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Basic studies on bioadhesive delivery systems for peptide and protein drugs

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Abstract

We have been evaluating the influence of different drying methods and of ionic crosslinkers on adhesive strength, cohesiveness as well as release behaviour of bioadhesive polymers. Chitosan-EDTA and carbomer were ionically crosslinked via 1,8-diaminooctane or L-lysine. The resulting polymers were either lyophilised or precipitated in acetone and air-dried. Tablets made of these pre-treated polymers (66.7%), mannitol (30%), and the model drug insulin (3.3%) were investigated in vitro. Whereas tablets containing the precipitated and air-dried chitosan-EDTA or carbomer exhibited under our experimental conditions an adhesive strength of 93.2 ± 15.6 and 93.1 ± 17.3 mN, it was determined to be 57.7 ± 9.5 and 56.1 ± 6.7 mN (mean $\pm$ S.D.; $n = 5$) for tablets of the same but lyophilised polymers, respectively. The use of ionic crosslinkers led also to a significant reduction in the bioadhesiveness of the dosage form. Furthermore, the stability of tablets could be strongly increased by using ionic crosslinkers and/or the precipitated and air-dried form of chitosan-EDTA or carbomer. Due to the use of ionic crosslinkers, the release rate of insulin was strongly reduced. The results represent helpful basic information for the development of peroral (poly)peptide delivery systems based on bioadhesive polymers.

Keywords: Bioadhesive polymers; Chitosan-EDTA; Poly(acrylic acid); Peroral administration of (poly)peptides; Crosslinking; Drug delivery systems

1. Introduction

In recent years bioadhesive polymers have gained considerable interest as auxiliary agents for the peroral administration of peptide and protein drugs (Lehr, 1994; Bernkop-Schnürch, 1997a). Due to the adhesive properties of drug delivery systems consisting of such excipients, the enzymatic degradation of therapeutic peptides and proteins between the dosage form and the mucosa can be reduced by the increased intimacy with the absorbing membrane. The protective effect towards a presystemic metabolism will even be im-

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proved by the use of certain bioadhesive polymers such as chitosan-EDTA and poly(acrylate) derivatives, as these polymers display an inhibitory effect towards numerous intestinal proteases per se (Lueßen et al., 1995, 1996a,b; Bernkop-Schnürch and Krajicek, 1998). The immobilisation of enzyme inhibitors to these polymers also provides an additional protective effect towards certain proteases which would otherwise not be inhibited (Bernkop-Schnürch, 1997b). Moreover, because of the strong binding affinity of poly(acrylate) derivatives and chitosan-EDTA towards divalent cations such as Ca^{2+}, they might open up tight junctions in mucosal tissues leading to an improved peroral absorption of peptide and protein drugs (Lueßen et al., 1997).

Besides these useful properties of poly(acrylate) derivatives and chitosan-EDTA for the peroral (poly)peptide administration, the dosage form itself has also a substantial influence on the bioavailability of perorally administered (poly)peptide drugs. Delivery systems for this route of administration include formulations such as microparticles, capsules and tablets (Akiyama et al., 1996; Bernkop-Schnürch and Dundalek, 1996; Mathiowitz et al., 1997). Above all, tablets have the major advantage that they provide an accurate dosage of perorally administered (poly)peptide drugs and that they are easy to manufacture and handle. In order to optimise the efficacy of tablets containing chitosan-EDTA or a poly(acrylate) derivative for the peroral (poly)peptide administration, it was the aim of this study to evaluate the influence of different drying methods and of ionic crosslinkers on such delivery systems regarding bioadhesiveness, disintegration and release behaviour.

Results obtained from these studies should form the basis for peroral (poly)peptide delivery systems providing an intimate contact with the absorbing membrane, ensuring controlled release of the therapeutic agent and exhibiting a high mechanical stability of the swollen carrier matrix in the intestine. In order to guarantee a protective effect towards an enzymatic attack, the polymers described in this study will be substituted by poly(acrylate)- and/or chitosan-EDTA-inhibitor conjugates in future investigations. Data obtained from this study will represent an essential prerequisite for the following steps.

2. Material and methods

2.1. Synthesis of chitosan-EDTA

Chitosan-EDTA was synthesised in a slightly modified way as described previously by Bernkop-Schnürch and Krajicek (1998). Thereby, 2 g of chitosan (poly-[1 → 4]-β-D-glucosamine; Sigma, St. Louis, MO) suspended in 180 ml of demineralised water were dissolved at pH 6 by continuously adding 5 N HCl. Demineralised water was added to this solution to make the final volume 200 ml. On the other hand, 73.2 g of EDTA (ethylenediaminetetraacetic acid; Sigma, St. Louis, MO) were dissolved in 120 ml of demineralised water and the pH-value adjusted to 6.0 by adding 5 N NaOH. Demineralised water was added to make the final volume of this solution 200 ml. Thereafter, both solutions were mixed together and in order to mediate the formation of amide bonds between amino groups of chitosan and carboxyl groups of EDTA, EDAC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; Sigma, St. Louis, MO) was added in a final concentration of 0.05 M. The reaction mixture was incubated at room temperature under permanent stirring for 12 h. The resulting conjugate was isolated by exhaustively dialysing against demineralised water, 0.05 N NaOH and once more against demineralised water. In order to remove all sodium ions from the polymer, the pH-value of the polymer solution was adjusted to 2.0 by adding 1 N HCl. The occurring suspension was stirred for 2 h and then exhaustively dialysed against demineralised water. The purified product was lyophilised and stored at room temperature until use.

2.2. Neutralisation of polymers

One gram of the chitosan-EDTA and carbomer (Carbopol 934P; BF Goodrich, Cleveland, OH) were swollen in 200 ml of demineralised water, respectively. Polymers were completely (1/1), half-

(1/2), quarter- (1/4), or not at all neutralised by adding 1,8-diaminooctane (DAO; Sigma, St. Louis, MO) or L-lysine (Sigma, St. Louis, MO). The pH-value of half-, quarter- and not at all neutralised polymers was thereafter adjusted to 7.0 by adding 1 N NaOH. The obtained polymers were: chitosan-EDTA +1/1, 1/2 and 1/4 DAO; chitosan-EDTA +1/1, 1/2 and 1/4 lysine; chitosan-EDTA; C934P +1/1, 1/2 and 1/4 DAO; C934P +1/1, 1/2 and 1/4 lysine; C934P. They were lyophilised (lyo.) or precipitated (prec.) by pouring the polymer solutions rapidly into an unstirred bath of non-solvent (acetone) at solvent to non-solvent ratio of 1:200, washed in acetone, and air-dried. Dried polymers were stored at room temperature until use.

2.3. Pre-treatment of insulin

In order to improve the solubility of insulin, the zinc content was removed from the polypeptide. One hundred milligrams of insulin from bovine pancreas (Sigma, St. Louis, MO) were therefore suspended in 10 ml of demineralised water. Three milligrams of Na_2EDTA were added to this suspension and the pH-value was adjusted to 7.0 with 1 N NaOH. The resulting solution was lyophilised and the polypeptide drug stored at −20°C until use.

2.4. Preparation of tablets

Insulin (3.3%), polymer as indicated (66.7%) and mannitol (30%) were homogenised in a mortar and pressed (Hanseaten, Type EI, Hamburg, Germany) to flat-faced tablets with a diameter of 5.0 mm. Each tablet exhibited a weight of approximately 40 mg. The pressing power was kept constant during the preparation of all tablets.

2.5. Tensile studies

Tensiometer studies with these tablets consisting of unmodified C934P or pre-treated polymers as listed in Table 1 were carried out on native porcine mucosa as described previously by Bernkop-Schnürch and Apprich (1997).

2.6. Disintegration and release studies

The in vitro release rate of insulin from the drug delivery systems was determined in a slightly modified way as described previously by Bernkop-Schnürch et al. (1997b). Tablets were placed in 25 ml beakers (Schott, Duran 25 ml, Germany) containing 10 ml release medium (20 mM Tris–HCl pH 7.8). The vessels were closed, placed on a waterbath-shaker (GFL 1092; 100 rpm) and incubated at 37 ± 0.5°C; sink conditions were maintained throughout the study; 0.7 ml samples of released insulin were withdrawn at 2 h intervals—until the swollen drug carrier matrices disintegrated—and replaced with an equal volume of release medium, preequilibrated to temperature. Samples were diluted 1:2 with 0.1% trifluoroacetic acid and the remaining polymer content was removed by centrifugation (20000 × *g*, 4°C, Hermle Z 323K). A portion (15 μl) of the supernatant fluid was directly injected for HPLC analysis (series 200 LC; Perkin-Elmer). Remaining traces of polymer were hold back on a precolumn (Nucleosil 100–10C_{18}, 40 × 4 mm). Insulin was separated on a C_{18}-column (Nucleosil 100–5C_{18}, 250 × 4 mm) at 40°C. Gradient elution was performed as follows: flow rate 1.0 ml/min, 0–15 min, linear gradient from 91% A/9% B to 39% A/61% B (eluent A: 0.1% trifluoroacetic acid in

Table 1
Comparison of the adhesive strength of tablets containing 66.7% polymer as indicated, 3.3% insulin and 30% mannitol

Test tablet	Maximum detachment force (mN) ($n = 5$; ± S.D.)
Prec. chitosan-EDTA	93.2 ± 15.6
Lyo. chitosan-EDTA	57.7 ± 9.5
Lyo. chitosan-EDTA +1/1 lysine	51.8 ± 10.9
Lyo. chitosan-EDTA +1/1 DAO	41.6 ± 9.8
Prec. C934P	93.1 ± 17.3
Lyo. C934P	56.1 ± 6.7
Lyo. C934P +1/1 lysine	34.2 ± 3.9
Lyo. C934P +1/1 DAO	20.1 ± 5.2
Control (no disc)	1.3 ± 0.1

Maximum detachment force was determined in 50 mM Tris–HCl (pH 8.0) containing 0.9% NaCl at 37°C.

water; eluent B: acetonitrile). The polypeptide was detected by absorbance at 210 nm as well as 285 nm with a diode array absorbance detector (Perkin-Elmer 235C). The amount of insulin released was quantified from the integrated peak area and calculated by interpolation from an according standard curve for insulin. Cumulative corrections were made for the previously removed samples in determining the total amount released.

3. Results

3.1. Disintegration behaviour

Results concerning the disintegration behaviour of tablets consisting of chitosan-EDTA or carbomer used as carrier matrix demonstrated that the mechanical stability of the dosage form could be significantly increased due to the use of ionic crosslinkers as well as precipitation and air-drying of the polymers prior to compression.

Regarding the influence of ionic crosslinkers, we could find a direct correlation between the degree of crosslinking and the mechanical stability of tablets. The more extensively the polymer was crosslinked by forming intramolecular linkages, the higher was the mechanical stability of the dosage form. In comparison to lysine, 1,8-diaminooctane had a greater influence on disintegration characteristics. Reasons for this observation can be seen in the additional carboxylic acid group of lysine, which might at least partially disturb ionic crosslinking between the primary amino groups of the amino acid and the carboxylic acid groups of the polymers. On the other hand, the lower influence of lysine on the stability of the dosage form can also be explained by its comparably much higher hydrophilic character, leading to faster hydration of the polymer and therefore to faster disintegration of the dosage form. Results concerning the disintegration behaviour of chitosan-EDTA and carbomer tablets are shown in Fig. 1 and Fig. 2, respectively. Divalent cations have already been used as ionic crosslinkers for polymers such as alginate (Remunan-Lopez and Bodmeier, 1997). However, these cations cannot be used for chitosan-EDTA and carbomer, as at least high concentrations of such crosslinking agents cause coagulation and/or precipitation of these polymers. In contrast, the crosslinking with diaminooctane or lysine leads to transparent gels of high stability displaying quick swelling properties in aqueous solutions.

Besides the use of ionic crosslinkers also the precipitation of polymers in organic solvents and air-drying had an important influence on the disintegration behaviour. The stability of all tablet formulations consisting of precipitated polymers were generally much higher than of corresponding lyophilised polymers. In particular, tablets consisting of precipitated chitosan-EDTA which was previously crosslinked with diaminooctane demonstrated a very high stability of the carrier matrix in artificial intestinal fluid. The results, as shown in Fig. 1 and Fig. 2, can be explained by the amorphous state of polymers obtained by the freeze drying procedure. In contrast, the cohesiveness of polymers which were precipitated in a non-solvent was much higher. Results are in good agreement with earlier investigations, demonstrating a much faster disintegration of tablets containing lyophilised carbomer instead of precipitated (Kaiho et al., 1996).

3.2. Bioadhesion

Tensile studies demonstrated a significant influence of freeze-drying as well as crosslinking on the bioadhesiveness of the dosage form. Precipitated and air-dried polymers exhibited in general a much higher detachment force than lyophilised ones. Ionic crosslinkers caused a decrease in bioadhesiveness to all tablet formulations. The use of diaminooctane as ionic crosslinker led to the strongest reduction in adhesive properties of tablets consisting of chitosan-EDTA or carbomer. Results of tensile studies are listed in Table 1. Additionally, we could show, that the cohesiveness of tablets has also a major influence on the capability of the dosage form to adhere on the mucosa. In contrast to all formulations as listed in Table 1, tablets containing unmodified commercial available C934P displayed an insufficient cohesiveness. Hence, the adhesive bond of such tablets did not fail between the mucus and the

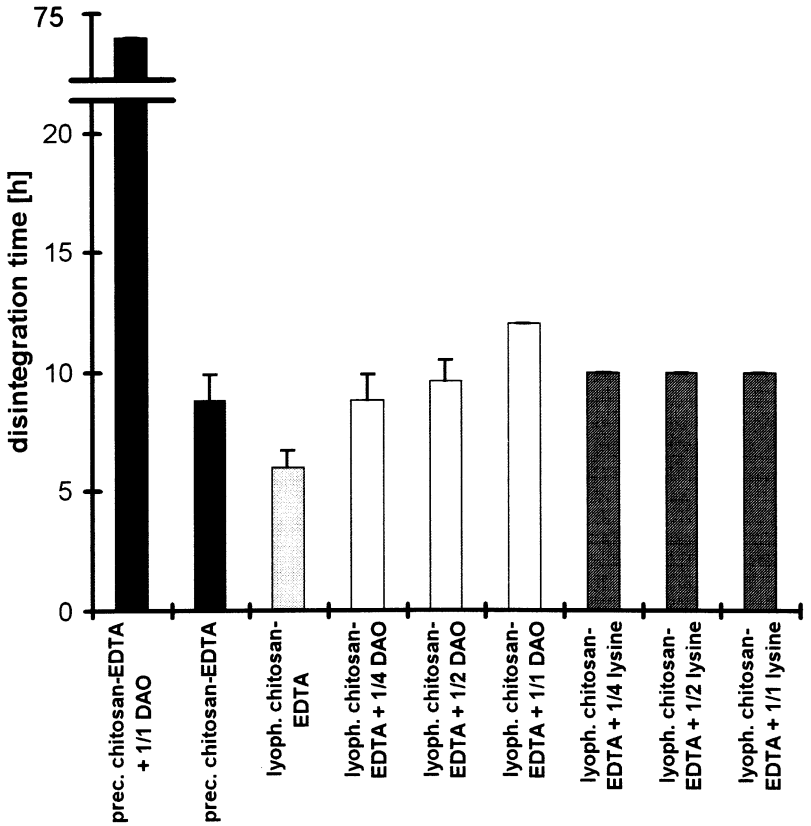

Fig. 1. Comparison of the disintegration behaviour of tablets consisting of 66.7% chitosan-EDTA, which had been pre-treated as indicated, 3.3% insulin and 30% mannitol. Dosage forms were incubated in 10 ml 20 mM Tris–HCl (pH 7.8) on a waterbath-shaker (100 rpm) at 37 ± 0.5°C. Indicated values are means $\pm$ S.D. of at least five experiments.

tablet but within the dosage form itself, leading to a comparably low apparent maximum detachment force of 16.2 ± 4.7 mN ($n = 5$; $\pm$ S.D.).

3.3. Release behaviour

The release rate from tablets could only be controlled as long as the cohesiveness of the swollen carrier matrix was guaranteed, demonstrating the importance of mechanical stability of this type of dosage form. Whereas the crosslinking of chitosan-EDTA with lysine had—apart from the stability of tablets—no effect on the release profile of insulin, it was in all other cases strongly dependent on the type of crosslinker as well as the extend of crosslinking. The release rate of insulin form the tablet generally decreased with an increasing degree of crosslinking. Carbomer tablets containing lysine instead of diaminooctane showed an approximately three times faster release of the therapeutic agent. Results of release studies are shown in Fig. 3, Fig. 4 and Fig. 5.

4. Discussion

As the bioadhesiveness, disintegration and release behaviour of dosage forms have a major influence on the bioavailability of perorally administered (poly)peptide drugs, we focused our research work on these parameters in order to optimise the efficiency of drug delivery systems containing chitosan-EDTA or carbomer.

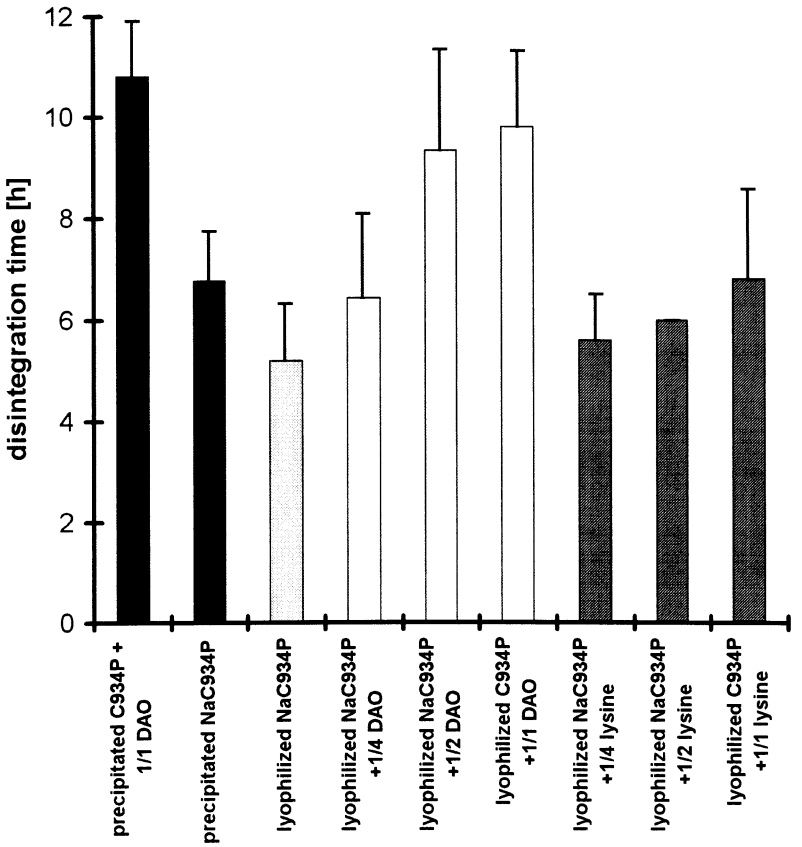

Fig. 2. Comparison of the disintegration behaviour of tablets consisting of 66.7% carbomer (C934P), which had been pre-treated as indicated, 3.3% insulin and 30% mannitol. Dosage forms were incubated in 10 ml 20 mM Tris–HCl (pH 7.8) on a waterbath-shaker (100 rpm) at 37 ± 0.5°C. Indicated values are means $\pm$ S.D. of at least five experiments.

The *bioadhesiveness* of the dosage form should guarantee an intimate and prolonged contact with the absorbing membrane. It should be as high as possible, so that additional effects of the polymers such as the inhibition of brush border membrane bound proteases (Bernkop-Schnürch and Marschütz, 1997; Bernkop-Schnürch et al., 1997a) or the opening of tight junctions (Lueßen et al., 1997) can take place. In connection with this, we could demonstrate that the precipitation and air-drying of polymers as well as the exclusion of ionic crosslinkers led to tablets of strong bioadhesiveness. The reduced adhesive properties of lyophilised polymers can be explained by their amorphous state obtained by the freeze drying procedure leading also to a strongly reduced cohesiveness. Due to the use of the ionic crosslinkers diaminooctane and lysine, the hydrophilic character of the whole system was strongly reduced, leading to a decrease in hydratability of the polymers. According to theories proposed to explain mucoadhesion on the basis of hydration and swelling of polymers followed by chain interpenetration with a hydrated mucus layer (Ponchel et al., 1987; Leung and Robinson, 1990; Jabbari et al., 1993), the reduced adhesive strength of crosslinked polymers can be explained by this decrease in hydratability.

Concerning the *mechanical stability of (poly)peptide drug delivery systems* based on bioadhesive polymers, it would be favourable to generate dosage forms displaying a very high stability in the intestine. The advantage of mechanically stable delivery systems displaying quick

swelling properties for the peroral administration of (poly)peptide drugs can be seen in the following reasons:

1. The inhibition of intestinal proteases in order to protect perorally administered peptide and protein drugs towards a presystemic enzymatic degradation will lead to a disturbed digestion of nutritive proteins. Furthermore, the inhibition of luminally secreted proteases induces a stimulation of enzyme secretion caused by a feed-back regulation, rapidly leading to both hypertrophy and hyperplasia of the pancreas. In long term therapy, the development of numerous neoplastic foci, frequently progressing to invasive carcinoma caused by this feed back-regulation, cannot be excluded (Melmed et al., 1976; McGuinness et al., 1982; Ge and Morgan, 1993). A reduction or even exclusion of this feed-back regulation might be possible by the development of drug delivery systems acting only in a very restricted area of the intestine. Tablets containing bioadhesive polymers displaying enzyme inhibitory activities and/or enzyme inhibitors should therefore not disintegrate and spread over the intestine.
2. (Poly)peptide drugs which are embedded in a bioadhesive polymer are partially protected towards an enzymatic attack of luminally secreted proteases, as the enzymes first of all have to penetrate into the polymer in order to degrade the drug (Bernkop-Schnürch and Göckel, 1997; Bernkop-Schnürch et al., 1997a). A rapid disintegration of the delivery system will certainly lead to a strong increase in the surface area of the dosage form thereby leading to a much higher accessibility of embedded (poly)peptide drugs for intestinal proteases. In this case, the protective effect of the dosage form will be reduced to a high extend.
3. Recent studies demonstrated that the inhibition of brush border membrane bound proteases can be achieved by polymers exhibiting a strong binding affinity and capacity towards certain divalent cations (Bernkop-Schnürch et al., 1997a). The inhibition can even be achieved without any direct

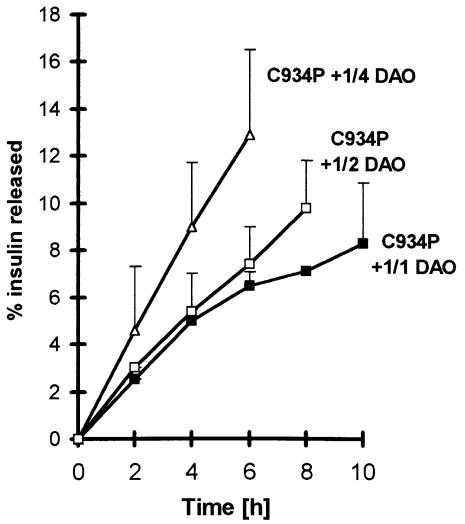

Fig. 4. Release profile of insulin from tablets consisting of 66.7% carbomer (C934P), which had been crosslinked with 1,8-diaminooctane (DAO), 3.3% insulin and 30% mannitol. Tablets were incubated in 10 ml release medium (20 mM Tris–HCl pH 7.8) on a waterbath-shaker (100 rpm) at 37 ± 0.5°C. Indicated values are means ± S.D. of at least three experiments.

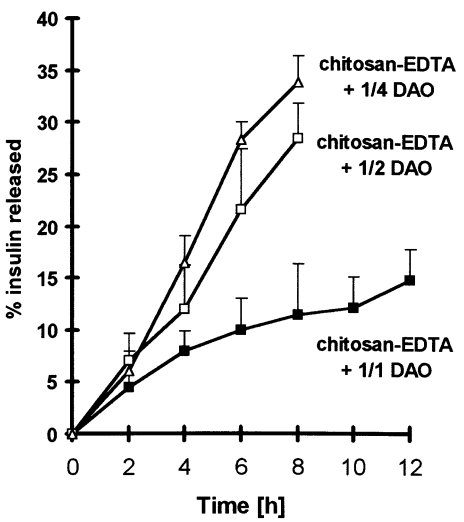

Fig. 3. Release profile of insulin from tablets consisting of 66.7% chitosan-EDTA, which had been crosslinked with 1,8-diaminooctane (DAO), 3.3% insulin and 30% mannitol. Tablets were incubated in 10 ml release medium (20 mM Tris–HCl pH 7.8) on a waterbath-shaker (100 rpm) at 37 ± 0.5°C. Indicated values are means ± S.D. of at least three experiments.

contact of the polymer with the membrane. However, this far distance inhibitory effect through the mucus layer covering gastrointestinal epithelia is only possible at comparably high polymer concentrations (Bernkop-Schnürch and Marschütz, 1997). A rapid disintegration and spreading of the polymer over the intestine will very likely undermine this effect. The development of dosage forms acting only in a very restricted area of the intestine is therefore essential.

The *release behaviour* of peptide and protein drugs embedded in bioadhesive polymers depends mainly on the molecular size of the therapeutic agent. According to the calculation of the diffusion coefficient, in which the radius of a molecule indirectly correlates with the diffusion coefficient, peptides will be faster released than proteins. Apart from the size and in some cases additional ionic interactions with the polymer, the release of poly(peptide) drugs from bioadhesive polymers such as chitosan-EDTA or carbomer can be controlled by the degree and kind of crosslinking. Additionally, as shown in earlier investigations, the release rate of a polypeptide drug can be reduced or enhanced by raising or lowering the share of the polymer in the delivery system, respectively (Bernkop-Schnürch and Dundalek, 1996). In dependence on the peptide and protein drug which should be perorally administered, the release rate of the therapeutic agent can therefore be adjusted in a very simple way by taking these parameters into account.

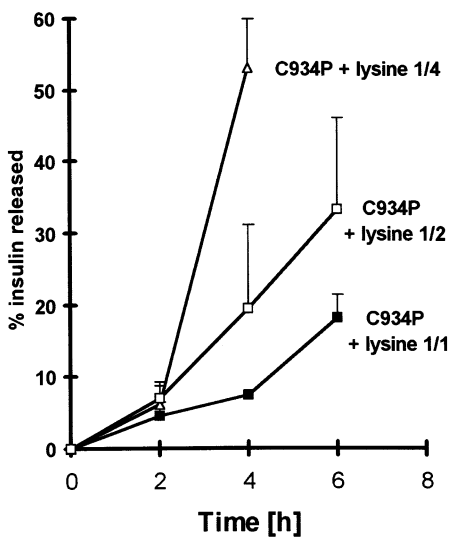

Fig. 5. Release profile of insulin from tablets consisting of 66.7% carbomer (C934P), which had been crosslinked with lysine, 3.3% insulin and 30% mannitol. Tablets were incubated in 10 ml release medium (20 mM Tris–HCl pH 7.8) on a waterbath-shaker (100 rpm) at 37 ± 0.5°C. Indicated values are means $\pm$ S.D. of at least three experiments.

5. Conclusions

The use of bioadhesive polymers exhibiting a protective effect towards a presystemic metabolism makes high demands on the delivery system, designed for the peroral administration of peptide and protein drugs. It has to display a high stability in the intestine while providing an intimate contact with the absorbing membrane as well as a controlled drug release. The results obtained within this study represent an important step towards fulfilling these demands and form the basis for the development of delivery systems of practical relevance.

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