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A novel in situ method to test polymers and coated microparticles for bioadhesion *

K.V. Ranga Rao and P. Buri

Laboratory of Pharmaceutical Technology, School of Pharmacy, University of Geneva, Geneva (Switzerland)

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Summary

A simple, quantitative and realistic in situ method to test the bioadhesive potential of polymers was developed. In this technique, the glass spheres or drug crystals were first coated with the polymers to be tested. Later, known amounts of these coated particles were placed on rat jejunum or stomach and kept in a humid environment. The tissue was then washed with phosphate buffer (for jejunum) or dilute HCl (for stomach) at a constant rate. The percent of particles retained on the tissue was considered as an index of bioadhesion. Among the polymers tested, polycarbophil and sodium carboxymethylcellulose-coated particles adhered stronger to the mucus than those with hydroxypropylmethylcellulose, methylcellulose and pectin. All the particles adhered better to the stomach than to the intestine.

Introduction

In recent years, hydrophilic polymers that bind to mucin or epithelial surfaces are becoming extremely popular in drug delivery. This is because these polymers help in prolonging the release of drug from a dosage form by localising it at a specific site like buccal (Kanig and Manago-Ulgado, 1965; Ishida et al., 1982; Gurny et al., 1984; Nagai and Machida, 1985; Merkle et al., 1985; Nagai, 1986) or nasal cavity (Nagai and Machida, 1985; Nagai, 1986), or GIT (Banker, 1980; Longer et al., 1985), bladder (Peppas et al., 1984), or vagina (Nagai and Machida, 1985; Nagai, 1986) etc. where mucus is present. To determine the bioadhesive potential of these polymers, several techniques were reported and they have been reviewed recently (Park et al., 1987; Duchêne et al., 1988). In majority of the techniques, the polymer sample is placed on one (Wang and Forrester, 1974; Reich et al., 1984) or between two soft tissue layers (Ch'ng et al., 1985) in an appropriate buffer or saline solution. The tissues used were either the mucosal surfaces of the rabbit stomach (Ch'ng et al., 1985) or mucous membranes of the oesophagus of various animals (Marvola et al., 1982 and 1983; Al-Dujaili et al., 1986; Robert et al., 1988) or mouse peritoneal membrane (Ishida et al., 1981) etc. Alternately, a glass plate is coated

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Correspondence: P. Buri, Laboratory of Pharmaceutical Technology, School of Pharmacy, University of Geneva, 30 quai Ernest-Ansermet, CH-1211 Geneva-4, Switzerland.

with the polymer and allowed to interact with mucus by dipping the glass plate in mucin solution (Smart et al., 1984). In all these methods, the force required to detach the polymer or polymer-coated glass plate from the tissue or mucin solution was measured, i.e., the adhesive bond between the polymer and the mucus or tissue is destroyed using shear/peeling/tensile forces. The results thus obtained are usually quantitative but indirect. To prevent the irritation of the dosage form to the tissue, microparticles coated with bioadhesive polymer(s) are preferred to the polymeric macromatrices for oral administration.

Therefore, we developed a simple, quantitative and realistic technique to screen both the soluble and insoluble polymers and polymer-coated microparticles for bioadhesion and the results are described in this paper.

Materials and Methods

Materials

Glass beads, 0.45 to 0.5 mm diam. (ABS, Geneva, Switzerland); crystals of acetyl salicylic acid, > 630 μm (Siegfried, Zofingen, Switzerland); Polycarbophil (PC), Blanose 7H4FD (Na CMC) and Methocel K4M Premium (HPMC) were kindly donated by BF Goodrich, Cleveland, U.S.A., Scheller, Zürich, Switzerland and Prochem, Zürich, Switzerland, respectively. Methocel MC 25 (MC), pectin (PT) and *N*-Acetyl-L-cysteine (AC) were from Fluka, Buchs, Switzerland.

Coating of the glass beads and aspirin crystals with the hydrophilic polymers

An apparatus reported earlier to coat tablets was adapted (Ranga Rao and Buri, 1987) to coat microparticles. Using this laboratory model, particles (1–15 μg) were fluidized by compressed air and coated by spraying the dispersions of the polymer from the top. To avoid the influence of core on the bioadhesive property of the polymer coat and for evaluating the mucoadhesion of polymers, glass beads were chosen as model particles. Due to low solubility (1 in 300) and its availability in granular form, aspirin crystals (> 0.63 mm) were also chosen as a model drug only. Hydrophilic polymers, viz., PC, Na CMC, HPMC, MC and PT were used to coat the particles. HPMC and MC were dispersed in a mixture of methylene chloride and ethanol (1 : 1) and the rest in 40% ethanol. Difference in weight of the particles before and after the coating was taken as the amount of the polymer coated over the particles. The amount of the polymer coated on glass beads and aspirin crystals was found to be 4 and 12.5% w/w, respectively.

Testing of coated particles for bioadhesion

Unfasted albino rats (450–500 g) were anaesthetised with pentobarbital (50 mg/kg i.p.). Stomach and intestine were dissected and placed in Sörensen phosphate buffer (pH 6.0) at room temperature (20 ± 1° C). Then jejunum was cut and washed with the buffer at a rate of about 5 ml/min for 10 min initially and later at about 30 ml/min for about another 20 min till the intestine was clean. The stomach was cut longitudinally and emptied of food. Then its inner surface was washed with 0.1 N HCl at a rate of about 20 ml/min for 5 to 10 min till the mucosa was clean. The tissue was used within 2 h after dissection.

Five cm length jejunum was cut, placed on a polyethylene support (a tube of 2 cm diam. cut longitudinally at its centre), cut longitudinally, spread and held in position with the help of pins as shown in Fig. 1. Uncoated or coated particles (100 mg glass and 75 mg aspirin) were placed uniformly on the mucosa of the intestine (4.5 × 1 cm). Similarly 50 mg of particles were placed on mucosa of stomach (ca. 2 cm^2) after fixing over the polyethylene support.

The tissue (stomach or jejunum with the particles) was then placed in a desiccator maintained at > 80% relative humidity and room temperature (20 ± 1° C) for 20 min to allow the polymer to hydrate and to interact with the glycoprotein and also to prevent the drying of the mucus.

After 20 min, the polyethylene support was introduced into a plastic tube cut in a similar manner and held in inclined position. The mucosa of stomach and intestine were washed for 5 min with 0.1 N HCl and Sörensen phosphate buffer (pH 6.0) respectively, at room temperature at the rate of 22 ml/min using a peristaltic pump. The

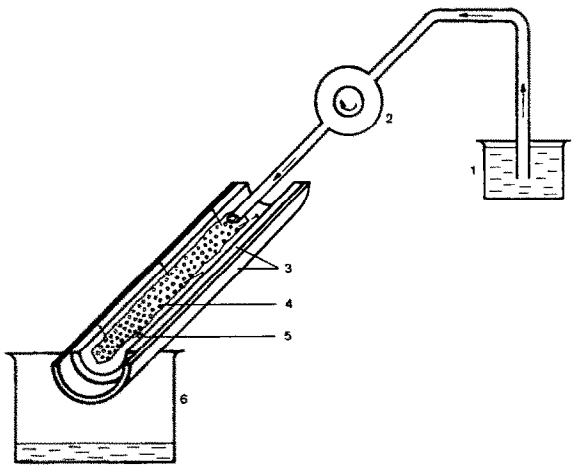

Fig. 1. Schematic diagram of the assembly used to test the bioadhesion of the microparticles. (1) Reservoir containing the washing solution; (2) peristaltic pump; (3) plastic support; (4) tissue; (5) pin; (6) receiver for collecting the washings.

tip of the tube carrying buffer solution or dilute HCl was placed 2–3 mm over the tissue so that the liquid flows evenly over the mucosa. The washings were collected into a beaker. After the washing, the liquid was decanted and the beaker dried in a hot air oven at 70 °C. The percent of beads washed away was determined by weighing the dried residue collected. Quantity of polymer dissolved during washing being very small compared to the weight of particles, it was considered as negligible.

In order to check the importance of the thickness of mucous layer for bioadhesion, mucus present on the intestine was gently scraped off before placing the particles.

Similarly the effect of the mucolytic agent, *N*-acetyl-L-cysteine (AC) was checked by adding 0.05 and 0.1 ml of 0.1 M freshly prepared solution of AC (pH adjusted to 9.0 with NaOH) to intestinal mucosa and kept aside for 1 h before placing the particles. Mucolytic activity of AC was reported to be maximum when its pH is 9.0 and also when incubated with mucus for 1 h (Sheffner, 1963).

Results and Discussion

The mean percent of the coated or uncoated particles adhered to the stomach or the intestine under various conditions are given in Table 1. Representative photographs showing the adhesion of particles to the intestine are shown in Fig. 2. Uncoated aspirin particles were entrapped in mucus primarily because of their irregular shape unlike the spherical glass beads. Swelling of the coat appears to promote its adhesion to mucus although swelling is not so apparent with a strong bioadhesive polymer like PC. Relaxation of the macromolecular chains facilitate their penetration and interlinking with the glycoprotein network of the mucus leading to better adhesion. During the initial trials, it was observed that when the amount of the cellulose ether coated on particles was 2% w/w, they did not bind to mucus indicating that some minimum thickness of the coat (i.e., minimum number of polymer chains) is needed for bioadhesion. Binding of the coated particles to the intestine was significantly less (HPMC, MC and PT) or equal (PC and Na CMC) compared to the stomach (Table 1) indicating that the affinity of the polymers to the stomach mucus was stronger than to the intestinal mucus. *N*-acetyl-L-cysteine is known to alter the conformation of the mucoproteins by exchanging its sulfahydryl groups with the disulphide bonds of the mucus (Sheffner, 1963). Due to this, the glass beads coated with Na CMC adhered to a much lesser extent to intestinal mucus in presence of AC. But the adhesion of drug crystals coated with Na CMC is unaffected by the presence of AC which may be because of the greater amount of the polymer in the coat. On the other hand, particles coated with HPMC or MC adhered better in the presence of AC. Since the viscosity of the mucus is decreased significantly in the presence of AC, polymer chains of HPMC and MC might have penetrated easily through the mucus and adhered to the epithelial cell surfaces. This indicates that the affinity of HPMC and MC polymer chains is greater to the epithelial cell surfaces compared to that of the mucus. Adhesion of glass particles was the same when either 50 or 100 μl of 0.1 M solution of AC was added. But HPMC, MC and PT coated drug crystals adhered to a lesser extent when the volume of AC was increased (Table 1). PC-coated particles adhered tenaciously to mucus and are unaffected by the presence of AC.

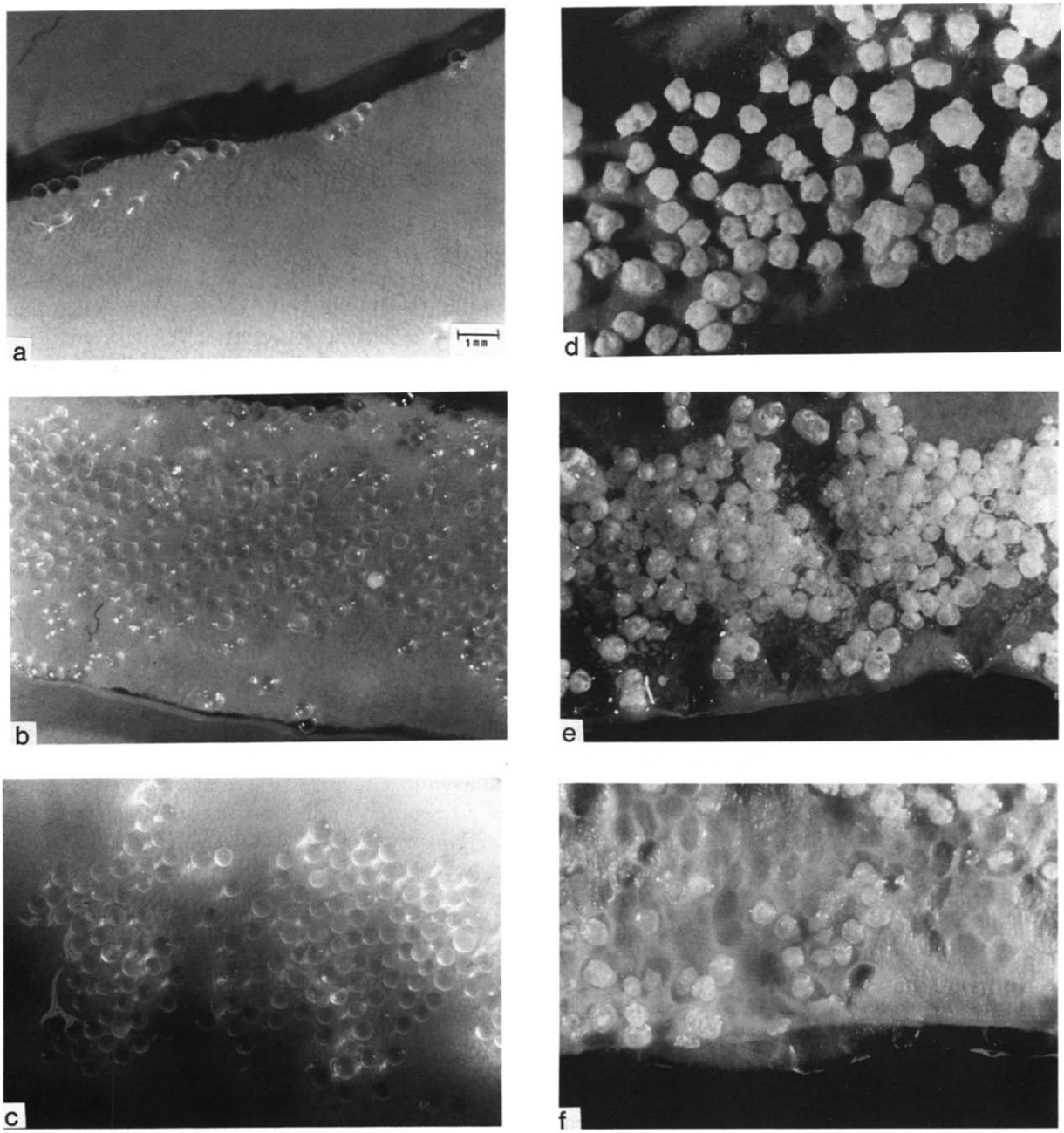

Fig. 2. Representative photographs taken after the experiment showing the adhesion of coated or uncoated glass beads or aspirin crystals to rat intestine. (a) Uncoated glass beads; (b) Na CMC-coated glass beads; (c) PC-coated glass beads; (d) PC-coated aspirin crystals; (e) HPMC-coated aspirin crystals; and (f) MC-coated aspirin crystals.

Adhesion of HPMC coated glass beads was much greater in the absence of mucus while the reverse was seen with Na CMC coated glass beads. This again supports the earlier observation that HPMC has greater affinity to the epithelial cell surfaces compared to the mucus. PC-coated par-

TABLE 1

*Mean ($n = 6$, * $n = 3$ or more) percent (w/w) of coated and uncoated particles adhering to stomach/intestine under various conditions*

Polymer	Glass beads					Drug particles				
	Stomach	Intestine				Stomach	Intestine			
	Normal *	Normal	+0.05 ml AC	+0.1 ml AC	−Mucus *	Normal *	Normal	+0.05 ml AC	+0.1 ml AC	−Mucus *
–	12 (±10.8)	0	0	0	0	72 (±9.0)	79 (±8.9)	89 (±2.3)	87 (±2.0)	76 (±10.3)
PC	93 (±9.1)	89 (±3.3)	89 (±7.0)	87 (±5.1)	76 (±3.7)	100	100	100	100	100
NaCMC	72 (±11.7)	74 (±10.5)	38 (±13.4)	31 (±11.3)	29 (±5.9)	96 (±1.5)	96 (±1.5)	100	100	100
HPMC	54 (±16.2)	6 (±3.2)	15 (±7.5)	25 (±6.9)	76 (±5.7)	98 (±1.2)	75 (±22.8)	92 (±5.0)	76 (±20.9)	89 (±6.5)
MC	45 (±11.4)	6 (±4.3)	14 (±11.1)	10 (±6.5)	18 (±2.3)	80 (±3.4)	58 (±20.4)	82 (±3.1)	69 (±13.7)	65 (±7.4)
PT	49 (±9.8)	25 (±14.8)	20 (±2.9)	19 (±2.6)	13 (±3.5)	89 (±11.0)	56 (±9.1)	63 (±6.5)	49 (±14.4)	79 (±8.6)

ticles adhered very strongly even when the mucus was minimum. Lesser binding of HPMC, MC and PT coated drug particles to the intestine compared to the uncoated drug crystals may be due to the greater flowability of the former than the irregular drug granules.

Conclusions

The bioadhesiveness of the coated particles to the intestine decreased in the following order: glass beads: PC > NaCMC > PT > MC > HPMC; drug crystals: PC > Na CMC > HPMC > MC > PT. In the stomach, the following order was seen: glass beads: PC > Na CMC > HPMC > PT > MC; drug crystals: PC > HPMC > Na CMC > PT > MC.

These results are in agreement with those of Ch'ng et al. (1985), that polyanions like PC and Na CMC adhere strongly to the mucus compared to the nonionic polymers like MC and HPMC. A minimum thickness of the coat is needed for their adhesion to mucus. HPMC has special affinity for the epithelial cell surfaces compared to the mucus. PC adheres tenaciously to mucus even in presence of 0.1 ml of 0.1 M AC and also when the mucus was minimum. AC alters the structure of the mucus and thereby affects the adhesion of Na CMC.

It may be concluded that this simple, realistic and quantitative technique using glass beads as core (compared to irregular aspirin particles) can be used for rating a series of polymers with respect to their bioadhesion properties.

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