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INTESTINAL TRANSIT OF BIOADHESIVE MICROSPHERES IN AN *IN SITU* LOOP IN THE RAT — A COMPARATIVE STUDY WITH COPOLYMERS AND BLENDS BASED ON POLY(ACRYLIC ACID)

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Two commercially available copolymers of acrylic acid, Carbomer (Carbopol® 934P) and Polycarbophil (Carbopol® EX-55 resin), and blends with Eudragit® RL 100 were screened for their mucoadhesive properties by determining the force needed to detach a polymer coated glass plate from porcine intestinal mucosa in vitro. *Microspheres of poly(2-hydroxyethyl methacrylate) (PHEMA) were synthesized by suspension polymerization and coated with candidate mucoadhesive polymers in an air-suspension process. A chronically isolated ileal loop model in the rat was used in order to study the intestinal transit of the microspheres. Bioadhesive properties of this potential drug delivery system were evaluated by recording the mean residence time of the microspheres when injected into the* in situ *perfused gut segment. Polycarbophil showed significantly improved mucoadhesive properties* in vitro *in comparison to Carbomer. In the* in situ *model, the residence time of Carbomer-coated microspheres was comparable to the non-coated controls, whereas Polycarbophil-coated spheres initially showed a marked bioadhesion.*

INTRODUCTION

It is a great challenge to develop bioadhesive drug delivery system (BDDS) for orally administered drugs. These may allow to control the gastro-intestinal transit and to improve drug absorption by very close contact with the intestinal mucosa. The brush border membrane of the enterocytes is covered by a water-insoluble gel of mucus, between 5 to 500 μm thick [1–3]. In order to adhere directly to the glycocalyx of the outermost cell layer — a mechanism used by several bacteria and viruses — the mucus gel layer must be penetrated. The BDDS of today, however, use this biological hydrogel as a connecting link between the delivery system and the epithelial cell surface. In this case the term *mucoadhesion* should be used to describe the phenomenon by which *bioadhesion* is achieved.

When the mucus gel-layer is to be exploited for purposes of controlled drug delivery, it is important to realize that both intestinal mucus and the outermost mucosal cell layer undergo an essential turnover [4]. Therefore, even for a relatively short-lasting fixation of a BDDS for several hours, the permanent renewal of the

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mucus may become an essential limiting factor. Unfortunately, there are no concise data available in literature about the renewal time of the mucus gel layer. Its formation is determined by the secretion and degradation of the constituting glycoproteins, both of which are regulated by complex processes which are still far from being completely understood [5,6].

Obviously, the development of an oral BDDS requires test methods which take mucus turnover into account. A recent review on methods to test bioadhesion is given by Duchêne et al. [7].

The best way to test possible bioadhesive dosage forms with relevance for the gastrointestinal route seems to be a direct approach *in vivo*. However, the experimental circumstances are often so complex and difficult to control, that it is impossible to determine critical physiological or technological factors.

In contrast, *in vitro* experiments play an important role for purposes of screening and gaining mechanistical insight in polymer–mucus interaction, because they allow to study bioadhesion under fairly controlled conditions. On the other hand, it is rather doubtful if they will record the properties of a candidate BDDS which become relevant when given orally to a volunteer or patient.

Probably the best compromise between the requirements of physiological integrity and the need to keep experimental parameters under control as far as possible is an experimental approach *in situ*. Here, the gut segment concerned remains in its natural environment under complete humoral and nervous supply by the test animal but is immediately accessible to the experimentator. Recently, Poelma and Tukker [8] have described a method for applying this technique to rats. An intestinal segment is isolated by surgery inside the animal which can be used for perfusion experiments over several weeks.

In a previous paper some preliminary results obtained with this *in situ* model were presented [9]. It was found that particles coated with a blend of Carbopol® 934P and Eudragit® RL 100 in a weight ratio of 9:1 showed a markedly delayed transit through the perfused gut segment in comparison to non-coated particles. The aim of the study presented here was to test the bioadhesive character of microspheres coated with different mucoadhesive polymers.

For some copolymers of acrylic acid promising effects have been reported with respect to a possible peroral administration [10]. Mucoadhesive polymers of that type have also been synthesized earlier in this laboratory [11] for buccal applications. In this study, two commercially available copolymers of acrylic acid – Carbomer and Polycarbophil – were used because of their known mucoadhesive properties. In order to improve the mechanical stability of coatings made from these polymers, the possibility of blends with another acrylate (Eudragit® RL 100) was also studied.

EXPERIMENTAL

Preparation of microspheres

Microspheres of poly(2-hydroxyethyl methacrylate) (PHEMA) were prepared by suspension polymerization as described by Mueller et al. [12] with a modification recently published by Robert et al. [13]. A polymerization solution consisting of 60 g of hydroxyethyl methacrylate (HEMA, Fluka, Buchs, Switzerland), 6 g of ethylene glycol dimethacrylate (EGDMA, Fluka) as cross-linker and 0.06 g of azobisisobutyronitrile (AIBN, Polyscience, Warrington, U.S.A.) as initiator was added to an aqueous suspension medium containing NaCl and a gel of $Mg(OH)_2$. These salts are essential to stabilize the droplets of the dispersed organic phase during the polymerization process in order to obtain regular-shaped, free-flowing microspheres. The suspension medium was stirred, purged with nitrogen and kept under reflux at 70°C for 3 hours and then at 90°C for 1 hour.

A sieved fraction in the range 315–400 μm was used for further experiments.

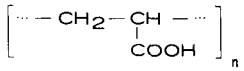
After extensive washing and drying, the beads were coloured for purposes of better visualization by soaking in ethanol containing 0.1% crystal violet for 4 hours at 50°C under light shaking. The coloured beads were washed with demineralized water and dried again. No visible leaching of the dye was observed when the beads were kept in aqueous media for several days. The density of the dried beads was 1.17 g/ml (Air Comparison Pycnometer, Model 930, Beckmann, U.S.A.).

Mucoadhesive polymers

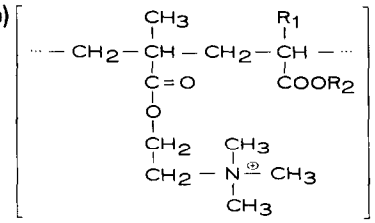
The tested polymers were Carbomer (Carbopol® 934P) and Polycarbophil (Carbopol® EX-55 resin), received as a gift from BF Goodrich (Cleveland, OH, U.S.A.). Chemically, the manufacturer describes both as polymers of acrylic acid (Fig. 1a).

Polymer blends were prepared by dispersing aliquot amounts of Carbopol® 934P and Eudragit® RL 100 in analytical grade methanol. The latter polymer was received as a gift from Roehm Pharma (Weiterstadt, F.R.G.). It is described by the manufacturer as a copolymer of esters of acrylic and methacrylic acid containing small amounts of quaternary ammonium groups. The molar ratio between ammonium groups and neutral ester groups is about 1:20 (Fig. 1b).

(a)



(b)



R_1 = H, CH_3

R_2 = CH_3, C_2H_5

Fig. 1. Chemical formulas of the tested polymers as reported by the manufacturers: (a) Carbomer and Polycarbophil, supplied as Carbopol® 934P and Carbopol® EX-55, respectively by BF Goodrich; (b) Eudragit® RL 100, supplied by Roehm Pharma AG.

Air suspension coating

In order to be able to coat very small amounts of microspheres in an air-suspension process we constructed a simple apparatus consisting of a 250 ml round glass flask in which a glass spray gun was mounted. The dimensions of the spray gun are essentially the same as described by Rao et al. [15]. A schematic picture of our apparatus is given in Fig. 2.

At the beginning of the coating process 1.0 g of the microspheres were fluidized in the flask with compressed air. 200 mg of the coating polymer were dispersed in 10 ml of methanol and sprayed onto the beads at a constant rate of 0.3 ml/min. To facilitate the evaporation of the solvent the bulb was placed in a beaker containing hot water (50°C).

Homogeneity of the coating was checked by microscopical inspection after swelling of the coated microspheres in water on a petri dish. Thickness of the coating was measured with an image-splitting eye piece (Vickers, A.E.I., England).

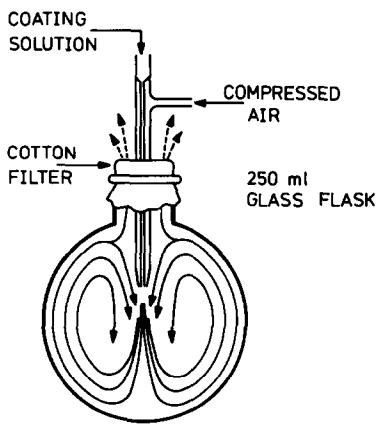


Fig. 2. A simple air-suspension coater.

Mechanical stability of the coating was tested in an USP XXI dissolution apparatus by dispersing 100 mg of the coated microspheres in 500 ml of isotonic saline (pH adjusted to 7.2–7.6 with 0.1 *M* NaOH). The paddle rotated at 100 rpm and temperature was kept at 37°C. Samples of the microspheres were pipetted from the test vessel and inspected under the microscope.

Testing of bioadhesive properties

Tensiometer method

Duodenum from freshly slaughtered pigs (either sex, 50–80 kg, in good state of health) was received from the Dutch National Veterinary Institute (CDI, Lelystad, The Netherlands) in oxygenated Krebs–Ringer buffer at room temperature and stored in the same medium at 5°C until use. All experiments were performed within 48 hours after killing the animals. The gut segment was cut in slices of about 5 cm and opened along the mesenteric border. Serosa and muscularis layers were removed by stripping with a pair of tweezers. This resulted in a flattening of the originally folded mucosal surface. Tissue pieces were gently washed and kept in Krebs–Ringer buffer.

A modified Du Nouy tensiometer was used in order to measure the strength of adhesive bonding of the coating polymers when brought in contact with porcine intestinal mucosa. The principle of the test apparatus is shown in Fig. 3. For an adhesion test a tissue of about 2 by 2 cm was fixed on a metal block with cyanoacrylate glue (Rapid-Kleber®, Henkel, F.R.G.). The mucosa was then covered with a Delrin® cap with a central hole of 1.0 cm diameter so that about 0.8 cm^2 of the mucosal surface was exposed. The block with the mounted tissue was transferred into a water-jacketed vessel containing isotonic saline (37°C, pH adjusted to 7.4). Cover glasses (22×22×0.15 mm) were coated with the polymers under investigation by pipetting 100 μl of a 1% (mass/volume) methanolic dispersion (i.e. 1 mg dry polymer) in the center of the glass platelet. After drying in air a thin polymer film remained. One cover glass was attached to the Delrin® support with its non-coated side by means of double-sided adhesive tape. The support was hung on the free arm of the tensiometer. By raising the water-jacketed vessel the support was imbibed in the test liquid until it almost touched the tissue. The scale was now set to zero and the arm locked. Further raising of the vessel brought the tissue in contact with the polymer and a slight pressure was exerted by the weight of the Delrin® support (1.5 g). This situation was maintained for 60 seconds. Then the lock was released and a vertically acting force was applied to detach the polymer from the mucosa. The force was increased continuously with the help of a small electric motor at a constant rate of 0.25 mN/s until the polymer was detached. The corresponding force was read from the scale. Linearity of the tensiometer was checked by suspending various weights between 100 and 1500 mg on its free arm. Cover glass and mucosa were only used once each measurement.

In situ perfusion studies

A detailed description and evaluation of the model is given elsewhere [8]. An intestinal segment of approximately 6–8 cm (about 15 cm proximal to the ileo-caecal junction) was isolated with intact blood supply. The loop remained in the peritoneal cavity. The head–tail connection of the intestine was restored by end-to-end anastomosis. Perfusion was possible through plastic tubes (3 mm i.d., 5 mm o.d.), connected to the intestinal segment via two Delrin® cannulas in the abdominal wall. After surgery, the rat was placed into a restraining cage and supplied with water and food. After recovery from the operation (2–4 days) the animal was ready for use in perfusion experiments.

The experimental set-up depicted in Fig. 4 was provided in four-fold, allowing to perform more experiments at the same time. The loop was perfused with isotonic saline (37°C, pH adjusted to 7.2–7.4) with a constant flow of 1.0

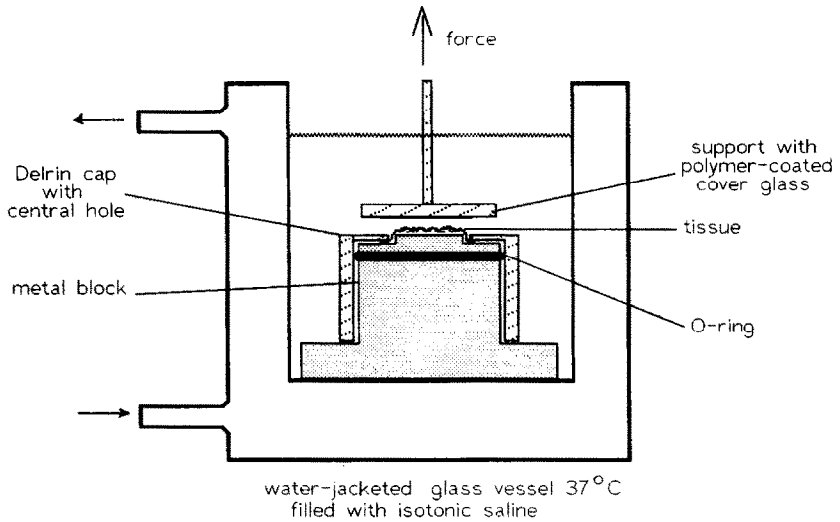


Fig. 3. Experimental set-up to measure the force of detachment of polymer-coated cover glasses from porcine intestinal mucosa.

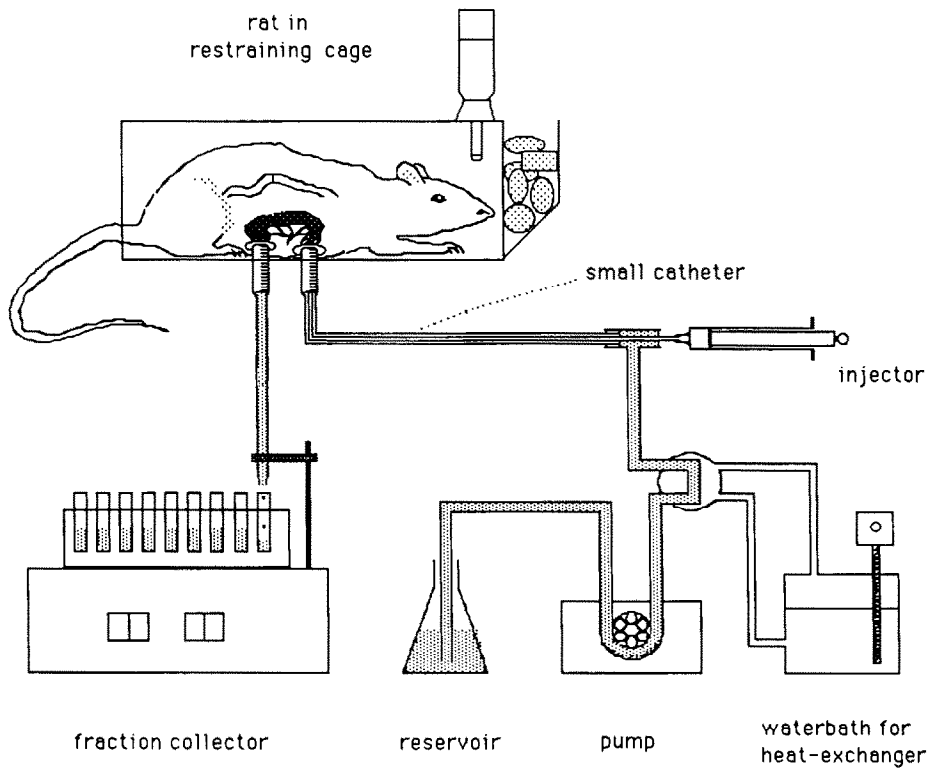


Fig. 4. Experimental set-up to study the intestinal transit of microspheres in a chronically isolated internal loop in the rat.

ml/min. Before the beginning of an experiment, the loop was rinsed by perfusing it for 30 minutes. At $t=0$ about 50 microspheres were injected with a small Teflon® catheter (1.1 mm i.d., 1.7 mm o.d.) without interrupting the perfusion. Fractions of the perfusate were collected over a period of 6 hours at intervals of 5 minutes. The particles in the fractions were counted. At the end of the experiment the last particles were removed by flushing the loop manually with a syringe.

RESULTS

Mucoadhesive properties of the coating polymers *in vitro*

The force of detachment as determined by the tensiometer method for Polycarbophil (Carbopol® EX-55) and blends of Carbomer (Carbopol® 934P) with various amounts of Eudragit® RL 100 is shown in Table 1. Pure Eudragit® RL 100 and glass plates without coating did not show any mucoadhesive properties.

As shown in Fig. 5 the force of detachment increases with increasing amounts of Carbomer in the polymer blends until at 90% Carbomer a maximum is reached. Polycarbophil shows markedly better mucoadhesive properties as indicated by a significantly increased force of detachment when compared with pure Carbomer ($P \leqslant 0.01$, Wilcoxon U-test). However, the values for Polycarbophil show a greater scatter than those for all other coatings investigated. The value for Polycarbophil was found to be in the same order of magnitude as reported by Ch'ng et al. [10], who measured with a similar method but in USP simulated gastric fluid (pH 1.2). Blends of Polycarbophil with Eudragit® RL 100 have not been studied in view of the insolubility of Polycarbophil.

Characterization of the coated microspheres

PHEMA microspheres coated with Polycarbophil (PCP), Carbomer (CBP) and a blend

TABLE 1

Mucoadhesive properties of different blends and commercially available copolymers of acrylic acid as measured by the tensiometer method. Asterisk indicates significant versus Carbomer ($P \leqslant 0.01$, Wilcoxon U-test)

Polymer weight ratio		Number of tests	Force of detachment [mN/cm^2] (mean ± S.E.M.)
Polycarbophil (Carbopol® EX-55)		11	9.19 ± 1.15*
Carbomer (Carbopol® 934P)		10	6.06 ± 0.24
Carbopol® 934P / Eudragit® RL 100	9:1	5	5.95 ± 0.29
	3:1	8	4.34 ± 0.51
	1:1	6	2.93 ± 0.17
	1:3	5	1.67 ± 0.18
Eudragit® RL 100		3	0
Cover glass without coating		2	0

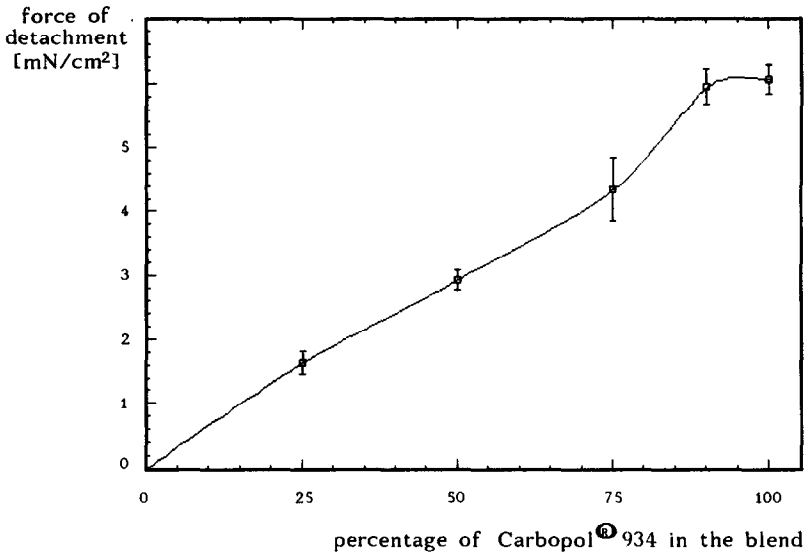


Fig. 5. Force of detachment as a function of increasing amount of poly(acrylic acid) in blends of Carbopol® 934P with Eudragit® RL 100. Points indicating mean ± S.E.M. as given in Table 1.

of 9 parts by weight of Carbopol® 934P with 1 part by weight of Eudragit® RL 100 (CRL) were tested, as well as non-coated microspheres (BLK) as control. The abbreviations in brackets refer to the four batches which have been used. After air-suspension coating the microspheres were surrounded by a very thin polymer film which swells within one minute up to an equilibrium thickness between 40 and 50 μm when exposed to water or saline. As shown in Fig. 6 there were no significant differences either in equilibrium thickness or in swelling velocity between the three coating polymers. A representative microscopical photograph is shown in Fig. 7. Under the conditions of the USP XXI dissolution test all coatings re-

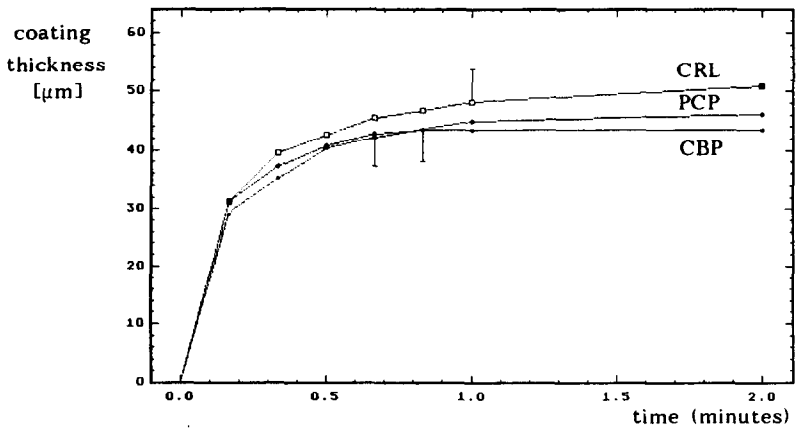


Fig. 6. Swelling of mucoadhesive coating surrounding the microspheres when immersed in demineralized water at room temperature. Shown are the averages of $N=6$ to 7. Error bars indicate S.E.M.

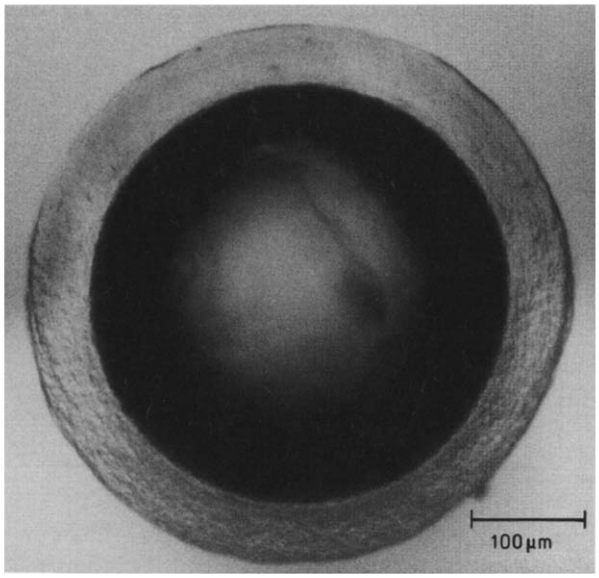


Fig. 7. Microscopical photograph of a PHEMA microsphere coated with mucoadhesive polymer (Polycarbophil) in swollen state.

mained intact over more than 24 hours. No dissolution or erosion was seen even for the non-blended Carbomer.

Intestinal transit of microspheres *in situ*

Bioadhesive properties of microspheres with different coatings were studied in a randomized block design with four rats on consecutive days. Due to leakage two runs were not evaluable and one rat had to be replaced during the study.

Residence curves, in which the sum of all particles collected in the fractions was normalized to be 50, were constructed as shown in Fig. 8. Individual residence curves can be characterized by the parameter mean residence time (MRT). This model-independent approach is based on the center of gravity of the area under the residence curves and has already been applied earlier in pharmacokinetics and biopharmaceutics [16]. MRT was calculated as

$$\mathrm{MRT} = \frac{\mathrm{AUC}}{N} \qquad (1)$$

where AUC is the area under the residence curve as calculated by the trapezium rule and N the total number of injected particles. As in most cases not all particles had left the intestinal loop before the end of the experiment, the corresponding residence curves do not approach zero. Therefore a certain period of time has to be defined for which the AUC and hence MRT is calculated. The MRT_{1h} was calculated for the first hour after injection.

Figure 9 shows the average MRT_{1h} values for the three coatings and the control. One-way ANOVA revealed significant differences at $p = 0.0186$. Normal distribution of the data was assumed, homogeneity of variances was checked by Cochran's C, Bartlett's and Hartley's test [17]. Based on Scheffé's multiple-range test the MRT_{1h} of PCP-coated microspheres was significantly increased when compared with all other polymers or control. Both CBP and CRL still gave an significant improvement versus control (BLK). Although statistically not significant, the average MRT_{1h} for CRL was higher than for CBP. So far, the results of the transit studies reflect the mucoadhesive properties of the three polymers as found by the tensiometer method. Furthermore, the MRT_{1h} value for the non-coated particles was practically identical with previous findings [9]. For particles coated with the blend of Carbopol® 934P and Eudra-

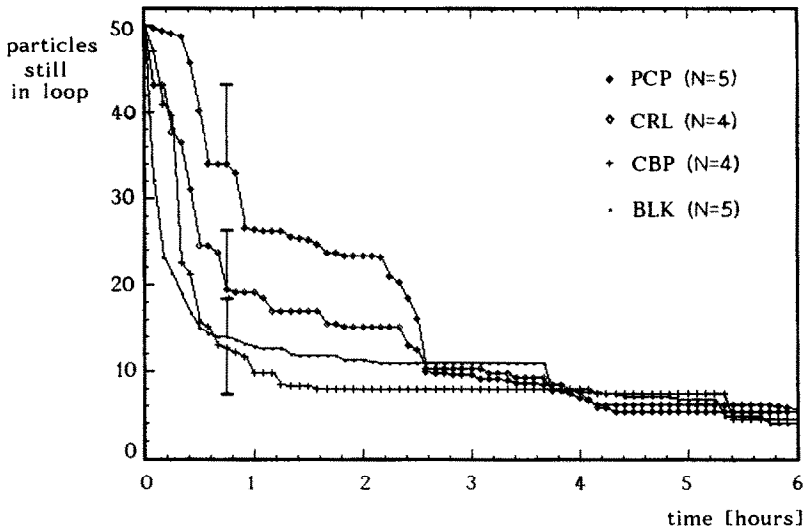


Fig. 8. Residence curves of PHEMA microspheres in *in situ* perfused isolated intestinal loop. Shown are the normalized mean curves with error bars indicating S.E.M.

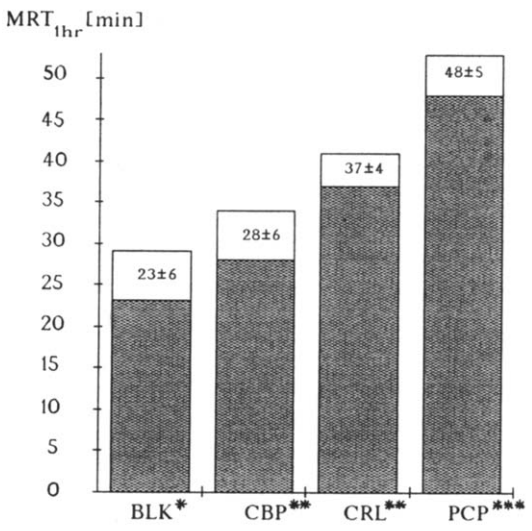


Fig. 9. Mean residence time for the first hour after injection as calculated by eqn. (1) (MRT_{1h}) of PHEMA microspheres with different coatings. Indicated values are mean ± S.E.M. For legend see text. The number of asterisks indicates homogenous groups as revealed by Scheffé's test based on 95% confidence intervals.

git® RL 100, a smaller value was found in the present study. The difference can be explained by the variation between the test animals.

However, one might wish to get a more absolute parameter for the transit velocity of the microspheres through the intestinal loop. From the curves in Fig. 8 it is evident that in general after 2 to 3 hours an asymptotic minimum had been reached, indicating that no more particles left the loop until the experiment was stopped. In perfusion experiments with opened abdomen under narcosis, it appeared that these remaining particles are withheld in the loop not by bioadhesion but due to wedging in folds and crevices of the gut segment. Under this premise an absolute MRT can be calculated by

$$MRT_{abs} = \frac{AUC - AUA}{N^*} \qquad (2)$$

where AUA is the area under the asymptotic minimum extrapolated to $t=0$, and N^* is the number of particles which had left the loop before the experiment was stopped. Average values of MRT_{abs} are shown in Fig. 10. PCP microspheres resided in the loop for the longest time, but any effect of the Carbomer coatings (CBP and CRL) had disappeared when compared with the control. Due to the relatively large standard deviations and the limited number of experiments, the differences lack statistical significance.

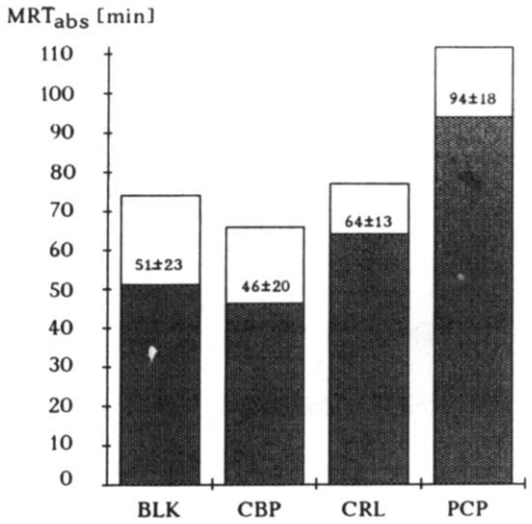


Fig. 10. Absolute mean residence times as calculated by eqn. (2) (MRT_{abs}) of PHEMA microspheres with different coatings. For legend see text. Differences statistically not significant.

DISCUSSION

In literature Polycarbophil and Carbomer are described as loosely crosslinked copolymers of acrylic acid [14]. Whereas in Polycarbophil the crosslinker is divinylglycol, Carbomer is crosslinked with allylsucrose. A more specified description of the chemical structure, especially the degree of crosslinking, was not available for both polymers. According to the manufacturer's information, Carbopol® 934P has an average molecular weight of approx. 3,000 kDa. For Carbopol® EX-55 resin this information was not given. Whereas Carbomer is known to form highly viscous aqueous solutions when neutralized with alkali hydroxides or amines, Polycarbophil is characterized as being practically insoluble in water, dilute acids, alkalies and common organic solvents [14]. However, it was possible to prepare homogenous dispersions of both polymer powders in methanol, which could be used to coat glass plates or PHEMA microspheres.

The reason for the obvious difference in mucoadhesive properties between Carbomer and Polycarbophil remains to be investigated. However, it can be speculated that the difference in crosslinking co-monomer might be responsible for the observed effects. As suggested earlier by Gurny et al. [18], there is a certain optimum in chain mobility which is needed for the interpenetration with the mucus. When this mobility is increased too much due to excessive swelling, the polymer/tissue interface becomes disentangled and the adhesive strength decreases. Such effects are of course time dependent and therefore may be less pronounced when mucoadhesion is measured only after a relatively short time of contact. On the other hand, for the development of a BDDS this dynamic aspect of bioadhesion might be of greatest importance. As the results from the transit studies indicate, the modified degree of crosslinking in Polycarbophil is advantageous. The state of optimal chain mobility yielding a maximum of mucoadhesive strength is obviously maintained for a longer period of time. However, the fact that a significant difference is only seen with MRT_{1h} and not with MRT_{abs} suggests that the bioadhesive effect remains limited to an initial period of time for all polymers tested. At present, it is unclear whether this is due to physiological reasons (mucus turnover) or due to shortcoming mucoadhesive properties of these polymers.

An interesting aspect of the results is the slight improvement of the bioadhesive properties of Carbomer when blended with 10% Eudragit® RL 100. In order to increase the mechanical stability of the Carbomer coating, this mucoadhesive but water-soluble polymer was blended with various amounts of Eudragit® RL 100. This polymer is swellable but not soluble in water and does not possess mucoadhesive properties itself. Although the force of detachment as measured by the tensiometer method is essentially the same for this mixture as for the pure mucoadhesive polymer, there seems to be a positive influence of Eudragit® in terms of an increased transit time. Possibly, Eudragit® acts as a "physical" crosslinker in the blend

which limits the overhydratation of the mucoadhesive polymer and hence provides a longer lasting mucoadhesive contact. In view of this favourable effect, the concept of polymer blending should be kept in mind, although it is not required with respect to the mechanical stability of these coatings.

The *in situ* perfused gut technique allows to study the transit of (bioadhesive) microspheres under controlled conditions close to the physiological reality. Dynamic changes in mucoadhesion become recognizable because mucoadhesion is studied as a time-dependent process. In contrast to "real" *in vivo* experiments, such a model may help to save animal lives in the earlier phase of dosage form development and does not require the use of radioactive markers. The selective investigation of both physiological and technological factors relevant for bioadhesion such as, for example, perfusion medium, loop length, particle size and mucus turnover is possible. The absorption of drugs delivered from microspheres can be determined by measuring plasma concentrations which allows the study of the influence of bioadhesion on bioavailability. This work is now in progress and will be published later.

CONCLUSIONS

Although chemically very similar, the two copolymers of acrylic acid, Carbomer (Carbopol® 934P) and Polycarbophil (Carbopol® EX-55 resin), show significant differences in mucoadhesive properties as evaluated by two different experimental methods. Blending of the (putative) water-soluble Carbomer with a water-insoluble, non-mucoadhesive polymer (Eudragit® RL 100) in a weight ratio of 9:1 parts did not reduce mucoadhesion in terms of force of detachment. Instead, the concept of blending might be useful in order to maintain mucoadhesive properties for a longer period of time. Sprayed on PHEMA microspheres in an air-suspension process, all three polymer formulations formed mucoadhesive coatings of excellent mechanical stability.

As the results of the perfusion experiments show, the approach to study the transit of particles in an isolated intestinal loop *in situ* is feasible. This technique is particularly useful in order to evaluate and differentiate between bioadhesive properties of candidate drug delivery systems.

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REFERENCES

1 S. Kerss, A. Allen and A. Garner, A simple method for measuring thickness of the mucus gel layer adherent to rat, frog, and human gastric mucosa: influence of feeding, prostaglandin, *N*-acetylcysteine and other agents, Clin. Sci., 63 (1982) 187–195.

2 M. Bickel and G.L. Kauffman, Gastric gel mucus thickness: effect of distention, 16,16-dimethyl prostaglandin E2 and carbenoxolone, Gastroenterology, 80 (1981) 770–775.

3 S.I. McQueen, P. Hutton, A. Allen and A. Garner, Gastric and duodenal mucus gel thickness in rat: effects of prostaglandines and damaging agents, Amer. J. Phys., 245 (1983) G388–G393.

4 M. Lipkin, Proliferation and differentiation of normal and diseased gastrointestinal cells, in: L.R. Johnson (Ed.), Physiology of the Gastrointestinal Tract, 2nd edn., Raven Press, New York, NY, pp. 255.

5 L.C. Hoskins, Degradation of mucus glycoproteins in the gastrointestinal tract, in: The Glycoconjugates, Vol. II, Academic Press, New York, NY, 1978, pp. 235.

6 M.R. Neutra and J.F. Forstner, Gastrointestinal mucus - synthesis, secretion and function, in: L.R. Johnson (Ed.), Physiology of the Gastrointestinal Tract, 2nd edn., Raven Press, New York, NY, 1987, pp. 975.

7 D. Duchêne, F. Touchard and N.A. Peppas, Pharmaceutical and medical aspects of bioadhesive systems for drug administration, Drug Dev. Ind. Pharm., 14 (1988) 283-318.
8 F.G.J. Poelma and J.J. Tukker, Evaluation of a chronically isolated internal loop in the rat for the study of drug absorption kinetics, J. Pharm. Sci., 76 (1987) 433-436.
9 C.-M. Lehr, J.A. Bouwstra, J.J. Tukker and H.E. Junginger, Design and testing of a bioadhesive drug delivery system for oral application, S.T.P. Pharma, 5 (1989) 857-862.
10 H.S.Ch'ng, H. Park, P. Kelly and J.R. Robinson, Bioadhesive polymers as platforms for oral controlled drug delivery. II. Synthesis and evaluation of some swelling, water-insoluble polymers. J. Pharm. Sci., 74 (1985) 399-405.
11 M.E. de Vries, H.E. Boddé, H.J. Busscher and H.E. Junginger, Hydrogels for buccal drug delivery: properties relevant for muco-adhesion, J. Biomed. Mat. Res., 22 (1988) 1023-1032.
12 K.F. Mueller, S. Heiber and W. Plankl, Process for preparing hydrogels as spherical beads of large size, U.S. Patent 4,224,427, 1978.
13 C.C.R. Robert, P.A. Buri and N.A. Peppas, Influence of the drug solubility and dissolution medium on the release from poly(2-hydroxyethyl methacrylate) microspheres, J. Controlled Release, 5 (1987) 151-157.
14 J.E.F. Reynolds, Martindale, The Extra Pharmacopoeia, 28th edn., The Pharmaceutical Press, London, 1982.
15 K.V.R. Rao and P.A. Buri, Design and in vitro testing of coated microparticles for bioadhesion, 47th Int. Congr. F.I.P., Amsterdam, 1987, abstract no. 281.
16 D. Brockmeier, Die Rekonstruktion der Freisetzungsprofile mikroverkapselter Arzneiformen durch den Mittelwert und die Varianz der Freisetzungszeiten, Arzneim. Forsch./Drug Res., 31 (1981) 1746-1751.
17 Statigraphics, V. 2.6, Statistical Graphics Corporation, Rockville, MD, U.S.A., 1987.
18 R. Gurny, J.-M. Meyer and N.A. Peppas, Bioadhesive intraoral release systems: design, testing and analysis, Biomaterials, 5 (1984) 336-340.