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RESEARCH PAPER

Anionic Mucoadhesive Polymers as Auxiliary Agents for the Peroral Administration of (Poly)Peptide Drugs: Influence of the Gastric Juice

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ABSTRACT

The incorporation of (poly)peptide drugs in mucoadhesive polymers is a promising strategy for their peroral administration. In this study, the protective effect of various polymers toward an artificial gastric fluid and the influence of an enteric coating on the adhesive properties have been investigated. Tablets containing 30 mg of carbomer (C934P), neutralized carbomer (NaC934P), or sodium carboxymethylcellulose (NaCMC), 0.1 mg of the model protein peroxidase, and 19.9 mg of mannitol were incubated at 37°C for 2.5 hr with a simulated gastric fluid with and without pepsin. All polymers—although anionogenic—displayed quick swelling behavior in the acid milieu, leading to an unintended protein release. Moreover, pepsin is capable of penetrating into the polymeric carrier systems, thereby rapidly degrading the embedded protein. Enteric coating, on the other hand, leads to strongly reduced adhesive properties. Only NaC934P tablets coated with polymethacrylate containing 9% triethylcitrate displayed no significant ($p < .05$) reduction in adhesive strength. Results give essential basic information for the development of peroral (poly)peptide dosage forms based on mucoadhesive polymers.

Key Words: *Enteric coating; Mucoadhesive polymers; Pepsin; (Poly)peptide drugs.*

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INTRODUCTION

The development of peroral delivery systems for peptide and protein drugs is highly desirable. On the one hand, the number of these therapeutic agents rapidly increases due to progress in biotechnology and gentechnology; on the other hand, oral dosage forms offer the greatest ease of application. However, considerable barriers encountered with the peroral route of administration are responsible for relatively poor bioavailability of therapeutic peptides and proteins. These barriers can be divided into the enzymatic barrier, the diffusion barrier, and the absorption barrier. A promising strategy to overcome these barriers is the use of bioadhesive polymers that offer the following advantages for the peroral administration of peptide and protein drugs:

1. The drug release from such polymers used as carrier systems can be controlled easily (1).
2. Bioadhesive polymers such as chitosan and polycarbophil display an additional permeation-enhancing effect (2).
3. Slightly modified bioadhesive polymers are able to guarantee a protective effect for embedded therapeutic peptides and proteins toward an enzymatic attack caused by gastrointestinal (GI) proteases (e.g., 3–5).
4. Furthermore, these auxiliary agents can provide intimate contact between the dosage form and the mucosa, thereby excluding a presystemic metabolism of the peptide and protein drug on the way between the delivery system and the absorption membrane.

Rapid swelling of the polymer on the intestinal mucosa leads to strong adhesion between the bioadhesive polymer and the mucus gel layer covering the absorption membrane. As many anionic polymers, such as polymethacrylates, cellulose acetate phthalate, or hydroxypropylmethylcellulose phthalate, do not swell at a pH value below 5, anionic bioadhesive polymers such as carbomer or sodium carboxymethylcellulose (NaCMC) might display similar features in the acid milieu. Based on an appropriate galenic, anionic bioadhesive polymers therefore should not be hydrated at all or only be hydrated partially during the gastric passage. In the small intestine, they should swell at pH values around 7, thereby providing strong adhesion. Moreover, during the gastric passage, peptide or protein drugs embedded in anionic polymeric carrier systems should not be degraded by pepsin or be released, making an enteric coating useless.

To verify or prove false this working hypothesis, tablets based on various bioadhesive anionic polymers with an embedded pepsin-degradable model protein (peroxidase) were generated. The swelling behavior, protein release, and protective effect toward an enzymatic attack caused by pepsin should be investigated in a simulated gastric fluid. A further aim of this study was the evaluation of the influence of enteric coatings on the adhesive properties of resulting dosage forms.

MATERIALS AND METHODS

Preparation of Polymers

Carbomer (Carbopol C 934P; B. F. Goodrich, Cleveland, OH) was neutralized by gradually adding 10 g of the polymer to 100 ml of a 4% (mass/mass [m/m]) methanolic sodium hydroxide solution while vigorously agitating with a magnetic stirrer. The precipitate of the resulting sodium salt was separated by filtration, washed with methanol until the pH value of the filtrate became neutral, and dried in a desiccator. The neutralized polymer (NaC934P) was stored at room temperature until use.

Unmodified carbomer (Carbopol C 934P) and NaCMC (Kwizda, Vienna, Austria) were used as received.

Preparation of Tablets

First, 30 mg of polymer, 19.9 mg of mannitol (Merck, Darmstadt, Germany), and 0.1 mg of horseradish peroxidase (Sigma, St. Louis, MO) were homogenized and pressed (Hanseaten, type EI, Hamburg, Germany) to tablets (diameter 5.0 mm, depth ~4 mm). The pressing power was kept constant during the preparation of all tablets.

Because of the small quantity of dosage forms, tablets were not spray-coated in a fluidized bed apparatus for economic reasons. Instead, enteric coating was achieved by dipping tablets three times in coating solutions as listed in Table 1 and air drying. Thickness of the enteric coating layer was determined under the microscope (Labophot 2, Nikon, Japan) from slices of coated tablets.

Table 1

Formulations Used for Enteric Coating of Dosage Forms

	Polymethacrylate (Eudragit L 100-55)	NaOH	Triethylcitrate
Coating A	90%	1%	9%
Coating B	99%	1%	—

Indicated compounds were dissolved in acetone.

Measurement of Peroxidase Activity in Tablets Incubated with a Simulated Gastric Fluid

To evaluate the protective effect of different anionic polymers in the gastric fluid, the degree of horseradish peroxidase degradation was determined in uncoated dosage forms. Tablets were therefore incubated with 10 ml of a simulated gastric fluid according to the Pharmacopoeia Europea (3.2 g of pepsin [0.9 Ph.Eur. units/mg; Chemag, Vienna, Austria] and 2.0 g of NaCl in 1000 ml of 0.08 N HCl) at 37°C ± 0.5°C on an oscillating water bath (GFL 1092; 60 rpm). Hydrated matrices were withdrawn at predetermined time points and frozen for 2 hr at −80°C. Aliquots of 30–90 mg were obtained by dividing the frozen matrices with a scalpel in four equal parts. These samples were diluted 1:20 with 200 mM phosphate-buffered saline (pH 6.6) to stop any further pepsinic degradation. In the case of unmodified carbomer, samples were also neutralized with 1 N NaOH before dilution. Aliquots of 200 µl were transferred to the first wells of a microtitration plate (96 well, not binding) and diluted in 1:2 steps with 100 µl of 200 mM phosphate-buffered saline (pH 6.6) in the following wells. Then, 100 µl of the substrate medium (24 mg *o*-phenylendiamine dihydrochloride, 2.4 ml 1 M phosphate buffer pH 6.6, 9.6 ml H_2O, and 24 µl 30% H_2O_2) were added, and the enzymatic reaction was allowed to proceed at room temperature for 5 min. Thereafter, the enzymatic reaction was stopped by adding 50 µl of 2 N HCl. Optical densities were read at 492 nm with a microtitration plate reader (Anthos reader 2001, Salzburg, Austria).

To evaluate the amount of protein released from tablets, the remaining activity of peroxidase was also determined in dosage forms, which were incubated in the same medium as described above, but without pepsin.

Determination of the Swelling Behavior and Changes in pH-Value

The swelling behavior and changes in pH value of tablets were evaluated by incubating the tablets with 10 ml of 0.08 N HCl containing 0.2% (m/v) NaCl on an oscillating water bath (GFL 1092; 30 rpm) at 37°C ± 0.5°C. Hydrated matrices were withdrawn at predetermined time points. If neither erosion nor disintegration occurred, the weight of the swollen delivery systems was determined with a precision scale (Sartorius MC 210 P, Vienna, Austria). Thereafter, each tablet was frozen at −80°C, and lateral parts (~50%, m/m) of the swollen dosage forms that might disturb an exact determination of the pH value of the polymeric carrier systems were removed with a scalpel. The remaining core was thawed at room temperature, diluted with 350 µl of demineralized water, and homogenized. The pH value was then determined with pH indicator strips (Merck, Darmstadt, Germany). Dilution had no marked influence on pH values.

Adsorption Studies

The capability of tested polymers to bind pepsin and horseradish peroxidase was investigated by adsorption studies. For the studies, 4.5 mg of each polymer were hydrated with 1.5 ml of the simulated gastric fluid containing 0.05% pepsin or horseradish peroxidase. After incubation at 37°C for 2.5 hr under permanent shaking, polymers were removed by centrifugation (20,000*g*, 30 min, 20°C). The remaining protein concentration in the supernatant fluid was determined spectrophotometrically by measuring the absorbance at 280 nm and 370 nm for pepsin and horseradish peroxidase, respectively. Samples, which were treated in the same way as described above but without polymers, served as references. The percentage of polymer-bound protein was calculated from the difference in protein concentration of samples incubated with polymers and the corresponding references.

Tensile Studies

Tensiometric studies with coated and uncoated tablets were carried out on native porcine mucosa. After a contact time between test disk and mucosa of 10 min in 100 mM Tris-HCl buffered saline (pH 6.8) or simulated gastric fluid without pepsin at 37°C, the maximum detachment force at which the adhesive bond between test disk and mucosa failed was recorded by pulling the mucosa at a rate of 2 mm/min from the disk (5).

Statistical Data Analyses

Statistical data analyses were performed using the Student *t* test, with $p < .05$ as the minimal level of significance.

RESULTS

Release Behavior of Tablets in a Simulated Gastric Fluid

Incubation of tablets with a simulated gastric fluid revealed strong swelling behavior for all tested anionic polymers in the acid milieu. Although it could be demonstrated that pure NaCMC displays very poor swelling be-

havior compared with poly(acrylic acid) derivatives (6), the water uptake of tablets containing NaCMC was in the range of all other tested polymers. Neutralized carbomer should be comparably more hydrophilic as a salt and therefore easier to swell than the corresponding acid form of the polymer. Results, however, showed no differences in the swelling behavior of carbomer and neutralized carbomer. A comparison of the swelling behavior of tablets based on carbomer and NaCMC is shown in Fig. 1. As the swelling of polymers is an essential parameter for bioadhesion, the adherence of these dosage forms on the gastric mucosa (and therefore an unintended, at least somewhat prolonged, gastric residence time) has to be taken into consideration.

Because all tested anionic polymers were hydrated and swelled in the acid milieu, the incorporated model protein was released rapidly from the delivery systems. Release studies showed that peroxidase is not completely released from each delivery system. Especially in tablets containing carbomer and neutralized carbomer, the liberation profile had reached its plateau phase already after a protein release of only approximately 60%. This observation can be explained by the high adsorptive binding properties of carbomer and neutralized carbomer. In the adsorptive binding studies described here, peroxidase was bound to carbomer and neutralized carbomer by

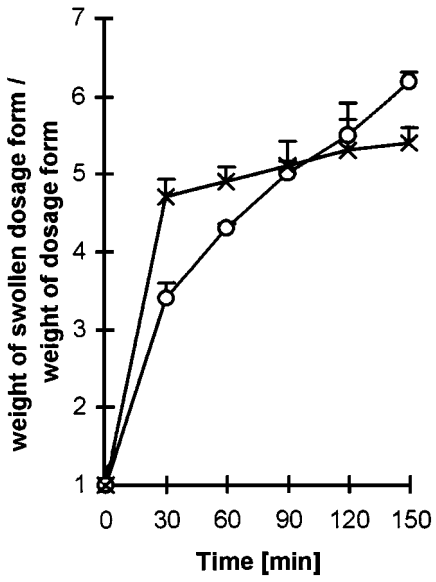

Figure 1. Swelling behavior of uncoated tablets containing 60% carbomer (○) and NaCMC (×) in a simulated gastric fluid at 37°C. Indicated values are means of at least three experiments ± SD.

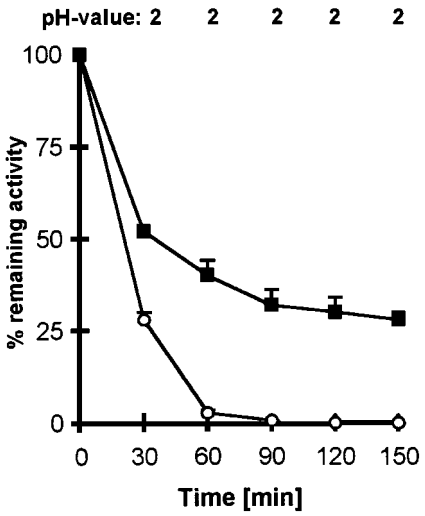

Figure 2. Decrease in peroxidase activity and changes of the pH value in uncoated tablets containing carbomer. Dosage forms were incubated with a simulated gastric fluid without (■) and with (○) pepsin (2.88 Ph.Eur. units/ml) at 37°C. Indicated values are means of at least three experiments ± SD.

85.1 ± 1.9% and 88.3 ± 1.9% (mean ± SD, $n = 3$), respectively. In contrast, the amount of peroxidase bound to NaCMC was negligible. Results of release studies are shown in Figs. 2–4. They demonstrate that, even if anionic mucoadhesive polymers could provide a protective effect toward a pepsinic degradation, an enteric coating will still be necessary to avoid drug release during the gastric passage.

Pepsinic Degradation of Peroxidase in Polymeric Carrier Systems

Due to the rapid swelling of dosage forms in the gastric fluid, pepsin can easily penetrate into the hydrated polymeric matrices. This penetration does not seem to be impeded by interactions of the protease with polymers as it could be demonstrated in adsorptive binding studies that pepsin is not at all bound to (neutralized) carbomer and NaCMC. Penetrating pepsin leads to a strong degradation of the incorporated model protein since the amount of intact peroxidase, which has not been released from the carrier systems, was strongly reduced in the simulated gastric fluid containing pepsin. This effect could be observed for tablets containing carbomer or NaCMC and is shown in Figs. 2–4. In contrast to carbomer, the corresponding neutralized polymer was the only one that could provide a protective effect against pepsinic degra-

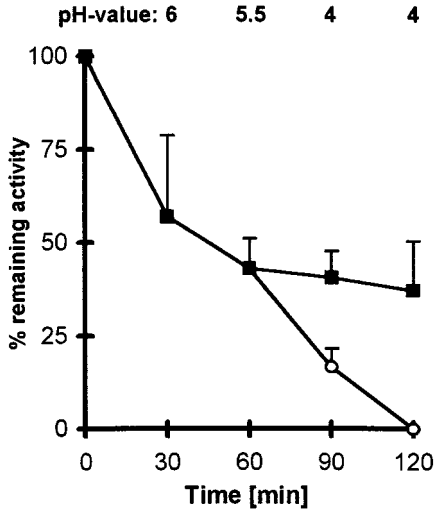

Figure 3. Decrease in peroxidase activity and changes of the pH value in uncoated tablets containing neutralized carbomer. Dosage forms were incubated with a simulated gastric fluid without (■) and with (○) pepsin (2.88 Ph.Eur. units/ml) at 37°C. Indicated values are means of at least three experiments ± SD.

dation within the first hour (Fig. 3). This enzyme-inhibitory capability of dosage forms containing neutralized carbomer can be explained by the comparably high buffer capacity of this polymer, which guarantees a pH value of the carrier system not below 5.5 within the first hour. Due to this high pH value within the delivery system, the enzymatic activity of penetrating pepsin seems to be inhibited completely as the protease displays no enzymatic activity at pH values above 5.5 (7). In comparison to neutralized carbomer, the buffer capacity of NaCMC is obviously not sufficient to maintain a pH value above 5.5 within the corresponding delivery system, leading to a rapid degradation of the incorporated model protein (Figs. 2 and 4). This observation is in good correlation with calculated data, demonstrating that NaCMC, in theory, can offer a maximum of only 4.1 mM sodium carboxylate groups per gram polymer, compared to carbomer with 10.6 mM/g.

Influence of Enteric Coating on Bioadhesive Properties

Within this study, we were able to demonstrate that both the enteric coating and the tablet core have a certain influence on the bioadhesive properties of the whole dos-

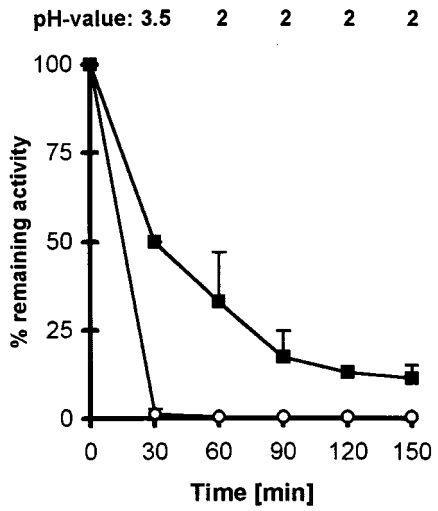

Figure 4. Decrease in peroxidase activity and changes of the pH value in uncoated tablets containing NaCMC. Dosage forms were incubated with a simulated gastric fluid without (■) and with (○) pepsin (2.88 Ph.Eur. units/ml) at 37°C. Indicated values are means of at least three experiments ± SD.

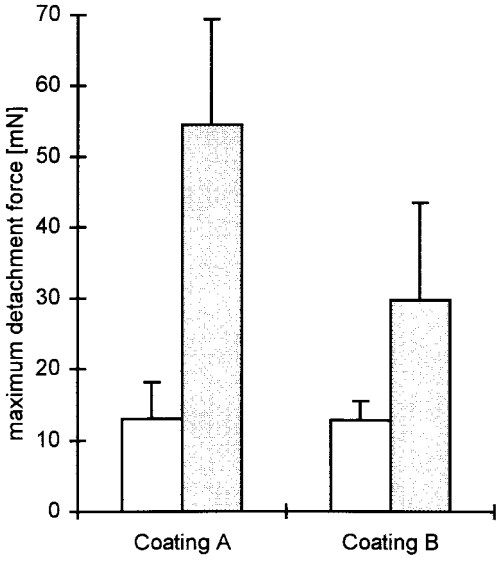

Figure 5. Comparison of the adhesive properties of tablets consisting of 100% mannitol (white bars) and 100% NaC934P (gray bars) with coating **A** (thickness of the coating layer: 65 μm) or **B** (thickness of the coating layer 50 μm), respectively. Indicated values are means of at least three experiments ± SD.

Table 2

Comparison of the Bioadhesive Properties of (Un)coated Tablets Containing Different Adhesive Polymers

Tested Tablet	Uncoated	Coating A (Thickness of the Coating Layer 65 μm)	Coating B (Thickness of the Coating Layer 50 μm)
NaC934P	49.9 ± 7.0	42.5 ± 9.8	17.9 ± 5.4
C934P	n.d.	6.7 ± 2.8	4.1 ± 1.6
NaCMC	30.2 ± 4.6	16.4 ± 4.0	10.6 ± 4.0

n.d. = not done.

age form. Tablets consisting of 100% mannitol with coating A, for instance, had significantly lower adhesiveness than tablets consisting of 100% neutralized poly(acrylic acid) with the same coating. On the other hand, tablets consisting of 100% neutralized poly(acrylic acid) displayed marked differences in their adhesive properties with coatings A and B. Results of this study are shown in Fig. 5.

In general, the enteric coating with polymethacrylate leads, in artificial intestinal fluid, to significantly reduced adhesive properties of tablets based on various mucoadhesive polymers. The only exception could be observed for NaC934P tablets with coating A, which demonstrated only slightly reduced adhesive properties. The determination of the maximum detachment force for tablets based on C934P was impossible as the adhesive bond did not fail between the mucus and the tablet, but within the dosage form itself. Results of these studies are shown in Table 2.

The reduction in adhesive properties due to enteric coating can be explained by the fact that the polymethacrylate represents a kind of ''isolating layer'' between the mucus and the mucoadhesive polymer. Adhesion studies in the gastric milieu showed a complete loss in mucoadhesive properties of all tablets. Accordingly, the gastric transit time should not be prolonged. After the gastric passage, however, the enteric coating seems to become a hindrance for bioadhesion of delivery systems, which should start swelling directly on the intestinal mucosa.

DISCUSSION

It is accepted beyond reasonable doubt that the presystemic metabolism caused by gastric and intestinal proteases is a major factor responsible for the low bioavailability of orally administered (poly)peptide drugs and antigens (8,9). An enteric coating, on the one hand, can guarantee a protective effect in the gastric fluid. On the other hand, mucoadhesive polymers that are slightly modified by the immobilization of enzyme inhibitors can strongly reduce the enzymatic degradation caused by intestinal enzymes (10,11). The intimate contact of an adhesive delivery system with the intestinal mucosa should also reduce proteolytic degradation of the (poly)peptide drug or antigen—in case of orally administered vaccines—on the way between the dosage form and the absorption membrane (12).

Duchêne et al. (13) proposed three essential stages in mucoadhesion. First, an intimate contact between the swelling mucoadhesive polymer and the mucus gel is required. In a second step, the polymer penetrates into the mucus gel network; this is followed by the formation of secondary chemical bonds between the mucoadhesive polymer and the mucus. Accordingly, if a mucoadhesive polymer is hydrated before it reaches the GI mucosa, the driving force for an interpenetration between polymer and mucus is already gone. Hence, the development of mucoadhesive delivery systems that do not start swelling until they have reached the intestinal mucosa seems to be highly desirable.

Carrier systems that do not start swelling until they have reached the intestinal mucosa might also lead to an enhanced absorption of peptide and protein drugs. Björk et al. (14), for instance, could demonstrate that the application of dry, swellable polymers, such as starch, induces dilatations of the tight junctions, leading to enhanced transport along the paracellular route. According to this adhesion-dehydration theory, the hydrophilic polymer, which is applied as a dry powder, absorbs water from the mucosal tissue in such a way that the epithelial cells are dehydrated and shrink until the normally tight intercellular junctions between the cells become physically separated (15). Hence, dosage forms with an enteric coating, providing a protective effect during the gastric passage as well as strong bioadhesive properties by directly swelling on the intestinal mucosa, might be a useful tool for the oral route of application. Although further improvements of dosage forms described here seem to be necessary, these systems represent an important first step in this direction.

A further advantage of systems described here might be the comparably high buffer capacity of neutralized carbomer in the gastric fluid, which were revealed in this study. The epidermal growth factor (EGF), for instance, is recognized as an important agent for acceleration of ulcer healing and has a peculiar biological property to

repair tissue damage by an enhanced proliferation and differentiation of epithelial tissues (16). Itoh and Matsuo (17) demonstrated, in a double-blind controlled clinical study, the enhanced healing of rat gastric ulcers after oral administration of EGF. This effect could be drastically increased by using the bioadhesive polymer hydroxypropyl cellulose as the drug carrier matrix (17). As EGF is strongly degraded by pepsin (18), the use of bioadhesive polymers to provide an additional protective effect toward pepsinic degradation might be helpful. A bioadhesive polymer-pepsin inhibitor conjugate, which guarantees a strong protective effect toward a pepsinic attack, has therefore already been generated (19). Results of the present study revealed that neutralized carbomer also displays a protective effect toward a pepsinic degradation due to its high buffer capacity. According to this, the development of delivery systems for EGF based on neutralized carbomer seems to be a promising novel strategy and should be the subject of further investigations.

Apart from this likely advantage for the (poly)peptide administration, the high buffer capacity of neutralized carbomer might also be highly beneficial in treatment of *Helicobacter pylori* infection in peptic ulcer disease, as common antibiotics such as amoxicillin or metronidazole display poor stability in the acidic pH of the stomach (20). The incorporation of these therapeutic agents in neutralized carbomer used as a carrier matrix might improve their stability in the acid milieu.

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