5. [d]Micronised Kollidon CL. [e]The value was characterised for the material as supplied and used for the three size fractions.

Compaction of tablets

All powders were mixed with magnesium stearate powder (0.5% w/w) in glass jars in a 2-L Turbula mixer (W. A. Bachofen AG, Basel, Switzerland) at 120 rev min^{-1} for 2 min and then stored at 40% r.h. and room temperature for at least 48 h before compaction. Tablets were made in an instrumented single punch press (Korsch EK0, Germany) at 100 MPa using 1.13-cm flat-faced punches. The upper punch pressure was obtained by keeping the distance between the punches constant (3 mm at zero pressure) and varying the amount of powder in the die. The powder was weighed on an analytical balance and manually filled into the die.

Preparation of binary interactive mixtures

Milled Ac-Di-Sol (medium size fraction, Table 1) was added to mannitol or DCP (both 180–355 μm) in varying proportions to obtain different concentrations of Ac-Di-Sol and surface area ratios (calculated according to Nyström et al (1982)). Kollidon CLM and coarser and finer particle size fractions of Ac-Di-Sol were also added to DCP (Table 2). Batches of approximately 15 g powder were mixed in 100-mL glass jars (thus the jar was filled by less than one-third of its inner volume) in a 2-L Turbula mixer (W. A. Bachofen AG, Basel, Switzerland) at 120 rev min^{-1} for 24 h. The mixture with the fine particle size was mixed for an additional 24 h to obtain an agglomerate-free mixture. Mixing was performed in accordance with previous studies (Westerberg 1992; Sundell-Bredenberg & Nyström 2001) and the mixture homogeneity was visually confirmed.

Bioadhesion measurements

Materials and characterisation of the mucosa

Fresh pig intestine was collected at a slaughterhouse (Swedish Meat AB, Uppsala, Sweden) and used while fresh or was frozen until required. Before use, the frozen intestine was thawed in buffer solution at 4 °C overnight. The buffer solution used was Krebs-Ringer Bicarbonate (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) pH 7.4.

To test the quality of the mucus layer and the effect of handling the mucosa, four representative tissue specimens were stained with Alcain blue, partly according to the method of Corne et al (1974). Both fresh and frozen tissues were then soaked for 2 h in TRIS (TRIZMAHydrochloride; Sigma-Aldrich Chemie GmbH, Steinheim, Germany) buffered sucrose solution (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) with Alcian blue 8 GX, (Certistain, Merck, Germany) (1 mg mL^{-1}). The tissues were rinsed in TRIS/sucrose buffer and visually studied.

Adhesion test

A TA-HDi texture analyser (Stable Micro Systems, Haslemere, UK) with a 5-kg load cell and associated software was used for the bioadhesion studies. The pig intestine was cut into approximately 2-cm^2 pieces and placed in a tissue holder. Either a tablet (using a cyanoacrylate adhesive (Loctite Super Attak; Loctite Sweden AB, Gothenburg, Sweden)) or powder (using double-sided tape (Scotch; 3M Svenska AB, Sollentuna, Sweden)) was attached to the upper probe. The application of the powder was performed by immersing the probe in to a powder bed and thereafter the probe was gently shaken to remove any excess, to achieve a monolayer of particles, which was visually validated. After spreading 30 μL of buffer with a pipette on the mucosa to standardise hydration, the studied material was brought into contact with the mucosa under a force of 0.5 N over 30 s. The probe was then raised at a constant speed of 0.1 mm s^{-1} and the detachment force was recorded as a function of displacement. The detachment force was measured at a sampling rate of 25 measurements per second throughout the measuring cycle. The maximum force monitored (i.e. the fracture force) was determined using the computer software Texture Expert Exceed (Stable Micro Systems, Haslemere, UK).

Since the surface areas of the tablets and the probe (i.e. the corresponding surface area of the powder specimen) were unequal, the tensile stress (N cm^{-2}) was obtained by dividing the detachment force by the area of either the tablet or the probe. Obviously, the actual powder surface area effectively in contact with the mucosa could theoretically be calculated (e.g. in analogy with the calculations of the bonding area within tablets (Olsson & Nyström 2001)). However, it was believed that the area of the probe gave a fair approximation of the actual cross-sectional area of

Table 2 Bioadhesive properties of mixtures containing DCP and a bioadhesive material of varying particle size.

Bioadhesive material mixed with DCP	Concn of bioadhesive material (% w/w)	Surface area ratio	Maximum tensile stress (N cm^{-2})
Ac-Di-Sol			
Coarse (>5 μm)	35.9	1.0	0.469 ± 0.142
Medium	20.8	1.0	0.845 ± 0.220
Fine (<5 μm)	12.3	1.0	0.460 ± 0.141
Kollidon CLM[a]	7.5	1.5	0.022 ± 0.002

Mean values ± s.d., n = 5. [a]Micronised Kollidon CL.

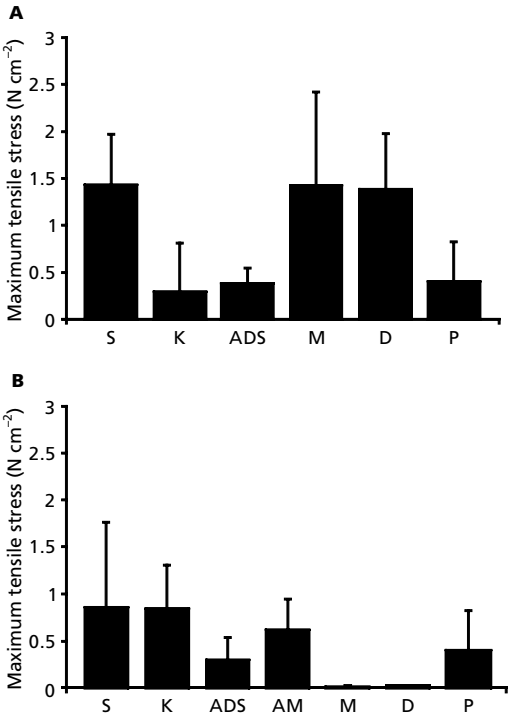

Figure 1 Maximum tensile stress in detaching tablet specimens (A) and powder specimens (B) from mucus membrane from pig intestine. Mean values ± s.d., n = 5. S, sodium alginate; K, Kollidon CL; ADS, Ac-Di-Sol; AM, Ac-Di-Sol milled; M, Mannitol; D, DCP; P, Probe.

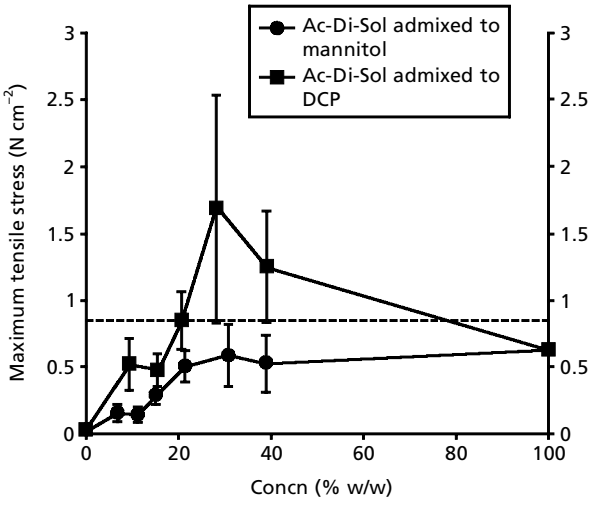

Figure 2 Maximum tensile stress in detaching powder specimens from pig intestinal mucus membrane as a function of concentration of milled Ac-Di-Sol (Medium) mixed with mannitol or DCP. The dashed line represents data for both pure sodium alginate and Kollidon CL in powder form. Mean values ± s.d., n = 5.

the tensile failure plane. The work of adhesion was calculated from the area under the curve of tensile stress versus probe displacement. Duchêne & Ponchel (1989) found

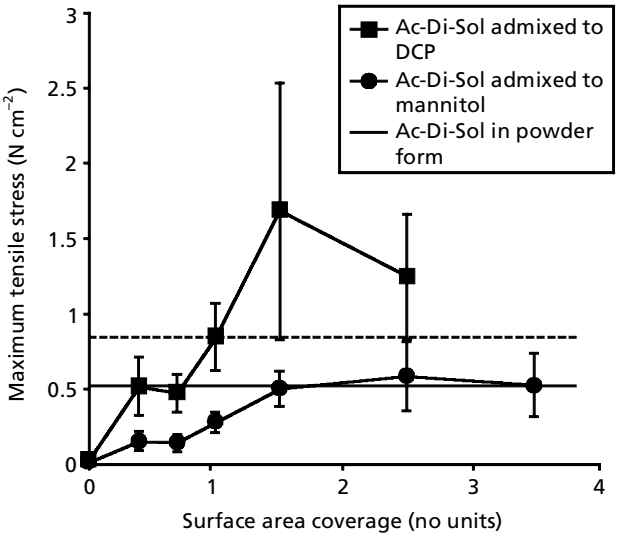

Figure 3 Maximum tensile stress in detaching powder specimens from pig intestinal mucus membrane as a function of surface area coverage of mannitol and DCP particles by milled Ac-Di-Sol (Medium) particles. Data for pure Ac-Di-Sol (Medium), sodium alginate and Kollidon CL in powder form are shown as reference lines (solid line for Ac-Di-Sol and dashed line for both sodium alginate and Kollidon CL). Mean values ± s.d., n = 5.

that adhesion work was a more reproducible and reliable parameter. They did not find any relationship between peak force and work of adhesion, while Tobyn et al (1995) found that there was some relationship between the two parameters, even though they stated that the two parameters should not be used interchangeably when comparing mucoadhesive results. However, since adding the data of tensile work did not influence the conclusions, only the values of maximum tensile stress are presented in this paper.

Statistical analysis

For all data presented in this paper, mean values and standard deviations were calculated. In Figure 1, the tensile stress for tablets and powders of different materials was compared with tablets and powders of sodium alginate using an unpaired, two-tailed *t*-test assuming unequal variances. This method was also used evaluating the mixtures in Table 2. For the mixtures in Figures 2 and 3, the influence of addition of bioadhesive component was evaluated using one-way analysis of variance followed by Newman–Keuls procedure to examine differences between mean values.

Results and Discussion

Primary characteristics of test materials and experimental factors

The primary characteristics of the test materials are presented in Table 1. Mannitol is freely soluble in water,

while DCP is practically insoluble (Wade & Weller 1994). Both materials are commonly used fillers and were not expected to have any pronounced bioadhesion. On the other hand, Ac-Di-Sol and Kollidon CL, which are both so-called superdisintegrants and which act primarily by extensive swelling as a result of water absorption, were assumed to have some bioadhesive properties. This assumption was based on the hypothesis of Mortazavi & Smart (1993), which suggests that water movement is involved in the mechanism of bioadhesion. Sodium alginate, a well-known bioadhesive component (e.g. Smart et al 1984; Robert et al 1988), was used in this study as a reference, representing a material with the greatest possible bioadhesive capabilities.

Instrumental settings, such as applied force and contact time, were investigated first to verify that parameters chosen from literature were applicable to the materials used in this study. Normally, for the type of bioadhesive measurements used in this study, a contact time of around 300 s has been used (e.g. Ponchel et al 1987; Tobyn et al 1997). However, a shorter duration of contact (30 s) was chosen for these studies, mainly because of the intention to reflect a fast disintegrating system, such as tablets for sublingual administration, as mentioned in the introduction, but also since the mechanism of water movement is believed to occur very rapidly (Mortazavi & Smart 1993). This shorter contact time has also been used previously by some workers (Reich et al 1984; Robert et al 1988). The removal speed of the probe was 0.1 mm s^{-1}, as has been used for similar measurements by others (Ponchel et al 1987; Tobyn et al 1995). For a measurement on the adhesive (no sample applied), the tensile stress was 0.22 N cm^{-2}. This indicated that the adhesive could not substantially have contributed to the bioadhesive effect of the materials, since both lower and higher values were obtained for the powders and mixtures.

On evaluating the quality of the mucus layer and the effect of handling the tissue, it was noted that neither the thawing process (in buffer solution at 4 °C) nor handling affected the quality of the mucosa (i.e. the mucus layer remained intact) and therefore both fresh and frozen mucosa were used in this study.

Comparison of tablets and powders as specimens for bioadhesion testing

Tablets made of sodium alginate, Kollidon CL, Ac-Di-Sol (all with particle size as supplied), mannitol and DCP (both 180–355 μm) were tested for bioadhesive strength by evaluating the force required to separate the formulation from the pig intestinal mucosa (Figure 1A). Powders of the same materials were also tested (Figure 1B). The bioadhesion of tablet formulations (Figure 1A) was high, as expected, for sodium alginate. However, DCP and mannitol had also high bioadhesive values (i.e. they did not differ significantly ($P > 0.1$) from sodium alginate) while Kollidon CL and Ac-Di-Sol tablets and the metal probe (i.e. no sample) had significantly lower ($P < 0.01$) bioadhesion values. Investigation of powders (Figure 1B) resulted in a ranking of materials that was closer to that expected; the values for DCP and mannitol were significantly lower ($P < 0.1$), than and those for Kollidon CL and Ac-Di-Sol were closer ($P > 0.1$) to, those of the bioadhesive sodium alginate.

The apparent high bioadhesive values for DCP and mannitol tablets were unexpected (i.e. the maximum tensile stress did not differ significantly ($P > 0.1$) from the tensile stress of sodium alginate tablets (Figure 1A)). Since neither of these materials has polymer chains likely to interact with mucin molecules, some other kind of attraction between the two smooth surfaces must have been involved. Dehydration of the mucosa may have occurred. This mechanism, described by Mortazavi & Smart (1993), is caused by water movement from the mucosa to the dry powder, resulting in adhesion between the two surfaces. However, as Mortazavi & Smart (1993) also concluded, adhesion involves more than just dehydration. For example, dehydration is obviously not involved in the results obtained with the metal probe. Another explanation could therefore be that adhesion forces due to surface tension may have been created between the mucosa and the materials (Mikos & Peppas 1989). Attraction forces due to surface energy effects have also been discussed as a possible bioadhesive mechanism by others (Smart 1999).

The porosity of tablets made of DCP, Kollidon CL and Ac-Di-Sol was relatively high (33–49%, see Table 1). Although there have been indications in the literature that tablet porosity does not affect bioadhesion (Ponchel et al 1987; Tobyn et al 1995), the possibility that a high tablet porosity could facilitate transport of water into the tablet and thereby cause adhesion cannot be excluded. This seems especially relevant for the test conditions used in this study (i.e. no pre-swelling of the tablets and a short contact duration).

Unexpectedly, instead of fracturing between the tablet and the mucus layer or through the mucus layer, Kollidon CL tablets fractured through the tablet itself. This material, which quickly absorbs large amounts of liquid, is usually used as a disintegrant (Gissinger & Stamm 1980). It is assumed that the fast absorption of liquid made the tablet weak, so that a fracture occurred at the interface of the wet and dry portions within the tablet. Ac-Di-Sol, which is also a superdisintegrant (Gissinger & Stamm 1980), showed the same tendency to fracture through the tablet, although to a lesser extent than with Kollidon CL (i.e. the fracture occurred nearer the surface of the tablet and was not seen with every measurement). These results indicate that it would be feasible to characterise the adhesive properties of materials using uncompacted powder specimens instead of a compressed tablet form.

The use of interactive mixtures (the addition of fine bioadhesive particles) to increase the bioadhesive properties of a carrier material

The coating of microparticles with a bioadhesive material has been used both to compare the bioadhesive properties

of materials (Ranga Rao & Buri 1989) and to enhance the bioadhesive properties of a material (Gåserød et al 1998). Alternatively, bigger carrier particles can be covered by smaller dry particles by dry mixing (i.e. by forming ordered or interactive mixtures). Interactive mixtures are commonly used to improve the content uniformity of low-dose preparations (Hersey 1975; Sundell-Bredenberg & Nyström 2001). The formation of interactive mixtures has also been used to promote the dissolution of drugs with low aqueous solubility (Westerberg 1992). When the freely soluble carrier particles rapidly dissolve, the drug is released as discrete, primary particles, thus increasing the dissolution rate. It was also considered feasible that this method could be used to increase the contact time of the carrier particle to the mucosa. If a bioadhesive material with a small particle size were mixed with the carrier particles, bonding of the bioadhesive material to the mucosa would prolong the time spent by the carrier particles at the absorption site, although the final duration of contact is also dependent on the solubility of the carrier particles. When drug particles are then added to the carrier particles using this technique, in contrast to the coated particle system (e.g. Gåserød et al 1998), the drug is released quickly as it is held at the absorption site. In this study, Ac-Di-Sol (medium fine particle size) was used as the bioadhesive material and was admixed to DCP or mannitol as carrier materials.

The effect of bioadhesive component proportions

Tensile stress between the mucosa and the nonbioadhesive carrier particles was improved ($P < 0.0001$) when the coarse DCP or mannitol was mixed with the medium fine particle size of Ac-Di-Sol (Figure 2). The bioadhesive properties improved ($P < 0.05$) initially with increases in the concentration of Ac-Di-Sol, as shown earlier with tablets containing bioadhesive materials (Ponchel et al 1987). Tobyn et al (1997), who also investigated tablets made from mixtures of formulation excipients and bioadhesive materials, stated that the excipients decreased the work of adhesion.

Interactive mixtures of DCP containing the two highest concentrations of Ac-Di-Sol (28.2 and 39.3% w/w) gave values for tensile stress significantly higher ($P < 0.05$) than for powders of pure Ac-Di-Sol (Figure 2). A synergistic effect on the strength of tablets composed of mixtures has also been reported (Mattsson & Nyström 2000); tablets formulated from mixtures were stronger than predicted from the individual materials. This was explained by an increase in fracture surface area in tablets containing the mixture of coarse and fine particles, compared with those containing the fine component alone. Similarly, when the fine bioadhesive particles are mixed with larger carrier particles, the surface area of the adhesive component in contact with the mucosa is greater than if only a flat, monoparticulate layer of bioadhesive component is exposed to the mucosa. This effect was, however, not seen with mixtures containing mannitol ($P > 0.1$), probably because of the higher water solubility of mannitol, as discussed below.

The effect of surface coverage of bioadhesive component

As seen in Figure 2, the increase in bioadhesive strength is significant ($P < 0.05$) up to a certain proportion of Ac-Di-Sol. When the surface area ratio exceeded unity (corresponding to concentrations > 20% w/w), the bioadhesive strength began to level off (i.e. an increase in amount of Ac-Di-Sol did not give a significant increase ($P > 0.1$) in tensile stress). The plateau in bioadhesion was possibly attributable to the surface area coverage of the bioadhesive component of the carrier (Figure 3). In this study, the surface area coverage is defined as the surface area ratio (Nyström et al 1982). For surface area ratios exceeding unity, an excess of fine powder coated on the coarse carriers probably increased the tendency for the fracture to go through, at least partly, a multiparticulate layer of adhering powder and thus a transition of the fracture path from mucus to powder specimen was anticipated for both mixture types at high concentrations of admixed Ac-Di-Sol.

The effect of carrier solubility

DCP mixtures were significantly more ($P < 0.02$) bioadhesive (had higher tensile stress) than mannitol mixtures. This may be a result of the higher water solubility of mannitol. Thus, the fracture for the mannitol mixtures might have gone through dissolved peripheral regions of the interactive mixtures and not entirely through the mucus layer. Additionally, Tobyn et al (1997) suggested that addition of a highly water-soluble additive reduces the water content when the material dissolves, and thus makes the water unavailable for the bioadhesive material, with decreased bioadhesion as a result.

The effect of particle size of the bioadhesive component

Table 2 summarises the results when varying particle size fractions of Ac-Di-Sol were mixed with DCP and when DCP was mixed with micronised Kollidon CL. The surface area coverage ratio is the same (1.0) for all samples of Ac-Di-Sol and the concentration varies as a result of the different particle sizes. If surface coverage of the carrier material is a dominating factor, a surface area ratio of unity would be expected to give the same bioadhesive results for all samples, since the same area of bioadhesive material and mucosa would be in contact. However, the intermediate size fraction had the strongest ($P < 0.02$) bioadhesive effect. This may have been a factor of the absolute amount of added bioadhesive component. Since absorption by Ac-Di-Sol is so fast, the dehydration of the mucosa (Mortazavi & Smart 1993) may have contributed to the mechanism of bioadhesion. It is possible, in that case, that the weight or volume concentration of added bioadhesive component is of greater import than the surface area ratio of the material. However, the coarsest size fraction of Ac-Di-Sol would then be expected to cause the strongest dehydration and thus the highest tensile stress and, as reported above, this was not the case. The

tensile stress associated with the coarser particle size of Ac-Di-Sol did not show any significant difference ($P > 0.1$) to that of the finer quality, despite a much higher concentration. This may have been due to weaker adhesive interactive forces between carrier and powder particles so that the fracture, at least in parts, went between the carrier and powder particles. Thus, the medium sized particles of Ac-Di-Sol gave the optimum mixture for a bioadhesive system composed of Ac-Di-Sol and DCP. Because of its small particle size, Kollidon CLM would also be expected to give a high tensile stress value if surface coverage was the limiting factor, but that did not occur. Despite the high surface area coverage of the carrier particles (1.5), the concentration of Kollidon CLM was too low to increase ($P > 0.1$) the bioadhesive capability of DCP. It is suggested that the low concentration of Kollidon CLM meant that the system absorbed little liquid, resulting in low tensile stress and work.

The fracture path and bioadhesion mechanisms

Chickering & Mathiowitz (1995) have discussed the shape of the deformation curve (i.e. the tensile force plotted versus distance) for microspheres in detail. They hypothesized that the shape of the curves is dependent on the adhesion mechanism of the material. In this study, a comparison between sodium alginate and DCP tablets showed that their curve profiles (height and width) and the maximum tensile stress were similar. In contrast, DCP powder had much lower maximum tensile stress than sodium alginate powder, and the morphology of the powder curves was different. According to the theory of Chickering & Mathiowitz (1995), a sharp peak, like the one for sodium alginate powder, could indicate a strong mechanical interpenetration between the mucosa and the material, while a more rounded curve, like the one for DCP powder, represents manifold weak bonds to the mucus layer. These data support the view that sodium alginate is capable of forming relatively strong interpenetrating attractions with the mucus layer, while DCP bonds by some kind of general surface energy mechanism. This could also explain the unexpectedly high values for DCP tablets.

Evaluation of the bioadhesive mechanisms of a material is generally a rather complex process, and it could be of interest to qualitatively indicate the dominating fracture path for each test specimen. To do this, the weakest plane of the system should be established, as discussed by others (e.g. Smart 1999; Hägerström & Edsman 2001).

Possible fracture paths for the specimens used in this study are presented in Figure 4. If bioadhesive forces (i.e. mechanical interpenetration) have been created between the mucosa and the specimen, the fracture should occur at position A (through the mucus layer) or, possibly, when water movement is a dominating adhesion mechanism, at position B (at the interface between specimen and mucus layer). As discussed above, the fracture went through the tablet (position C) for tablets made of Kollidon CL and Ac-Di-Sol rather than occurring at positions A or B. For powder specimens, a monolayer of particles must be applied to the metal probe to reduce the risk that multiparticulate layers will cause the fracture to follow a path between the particles (position C). The adhesive interactive forces between the carrier (in this study, mannitol or DCP) and the bioadhesive material in interactive mixtures must be stronger than the forces between the mucosa and the mixture. The latter is dependent both on the ability of the materials to create these adhesive forces and on the particle size, as discussed above.

In this study, taking into account all data generated for tablets, powders and mixtures, there seemed to be a maximum tensile stress of approximately $1.5\,N\,cm^{-2}$. This value is thought to reflect the intrinsic strength of the mucus layer (path A in Figure 4) since that would be the strongest part of the system, for these specific materials and test conditions. Lower tensile stress values would then reflect a weakening of the bioadhesive joint. This could occur if the material has a low ability to create bioadhesive interactions (fracture path at position B in Figure 4) or if there is a problem with the specimen (fracture path

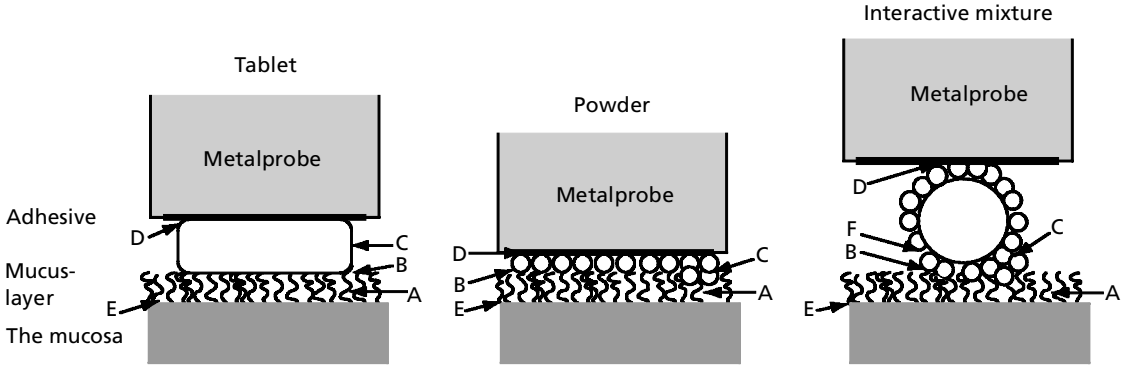

Figure 4 Schematic model of possible regions of failure during measurements of bioadhesion between the mucosa and tablets, powders and interactive mixtures. The possible regions are: A, through the mucus layer; B, at the interface between the specimen and the mucus layer; C, within the specimen; D, at the interface between the bioadhesive component and the specimen; E, between the mucosa and the mucus layer; and F, between particles within the interactive mixtures.

at C or F in Figure 4). Further, if strongly bioadhesive materials, with no specimen problems are tested, there should be no differences in the relative values between the tested materials. This indicates that ranking of bioadhesiveness is probably less feasible using solid bioadhesive specimens such as tablets.

Conclusions

The results indicate that it is feasible to characterise the adhesive properties of materials using uncompacted powder specimens instead of using a compressed tablet form. In the comparison of test specimens, tablets of the non-bioadhesive DCP were unexpectedly bioadhesive, while powder specimens were, as expected, nonbioadhesive. The presence of these unexpected adhesive properties may be explained by movement of water from the mucosa into the porous tablet structure, which would promote adhesion between the two surfaces. This phenomenon could also occur for other materials if tablets are used as a model for evaluating the bioadhesion of the material. When the measured bioadhesion is linked to the properties of the specimen rather than to the material itself, erroneous values for dispersible tablets are possible. Thus, powder specimens would appear to better reflect the bioadhesive properties of some materials.

Further, when superdisintegrants such as Ac-Di-Sol and Kollidon CL were tested in the form of tablets, the fracture went through the tablet (position C in Figure 4) rather than between the tablet and the mucosa or through the mucus layer, (positions B and A, respectively, in Figure 4) and erroneously low values of both tensile work and maximum tensile stress were obtained. However, when powder particles were used instead of tablets, the bioadhesive strength increased relative to sodium alginate (i.e. the tensile stresses of Ac-Di-Sol and Kollidon CL did not differ significantly from sodium alginate powders). Thus, particulate test systems for bioadhesion measurements also appear to be superior to tablets for materials with disintegrating properties.

Both tensile stress and tensile work between the mucosa and the coarser mannitol or DCP powders were improved when these were mixed with the fine particulate Ac-Di-Sol. This indicates that addition of materials with a higher adhesion tendency will increase the adhesion of another, less bioadhesive material. However, a prerequisite is that the adhesive interactive forces between the materials are strong enough so that adhesion is measured between the mixture units and the mucosa, rather than between the materials within the mixture units. Thus, it is concluded that such interactive mixtures could also be an interesting formulation tool in the development of bioadhesive formulations such as instant-release formulations for sublingual administration.

By testing different types of specimens and comparing the data it was speculated that the maximum possible bioadhesion (the intrinsic mucus strength, position A in Figure 4) was 1.5 $N\,cm^{-2}$ as obtained for sodium alginate tablets and Ac-Di-Sol interactive mixtures with DCP at optimal surface coverage. For other combinations (Ac-Di-Sol/Kollidon CL tablets, Ac-Di-Sol interactive mixtures with mannitol, and Ac-Di-Sol interactive mixtures with DCP at low or high surface coverage), a lowering of the fracture path from the mucus layer to the test specimen was obtained, as exemplified by Ac-Di-Sol/Kollidon CL tablets (position C in Figure 4), Ac-Di-Sol interactive mixtures with mannitol (position F in Figure 4) and Ac-Di-Sol interactive mixtures with DCP at low (position B in Figure 4) and high surface coverage (position C in Figure 4). Further, the use of interactive mixtures of bioadhesive powders with water-insoluble carriers at a proportion close to monoparticulate surface coverage could thus represent a generally applicable means of standardising the testing of bioadhesive capacity.

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