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RESEARCH PAPER

Development of a ''Continuous-Flow Adhesion Cell'' for the Assessment of Hydrogel Adhesion

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ABSTRACT

The purpose of this study was to develop an in vitro perfusion technique or ''continuous-flow adhesion cell'' model to predict the in vivo performances of different mucoadhesive drug delivery systems based on hydrogels. Two studies were performed, either using a rabbit small intestine or a polyethylene surface; the adhesion of four gels—two poly(acrylic acid)s (PAAs) (carbomer [CM] and polycarbophil [PC]), an ethyleneoxide-propyleneoxide block copolymer (Poloxamer® 407 [PM]), and a polysaccharide (scleroglucane [SG])—were evaluated. In this respect, scleroglucane was used as a control. The adhesiveness of the different gels for both supports is in accordance with that described in the literature, that is, polycarbophil adhered more strongly than carbomer, which itself adhered more strongly than poloxamer. This study proved that the gels adhere more strongly to the polyethylene tube than to the rabbit small intestine, thus indicating that evidence for adhesion properties does not need any presence of mucus. Therefore, our in vitro model could be a good method, more precise and more simple than an ex vivo technique, to predict the bioadhesion of gelified devices.

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INTRODUCTION

This work is a comparative study of the adhesiveness ex vivo and in vitro of hydrogels using a dynamic ''continuous-flow adhesion cell'' model developed in our laboratory (Fig. 1). The different methods used to predict bioadhesion are mainly adhesion strength tests (1–12), perfusion techniques (1,13,14), and rheological tests (1). Among them, only perfusion techniques may assess the duration of adhesion, and they appear as a more realistic measurement of adhesion performances since adhesion strength and rheological tests are indirect methods for bioadhesion evaluation. Three types of perfusion techniques are used (1): with a bed of mucus in the ''flow channel'' method, with a section of excised tissue cut lengthwise, and with an entire segment of intestine. The last two are so-called falling liquid film methods. We proposed to develop a falling liquid film test, namely, the continuous-flow adhesion cell method, using a rabbit small intestine or an inert polyethylene support for the assessment of ex vivo and in vitro adhesion.

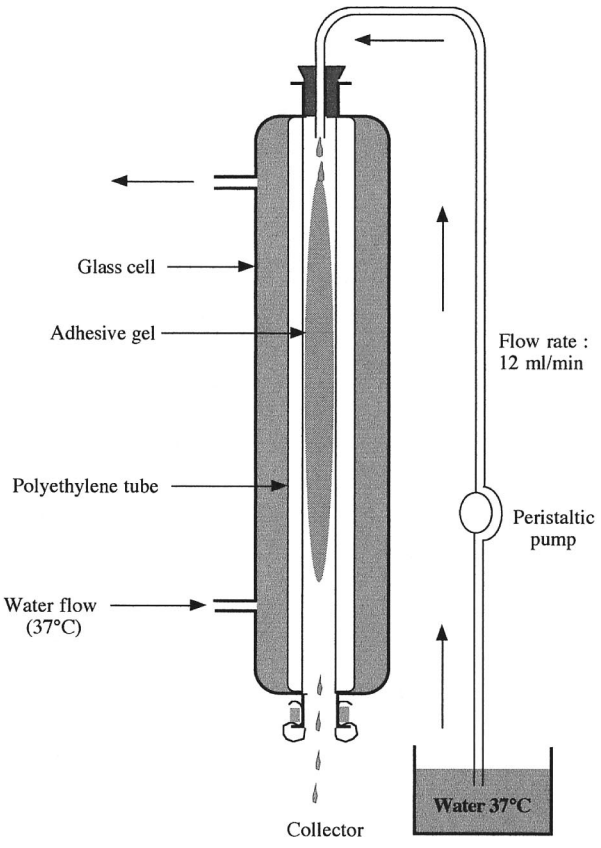

Figure 1. Continuous-flow adhesion cell.

The different polymers reported as bioadhesive hydrogels and used in oral (15,16), nasal (12,17), topical (18,19), rectal (11,20), ocular (19,21–25), and vaginal or uterine applications (2,15) are essentially the poly(acrylic acid) (PAA) polymers (15,20–22), carbomers (CMs) (Carbopol®) (12,17,21,24,25), polycarbophil (PC) (2, 15,24), and polyoxyethylene-polyoxypropylene block copolymer (Poloxamer®) (PM) (11,19,24). Other polymers (15,21,25,26) such as hyaluronic acid, hydroxypropyl methylcellulose, methylcellulose, polyvinyl alcohol, polyethylene glycol, and sodium carboxymethyl cellulose are used to a lessen extent, so we decided to focus our study on the properties of carbomer, polycarbophil, and polyoxyethylene-polyoxypropylene block copolymer.

The first part of this work represents an evaluation of the tracer diffusion throughout the different color gels tested in order to validate their further characterization on the bioadhesive model. The second part is the study of the polymer bioadhesion using a rabbit small intestine to compare our results with those already described in the literature. The third part deals with the assessment of intrinsic adhesion using an inert support, here a polyethylene surface, to develop a simple model that could predict further in vivo bioadhesion.

EXPERIMENTAL

Materials

The following polymers were studied: Carbopol 974P ($\overline{MM}$ 3,000,000) and Noveon® AA-1 (polycarbophil) (reticulated polymer with an estimated $\overline{MM}$ of 2,000,000), which are PAA derivative polymers (B. F. Goodrich, Rueil-Malmaison, France); Lutrol® F127 (Poloxamer 407) ($\overline{MM}$ 12,500), an ethyleneoxide-propyleneoxide block copolymer (BASF, Levallois-Perret, France); and Actigum® CS 11 (scleroglucane, SG) ($\overline{MM}$ 540,000), a polysaccharide polymer (SBI, Isle sur Sorgue, France).

Ponceau S (PS) was provided by Sigma (Saint Quentin Fallavier, France). Sodium hydroxide (NaOH) 0.1 N (Normapur grade) was supplied by Prolabo (Paris, France).

Gel Formulations

Each gel was prepared as follows. The polymer was gently poured into distilled water under rotating paddle

agitation, and the mixture was kept at room temperature until polymer swelling was complete, except for Poloxamer 407, which was prepared in an ice bath at 5°C according to the cold technique previously described (18). Ponceau S was added as a marker substance to the gels at a concentration of 2 mg/g. Each preparation was finally adjusted to pH 5.5–6 (Consort P600 pHmeter, Bioblock Scientifique, Illkirch, France) with 0.1 N NaOH.

The different polymer concentrations in water (w/v) for the preparation of CM, PC, SG, and PM gels were 0.5%, 1%, 1%, and 15.5%, respectively. These values were so chosen to match closely the concentrations reported in previous works dealing with the bioadhesive properties of these polymers. Indeed, PAA has already been used at 0.6% for ocular administration of pilocarpine (21,22); Carbopol 941 was administered nasally associated with insulin or calcitonin at a 0.1% or 1% concentration (12,17) and PM was described for ocular and rectal applications alone or when associated with pilocarpine, dexamethasone, phenylephrine, indomethacine, or sodium chloride and in concentrations varying from 10% to 25% (11,19). In fact, aqueous PM solution has the particularities of remaining liquid below 25°C and gelifying at a minimum concentration of 20% at skin temperature (18,20,27). In our particular case, we determined that a 15.5% concentration was suitable to induce gelation at 37°C.

The viscosity of the tested solutions was measured at 37°C. Viscosity experiments were performed with a rotational viscometer (Brookfield LV Viscometer, Brookfield Engineering Laboratories, Stoughton, MA).

The PS tracer was chosen for its solubility in hydrogels (solubility in water 1 g in 10 to 30 ml), for economic reasons, and to allow a simple colorimetric analytical detection.

In Vitro Ponceau S Diffusion Study

In vitro diffusion experiments were performed by dialysis with distilled water (800 ml) as the dissolution medium and at a constant temperature of 37°C. About 10 g of colored gel were inserted into a cellulosic dialysis tube with a cutoff of 6000–8000 (Polylabo, Strasbourg, France), which was then introduced into the vessel of an EP dissolution apparatus (Sotax Dissolutest AT 6, Basel, Switzerland) and subjected to a 75-rpm paddle rotation. Samples were taken for 3 hr as follows: 5 ml of the colored solutions were withdrawn and replaced with fresh distilled water to maintain the same total volume throughout the study. The PS concentrations of the samples were measured using a Philips spectrophotometer (PU8730, Philips Analytical, Cambridge, UK), operating at $\lambda = 520$ nm. All experiments were performed in triplicate.

Adhesion Evaluation Test

Continuous-Flow Adhesion Cell

The adhesion cell (Fig. 1) consisted of a glass tube (25 cm in length and 5 cm in diameter) with two 2.2-cm diameter openings at its top and bottom. Two lateral apertures allowed the free circulation of the thermostated liquid. A rabbit small intestine or a flexible polyethylene tube was introduced into the glass tube and fixed at its upper and lower ends with a perforated rubber stopper. This system permitted the liquid to flow through the internal tube at a desired rate.

Ex Vivo Study

For ex vivo study, the mucosal surface of a rabbit small intestine was used to test the mucoadhesion properties of the polymers. An approximately 30-cm segment of small intestine was removed from a rabbit and stored in Tyrode's solution until use (within 4 hr after the animal was killed). The operating procedure was as follows. About 10 g of colored gel were introduced with a syringe into the intestine. The intestinal loop was perfused with oxygenated Tyrode's solution (37°C) to maintain the viability of the gut segment. After 10 min, the apparatus was returned to a vertical position. A continuous flow of distilled water (37°C) at a constant rate of 12 ml/min was passed through the loop using a peristaltic pump. Colored solutions were then collected at fixed times for 3 hr. The absorption intensities were determined spectrophotometrically at $\lambda = 520$ nm.

Results are expressed as the retained PS percentage of the total amount recovered as a function of time. The rate of PS appearance at the bottom of the cell was a function of its cell residence time, which itself was proportional to both gel adhesion and PS diffusion throughout the gel. All studies were performed in triplicate.

In Vitro Study

A nonporous, inert, mucus-free material (polyethylene type) was used to determine the intrinsic adhesion. The bent flexible polyethylene tube (0.03 mm in width) was

maintained at 37°C in water. The procedure and expression of results were the same as for the ex vivo study.

RESULTS

Gel Formulations

Viscosity results at 37°C are summarized in Table 1. Polycarbophil viscosity was markedly higher (820,000 mPa · s) than those exhibited by the other hydrogels (5-fold higher than PM and 10-fold higher than CM). The viscosity of SG was found to be the lowest at 19,200 mPa · s.

In Vitro Ponceau S Diffusion Study

Figure 2 represents the PS diffusion from the polymer devices as a function of time. These diffusions were 22.1% ± 2.3% and 12.8% ± 0.5% at 180 min for PC and CM, respectively. In the case of SG or PM, tracer release was much lower (6.3% ± 0.2% and 4.0% ± 0.6% at 180 min, respectively).

Adhesiveness Evaluation Test

Ex Vivo Study

In this study (Fig. 3), the bioadhesion decreased from PC to CM and then to PM. The PC had the best adhesion, with 22.2% ± 7.4% of PS remaining in the small intestine after 180 min. After a similar time period, CM was completely removed (at 170 min, only 0.3% ± 0.6% of the tracer still remained in the small intestine), whereas PM showed complete removal after 45 min (0.5% ± 0.3%).

In Vitro Study

The in vitro adhesion assays showed the same profiles as those obtained for the ex vivo study (PC > CM > PM) (Fig. 4). The PC appeared to be the most adhesive gel since 59.2% ± 9.1% of PS remained in the polyethylene tube after 3 hr. The tracer percentage was lower for carbomer (19.3% ± 5.0% at 180 min) and considerably lower for other polymers. The PM adhered for 80 min, but 66.9% of PS had already been eliminated after 15 min. As expected, the control (SG) showed the poorest adhesiveness, with 84% of PS recovered from the collected solution after only 2 min. Afterward, the amount remaining on the inner surface of the polyethylene tube gradually decreased, giving the lowest measured concentration (0.5% after 25 min).

Table 1

Hydrogels Viscosity at 37°C

Polymer	Concentration (%)	Viscosity (mPa.s)
PC	1	820,000
PM	15.5	166,000
CM	0.5	85,000
SG	1	19,200

DISCUSSION

In Vitro Ponceau S Diffusion

The diffusion of PS varies from 4.0% ± 0.6% (PM) to 22.1% ± 2.3% (PC) after 180 min. These results indicate that diffusion strongly differs from one polymer to another. This observation has already been described in the literature (12,17,23,28–30), and the phenomenon seems to depend on molecular mass, concentration, and viscosity of the gels. Peppas and Sahlin (30) reported that the rheological properties of several bioadhesive formulations were correlated to the drug release rate. As far as this work is concerned, it could be said that the diffusion is related to the molecular mass. The higher PS diffusions were observed for PC (22.1% ± 2.3%) and CM (12.8% ± 0.5%), which have the greatest molecular masses (2,000,000 and 3,000,000, respectively). The lowest PS diffusions were observed for SG (6.3% ± 0.2%) and PM (4.0% ± 0.6%), which have the lowest molecular masses (540,000 and 12,500, respectively). As this diffusion phenomenon could be exploited to develop controlled delivery bioadhesive systems, it represents a limit for a tracer in the adhesiveness evaluation in further studies. In the present work (i.e., a comparative adhesion study of two supports, a rabbit small intestine and a polyethylene tube), these differences were not considered a problem. However, for further bioadhesion characterization, it would be preferable to follow radiolabeled polymers or to dose each one with an appropriate analytical method. A gravimetric assay could also be developed, but our own experience very rapidly showed the limits in sensibility of this method.

Adhesion Evaluation Test

Ex Vivo Study

The ex vivo study confirmed the higher bioadhesion of PAA gels as described in the literature. The best adhesion

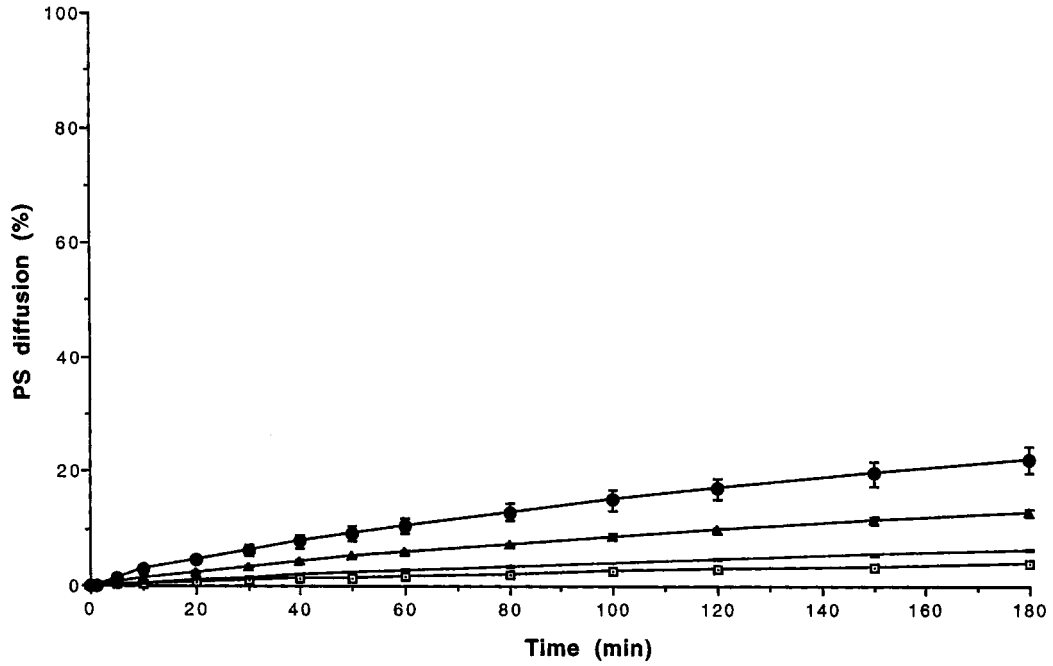

Figure 2. Ponceau S diffusion from gels as a function of time.

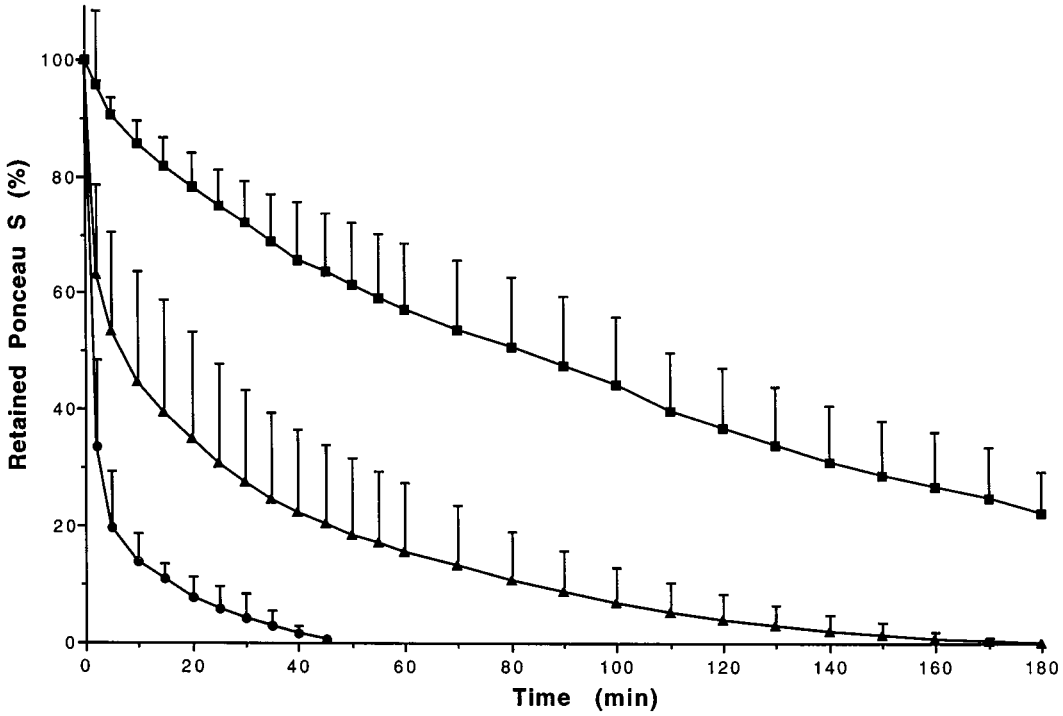

Figure 3. Ex vivo Ponceau S retained in gels as a function of time.

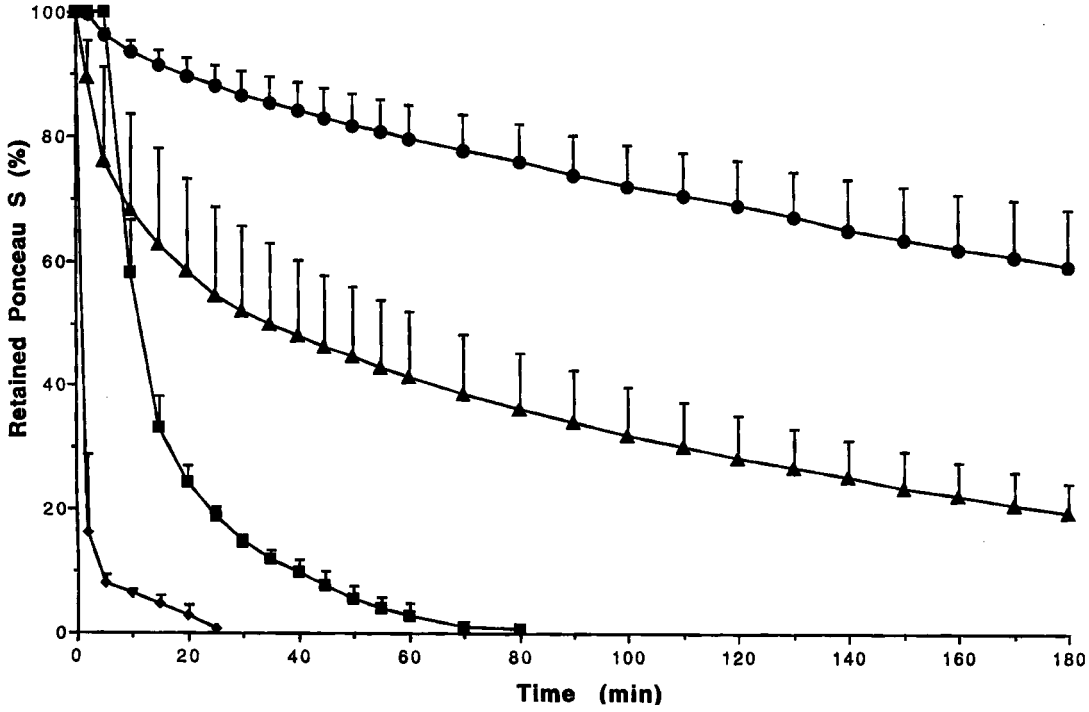

Figure 4. In vitro Ponceau S retained in gels as a function of time.

property was observed with PC (over the experiment time). The CM showed lower mucoadhesion (only for a 3-hr period), with removal of 50% of this gel after 8 min. These results could be correlated with a previous evaluation of the average residence time study in an in situ perfused gut segment model, which showed that PC significantly improves mucoadhesive properties compared to CM (31). In this work, the residence time of CM-coated microspheres was comparable to that observed with noncoated controls, whereas PC-coated spheres initially showed marked bioadhesion. The mucoadhesive properties of PAAs were described previously as a result of the interactions of their carboxylic groups with mucus functional groups (2,32–35). In fact, the bioadhesion is maximal at a pH less than 6 (32,34), at which most carboxyl groups of PAA are un-ionized and thus available for the formation of hydrogen bonds. Above pH 6 (2,32), hydrogen bonds disappear, and adhesion decreases. It has also been suggested that mucoadhesion occurs by a process of interpenetration of the mucoadhesive polymer with the mucus gel (30,33,36).

Concerning Poloxamer 407, it has been difficult to obtain reproducible adhesion results. Many of our experiments were invalidated by blocking problems at the beginning of or during the study. This could be the consequence of an increase in both PM concentration and temperature. It has been reported (11,19,37) that above a 10% (w/v) concentration, within a 35°C to 45°C temperature range, both mean micellar size and polydispersity may be augmented, thus inducing the gel formation by the way of aggregation between the micelles. This has a consequence on the gel flowability. The polymer-water mixture obeys Newton's law above the sol-gel transition temperature, whereas non-Newtonian behavior appears after gel formation. The influence of the temperature may be related to the chemical structure of PM: a central hydrophobic nucleus (polyoxypropylene) surrounded by hydrophilic sequences (polyoxyethylene, 70%) (11,38). In cold water, solubility of PM is due to the formation of hydrogen bonds between the water and the oxygen atoms of the polymer. When the temperature increases, the bonds break, producing interactions between the hydrophobic fractions, thus decreasing solubility. So, this structure gives the polymer the same properties that the mucin exhibits: adhesiveness and water washability (11).

After dealing with rejected assays, if only the most recurrent results are considered, PM was less adhesive on mucus than PAA. In fact, after 45 min, 100% of the

gel was removed. Any ex vivo comparison could be done with results given in the literature as only in vivo studies were performed (19,39). Nevertheless, if compared to Koller and Buri's works (19), a less important pharmacological answer was observed on rabbit intraocular pressure when adrenaline was administered in a Pluronic F127 gel (25%) than in a Carbopol 934 gel (2%). This could indicate that the contact time, and consequently the adhesion properties, with Pluronic F127 was less important.

In Vitro Study

Our in vitro results clearly showed that PC and CM are the best adhesives in the in vitro testing. These results for PAA are in agreement with those of Helliwell (1), Ranga Rao and Buri (13), and Lehr et al. (31), who showed that CM and PC are consistently the best adhesives in the in vitro assays, with PC performing slightly better than CM. In our own work, PC also had a substantially longer polyethylene transit time than CM (59.2% of the gel remained at 180 min). In the case of CM, the polyethylene transit time was lower, although 50% of the gel was eliminated at 35 min. In fact, the PS release began more quickly for CM than for PC.

Considering the same remarks as those for Poloxamer 407 in the ex vivo results, it may be noticed that its adhesion time was not negligible (90 min). Unfortunately, the adhesion profile was not linear since at 15 min only 33.1% $\pm$ 5.0% of PS still remained in the polyethylene tube.

The low adhesiveness obtained with the control (SG) was expected since this polysaccharide is known for its gelling properties (it is used as an excipient for the development of sustained-release matrices), but not for its adhesive properties.

CONCLUSION

Our ex vivo and in vitro results harden the idea that the adhesion of hydrocolloids containing carboxylic groups is greater than that of hydrocolloids with neutral groups (17).

It may be noticed that the ex vivo intestinal adhesion of hydrogels always decreased when compared to the in vitro results. Each polymer seemed to adhere better on a polyethylene support than on a mucus support. The less important interactions between PC and mucus than those observed on a polyethylene support have already been explained (13,32,41,42). It seemed that there is a higher strength in PC-mucin than in mucin-mucin interactions. Thus, PC seemed to carry the mucin. In the same way, Mortazavi and Smart (10) determined that CM (Carbopol 934) adhesion on a nonadhesive polyvinyl chloride surface was greater than on a rat intestinal mucosa. They showed that the presence of mucus or mucous glycoproteins is apparently unnecessary for adhesion, and that adhesive strength tends to be weaker when more mucus is present. This fact is consistent with the lubricant role of the mucus within the gastrointestinal tract.

It may be concluded, as Mortazavi and Smart (10) previously said, that these mucoadhesive hydrogels are able to adhere to different surfaces, and that a specific mucus/mucoadhesion interaction is not an important factor. In fact, the bioadhesion is often described as the result of three steps (2,36): an intimate contact (good wetting) between the bioadhesive and the substrate, an interpenetration, and chemical interactions (electrostatic forces, Van der Waals forces, hydrogen and hydrophobic bonds). Our work showed that adhesion can occur without interpenetration processes.

The model developed here was suitable for measuring gel adhesion and mucoadhesion. An in vitro dynamic model could be used as a comparative method for the assessment of hydrogel adhesion. Ex vivo study could be necessary to confirm and predict in vivo bioadhesion of polymers. However, it will be necessary for further investigations to find a tracer closely bound to each polymer.

REFERENCES

1. M. Helliwell, Adv. Drug Deliv. Rev., 11, 221–251 (1993).
2. S. H. S. Leung and J. R. Robinson, Polym. News, 15, 333–342 (1990).
3. D. Duchene, G. Ponchel, D. Wouessidjewe, F. Lejoyeux, and N. A. Peppas, STP Pharma, 4(8), 688–697 (1988).
4. M. Szycher, *High Performance Biomaterials: a Comprehensive Guide to Medical and Pharmaceutical Applications*, Technomic, Lancaster, PA, 1991, p. 345.
5. F. Thermes, J. Grove, A. Rozier, B. Plazonnet, A. Constancis, C. Bunel, and J. P. Vairon, Pharm. Res., 9(12), 1563–1566 (1992).
6. B. Parodi, E. Russo, G. Caviglioli, S. Caffagi, and G. Bignardi, Drug Dev. Ind. Pharm., 22(5), 445–450 (1996).
7. M. E. De Vries, H. E. Bodde, H. J. Busscher, and H. E. Junginger, J. Biomed. Mater. Res., 22, 1023–1032 (1988).
8. Y. Jacques and P. Burri, Eur. J. Pharm. Biopharm., 38(6), 195–198 (1992).
9. S. Burgalassi, L. Panichi, R. Bernicchi, U. Urbano, and M. F. Saettone, STP Pharma Sci., 7(2), 129–134 (1997).

10. S. A. Mortazavi and J. D. Smart, Int. J. Pharm., 116, 223–230 (1995).
11. F. Garcia Sagrado, M. Guzman, J. Molpeceres, and M. R. Aberturas, Pharm. Technol. Eur., 5, 46–57 (1994).
12. K. Morimoto, K. Morisaka, and A. Kamada, J. Pharm. Pharmacol., 37, 134–136 (1985).
13. K. V. Ranga Rao and P. Buri, Int. J. Pharm., 52, 265–270 (1989).
14. D. Tuffier, Etude expérimentale de la bioadhésion de certains excipients au niveau intestinal, Doctor of Pharmacy thesis, Poitiers, 1986.
15. K. Knuth, M. Amiji, and J. R. Robinson, Adv. Drug Deliv. Rev., 11, 137–167 (1993).
16. T. Nagai and Y. Machida, Adv. Drug Deliv. Rev., 11, 179–191 (1993).
17. D. Duchêne and G. Ponchel, STP Pharma, 5(12), 830–838 (1989).
18. I. Schmolka, J. Biomed. Mater. Res., 6, 571–582 (1972).
19. C. Koller and P. Buri, STP Pharma, 3(2), 115–124 (1987).
20. K. Morimoto, H. Akatsuchi, R. Aikawa, M. Morishita, and K. Morisaka, J. Pharm. Sci., 73(10), 1366–1368 (1984).
21. S. Kumar and K. J. Himmelstein, J. Pharm. Sci., 84(3), 344–348 (1995).
22. F. Thermes, A. Rozier, B. Plazonnet, and J. Grove, Int. J. Pharm., 81, 59–65 (1992).
23. S. D. Desai and J. Blanchard, J. Pharm. Sci., 87(2), 226–230 (1998).
24. J. R. Robinson, STP Pharma, 5(12), 839–846 (1989).
25. A. K. Zimmer, P. Chetoni, M. F. Saettone, H. Zerbe, and J. Kreuter, J. Controlled Release, 33, 31–46 (1995).
26. M. F. Saettone, P. Chetoni, M. T. Torraca, S. Burgalassi, and B. Giannaccini, Int. J. Pharm., 51, 203–212 (1989).
27. M. Ishida, N. Nambu, and T. Nagai, Chem. Pharm. Bull., 31, 1010–1014 (1983).
28. N. Garcia-Gonzalez, H. Blanco-Fuente, S. Anguiano-Igea, B. Delgado-Charro, F. J. Otero-Espinar, and J. Blanco-Mendez, STP Pharma Sci., 2(6), 494–499 (1992).
29. P. Colombo, Adv. Drug Del. Rev., 11(1–2), 37–57 (1993).
30. N. A. Peppas and J. J. Sahlin, Biomaterials, 17(16), 1553–1561 (1996).
31. C. M. Lehr, J. A. Bouwstra, J. J. Tukker, and H. E. Junginger, J. Controlled Release, 13, 51–62 (1990).
32. H. S. Ch'ng, H. Park, P. Kelly, and J. R. Robinson, J. Pharm. Sci., 74(4), 399–405 (1985).
33. G. Ponchel, F. Touchard, D. Duchêne, and N. A. Peppas, J. Controlled Release, 5, 129–141 (1987).
34. H. Park and J. R. Robinson, J. Controlled Release, 2, 47–57 (1985).
35. H. Park and J. R. Robinson, Pharm. Res., 4(6), 457–464 (1987).
36. D. Duchêne, F. Touchard, and N. A. Peppas, Drug Dev. Ind. Pharm., 14(2,3), 283–318 (1988).
37. S. C. Miller and B. R. Drabik, Int. J. Pharm., 18, 269–276 (1984).
38. D. Duchêne and G. Ponchel, STP Pharma Sci., 3(4), 277–285 (1993).
39. S. Miyazaki, T. Tobiyama, M. Takada, and D. Attwood, J. Pharm. Pharmacol., 47, 455–457 (1995).
40. F. Garcia Sagrado, M. Guzman, J. Molpeceres, and M. R. Aberturas, Pharm. Technol. Eur., 6, 38–44 (1994).
41. J. D. Smart, Int. J. Pharm., 73, 69–74 (1991).
42. J. D. Smart, Drug Dev. Ind. Pharm., 18(2), 223–232 (1992).