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European Journal of Pharmaceutics and Biopharmaceutics 57 (2004) 219–224
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

Preparation and characterisation of thiolated poly(methacrylic acid)–starch compositions

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Received 3 June 2002; accepted in revised form 4 November 2002

Abstract

The development of mucoadhesive polymer systems, which start swelling in the intestine after oral administration without an enteric coating, might be the key for drug delivery systems exhibiting a prolonged intestinal residence time. The preparation and characterisation of such polymeric excipients was therefore the aim of this study. A poly(methacrylic acid)–cysteine conjugate (thiolated PMAA) exhibiting 747.8 ± 30.9 μmol thiol groups per gram polymer was co-precipitated with starch at pH 3. The resulting thiolated PMAA–starch composition consisting of 24% thiolated PMAA and 76% starch was lyophilised and analysed with regard to its swelling behaviour as well as to its cohesive and mucoadhesive properties. Results demonstrated that the thiolated PMAA–starch composition does not swell at all in a simulated gastric fluid. In contrast, a 4- and 6-fold increase in weight by water uptake was observed at pH 5 and 7, respectively. Disintegration studies demonstrated improved cohesive properties due to the immobilisation of thiol groups on PMAA, which are involved in the formation of stabilising inter- and/or intrachain disulfide bonds. Tensile studies demonstrated a total work of adhesion of 90.2 ± 15.2 and 27.5 ± 2.9 μJ for thiolated PMAA–starch and PMAA–starch, respectively. These results were confirmed by mucoadhesion studies utilising the rotating cylinder method. Thiolated PMAA–starch represents therefore a promising novel mucoadhesive excipient, which might provide a prolonged residence time of various delivery systems in the intestine.

Keywords: Poly(methacrylic acid); Starch; Thiolated polymers; Mucoadhesion; Oral drug delivery

1. Introduction

Since their introduction, mucoadhesive polymers have proved successful in various delivery systems as a means to prolong the residence time of drugs on mucosal absorption membranes. Polyacrylic acid used as mucoadhesive polymer in ocular inserts, for instance, was shown to strongly prolong the residence time of the delivery system on the surface of the eye [1]. Buccal tablets based on mucoadhesive polymers were shown to adhere to the gingiva for approximately 10 h [2]. In nasal drug delivery, chitosan was found to lengthen the residence time of formulations on the nasal mucosa and subsequently to enhance significantly the bioavailability of various drugs [3]. Thus, however, the concept has not been useful for peroral drug delivery systems [4,5] although the benefits and potential would be tremendous.

In contrast to the applications mentioned above, the mucoadhesive delivery system cannot be applied on the mucosa in its dry form when administered orally. The swelling process on the mucosa, however, seems crucial in order to provide interpenetration between the mucoadhesive polymer and the mucus gel layer guaranteeing a strong adhesion, i.e. 'wetting' as cause of adhesion [6]. Given orally, however, the polymeric drug carrier matrix reaches the gut already in a pre-swollen form which is much less adhesive. On the other hand, the concept of enteric coats to postpone the swelling process from the stomach to the gut has also failed, as the hydrated polymeric coating material remains as a kind of 'isolating layer' between the mucoadhesive polymer and the mucus gel layer [7].

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doi:10.1016/j.ejpb.2002.11.001

In order to solve these problems, it was the aim of this study to generate a polymeric excipient exhibiting both features: swelling only at pH values above the gastric range (**I**) and strong mucoadhesive properties (**II**). To reach that goal, a recently generated poly(methacrylic acid) (PMMA)–starch composition [8], which does not swell in acidic media, was slightly chemically modified in order to make it more mucoadhesive. As known for many other polymers, due to the immobilisation of thiol groups, their mucoadhesive properties are strongly improved [9–11]. The mechanism responsible for this effect is based on the formation of disulfide bonds between the thiolated polymer and cysteine-rich subdomains of the mucus gel layer [11]. In this study, a thiolated PMMA–starch composition as shown in Fig. 1 was generated and evaluated with regard to its swelling behaviour and mucoadhesive properties.

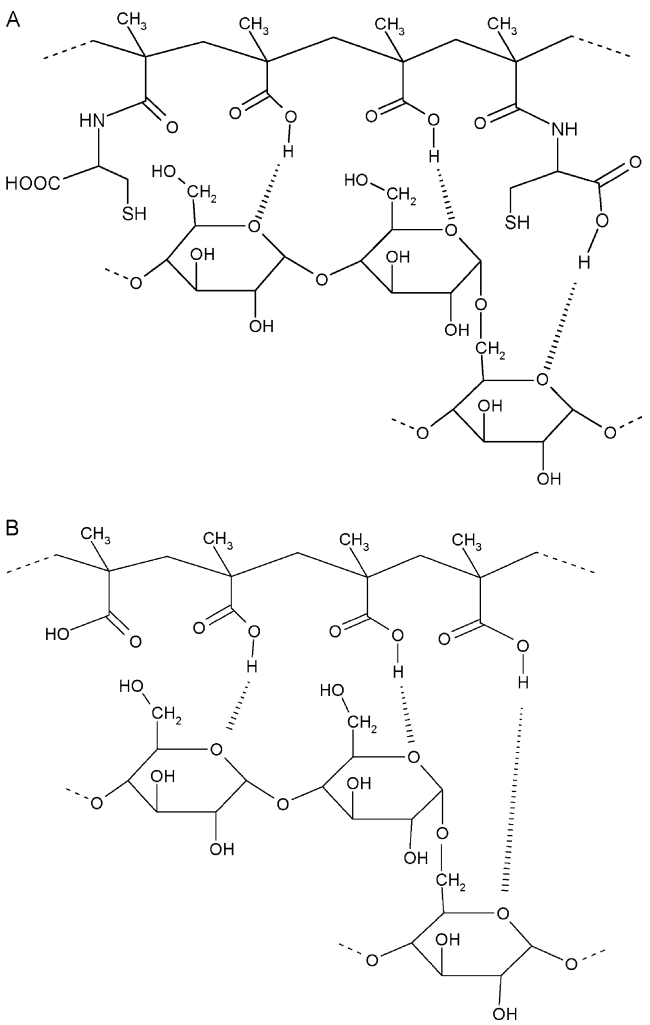

Fig. 1. Assumed substructure of the resulting poly(methacrylic acid)–cysteine–starch composition (A) and of the unthiolated PMAA–starch conjugate (B).

2. Materials and methods

2.1. Materials

PMAA (9.5 kDa, 30% (w/w) aqueous, $d = 1.18$) was purchased from Sigma, St Louis, MO; starch (amylum solani) was obtained from GATT, Innsbruck, Austria. Thiolated poly(methacrylic acid) [poly(methacrylic acid)–cysteine conjugate] was supplied by MucoBiomer (Leobendorf, Austria).

2.2. Characterisation of thiolated PMAA

2.2.1. Determination of immobilised thiol groups

The amount of immobilised thiol groups was determined with Ellman's reagent according to a method described previously [11].

2.2.2. Determination of remaining unbound cysteine

The quantification of remaining unbound cysteine in the conjugate was achieved by utilising 2,4,6-trinitrobenzene-sulfonic acid (TNBS, Sigma, St Louis, MO) reagent. Thiolated PMAA (1.0 mg) was hydrated in 990 μl of 4% $NaHCO_3$. After the addition of 10 μl of 5% TNBS the sample was incubated at 37 °C for 2 h. The absorbance was measured at 450 nm (Lambda 16; Perkin–Elmer, Vienna, Austria). The amount of unconjugated cysteine was calculated using a standard curve obtained by the primary amino-group determination of unmodified PMAA with increasing amounts of cysteine.

2.3. Preparation of the thiolated PMAA–starch composition

The aqueous 30% PMAA solution was purified by exhaustive dialysis against 0.2 mM HCl and dried by lyophilisation at −30 °C and 0.01 mbar (Christ Beta 1-8K; Osterode am Harz, Germany). Lyophilised PMAA and thiolated PMAA were dissolved in demineralised water at a final concentration of 1% (m/v). The pH of both solutions was adjusted to 3. To 100 ml of each solution, 138 ml of a 1% (m/v) aqueous starch solution was added. After continuous stirring for 30 min the resulting precipitates were separated by centrifugation for 3 min at 13 500*g* (RC5C, Sorvall Instruments, INULA, Austria). The dried compositions were then pulverised by a mortar and stored at 4 °C until further use.

2.4. Preparation of tablets

Tablets of 30 mg weight based on the PMAA–starch composition and the thiolated PMAA–starch composition were compressed (Hanseaten Type EI, Hamburg, Germany) into 5.0 mm diameter flat-faced discs. The compaction pressure was kept constant during the preparation of all tablets.

2.5. Determination of the swelling behaviour

The water absorbing capacity was determined by a gravimetric method as described previously [9]. In brief, tablets as described above were fixed on a needle and incubated in simulated gastric fluid (0.1 M HCl, pH 1.2) as well as in 100 mM phosphate buffer solution pH 5.0 and 7.0 at 37 °C. At predetermined time intervals the hydrated test discs on the needle were taken out of the incubation medium. After removing unbound water, the amount of water uptake was determined gravimetrically.

2.6. Disintegration test of PMAA–starch tablets

The stability of tablets in simulated gastric fluid (0.1 M HCl, pH 1.2), 100 mM phosphate buffer pH 5.0 and 100 mM phosphate buffer pH 7.0 was analysed with a disintegration test apparatus according to the USP XXIII at 37 °C. The oscillating frequency was adjusted to 0.5 s^{-1}.

2.7. Tensile studies

In order to compare results obtained by tensile studies to those of previous studies carried out with thiolated polymers [9–12], the same analytical method was used. Test tablets as described in Section 2.4 were attached to a stainless steel flat disc (0.3 g of weight in system), which was hung by a nylon thread (15 cm) from a laboratory stand. Utilising a cyanoacrylate adhesive, native porcine mucosa (supplied by a local slaughterhouse) was fixed to a glass tissue mount. The tissue mount and fixed mucosa were placed in a beaker and 100 mM phosphate buffered saline pH 6.8 was added to immerse mount and tissue. The beaker was placed on a balance and carefully raised by a mobile platform until the mucus came into contact with the test tablet. After 30 min incubation at 25 °C, the mucosa was pulled at a rate of 0.1 mm s^{-1} from the tablet. Data points were collected every second by a personal computer, which was connected with the balance, using the WINWEDGE software (TAL Technologies, Inc., Philadelphia, PA). Data were transferred to EXCEL 5.0 (Microsoft) and the total work of adhesion (TWA) representing the area under the force/distance curve and the maximum detachment force (MDF) were determined.

2.8. Mucoadhesion studies on the rotating cylinder

In order to evaluate the mucoadhesive properties of thiolated PMAA–starch and unmodified PMAA–starch by a second test system, mucoadhesion studies were also performed on the rotating cylinder [12]. In brief, test tablets were attached to freshly excised intestinal porcine mucosa, which had been fixed on a stainless steel cylinder (diameter, 4.4 cm; height, 5.1 cm; apparatus four-cylinder, USP XXIII). The cylinder was placed in the dissolution test apparatus containing 100 mM phosphate buffer pH 7.0 at 37 ± 0.5 °C (according to the USP). The fully immersed cylinder was agitated at 125 rev./min. The detachment of the test tablets was determined visually.

2.9. Statistical data analyses

Statistical data analyses were performed using the Student's *t*-test with $P < 0.05$ as the minimal level of significance.

3. Results and discussion

3.1. Characterisation of thiolated PMAA

The PMAA–cysteine conjugate used in this study has a chemical structure as illustrated in Fig. 1. A detailed chemical analysis of the polymer showed that in average 747.8 ± 30.9 μmol thiol groups are immobilised per gram polymer. After the preparation of the PMAA–cysteine–starch complex, the thiol groups were quantified again demonstrating that no thiol oxidation took place during this process (data not shown). The remaining traces of unbound cysteine were determined with TNBS-reagent to be less than 0.26% (m/m) of the total mass.

3.2. Preparation of (thiolated) PMAA–starch compositions

The PMAA–starch and thiolated PMAA–starch composition were prepared by a method described previously [8]. Accordingly, the compositions contained 24% (m/m) thiolated or unmodified PMAA and 76% (m/m) starch. The presumptive complex formed between thiolated or unmodified PMAA and starch is illustrated in Fig. 1. Both the unmodified and thiolated PMAA–starch composition were easy to grind after lyophilisation and appeared as white odourless powders soluble in aqueous solutions above pH 5. The compositions were furthermore directly compressible into tablets.

3.3. Evaluation of the swelling behaviour

The swelling behaviour of tablets comprising the (thiolated) PMAA–starch composition was evaluated at pH 1.2, 5 and 7. Results demonstrated that at pH 1.2—mimicking the situation in the stomach—neither the thiolated PMAA–starch composition nor the unmodified PMAA–starch composition swelled at all. This effect can be partly explained by numerous stabilising hydrogen bonds between the carboxylic acid groups of (thiolated) PMAA and the ring oxygens of starch being favoured at low pH values as illustrated in Fig. 1. On the other hand, the share of protonated carboxylic acid groups increases at low pH values leading to a relatively more hydrophobic polymer, which is less wettable. In contrast, at pH 5 and 7 water uptake by the tablets was observed, which was more

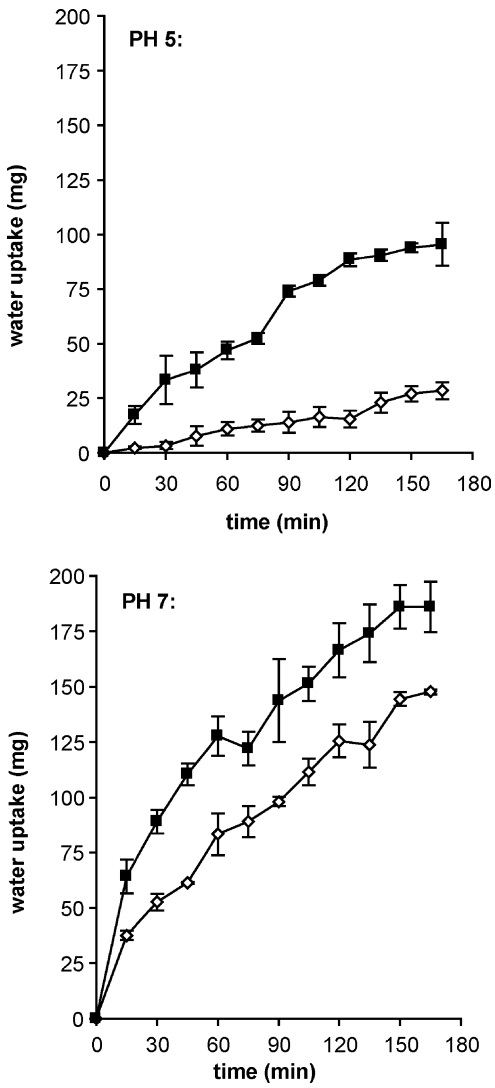

Fig. 2. Comparison of the water uptake of compacts (30 mg) comprising PMAA–starch (◇) and thiolated PMAA–starch (■) at pH 5 and 7 both at 37 °C. Indicated values are means ± SD of three experiments.

pronounced at the higher pH. The results of this study are shown in Fig. 2. A comparison of the swelling behaviour of the thiolated PMAA–starch composition with the unmodified PMAA–starch composition revealed a more pronounced water uptake of the thiolated version.

3.4. Disintegration studies

The disintegration behaviour also has a great impact on the mucoadhesive properties of polymers. A strong adhesion of a delivery system on the mucosa is ineffective if the adhesive bond fails within the delivery system itself [13]. Moreover, a controlled drug release out of a mucoadhesive polymeric carrier matrix, which is based on a simple diffusion process, will fail, if the polymeric network disintegrates too early. Accordingly, disintegration studies of tablets comprising thiolated and unmodified PMAA–starch were carried out within this research work. Results demonstrated a significantly higher stability of the thiolated version. This observation is in good agreement with former studies showing improved cohesive properties of thiolated polymers in comparison to the corresponding unmodified polymers [10,14]. At pH 7, the thiolated PMAA–starch composition exhibited a 2-fold improved stability versus the unmodified PMAA–starch composition, which is less pronounced in comparison to improvement ratios obtained by the thiolation of polycarbophil, sodium carboxymethylcellulose and alginate. A reason for this observation seems to be the high proportion of starch within the test tablets, strongly limiting the formation of disulfide bonds within the thiolated PMAA chains. In case of thiolated polycarbophil, sodium carboxymethylcellulose and alginate, however, no additional polymer was added providing a much closer location of thiol groups to each other, which is essential for the formation of stabilising inter- and intrachain disulfide bonds within the polymeric network.

Apart from thiolation, the pH also had a great impact on the stability of the tablets. The lower the pH, the more stable were the test tablets. These results are in good agreement with the determined swelling behaviour of the polymer compositions at increasing pH values. Results of disintegration studies are shown in Fig. 3.

3.5. Mucoadhesion studies

In order to evaluate the mucoadhesive properties of the polymer compositions, mucoadhesion studies were

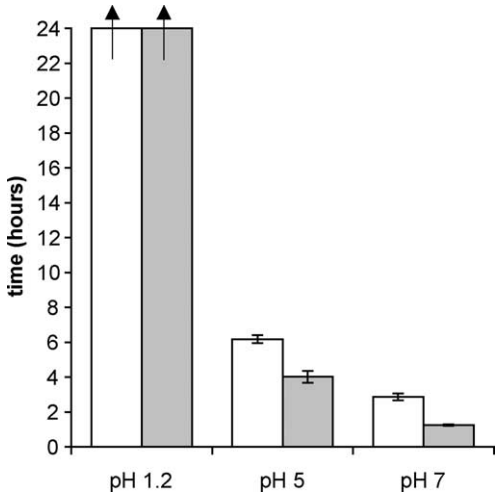

Fig. 3. Comparison of the disintegration behaviour of matrix tablets (30 mg; 5 mm i.d.) containing the thiolated PMAA–starch composition (white bars) or the unmodified PMAA–starch composition (grey bars); studies were carried out with a disintegration test apparatus in simulated gastric fluid 0.1 M HCl (pH 1.2), in 100 mM phosphate buffer pH 5.0 and 100 mM phosphate buffer pH 7.0 at 37 °C. At pH 1.2, test tablets did not disintegrate within an observation period of 24 h. Indicated values are means ± SD of three experiments.

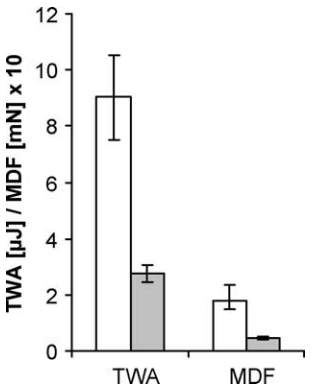

Fig. 4. Comparison of the total work of adhesion (TWA) and maximum detachment force (MDF) of test discs comprising the thiolated PMAA–starch composition (white bars) and the unmodified PMAA–starch composition (grey bars); indicated values are means ± SD of at least five experiments.

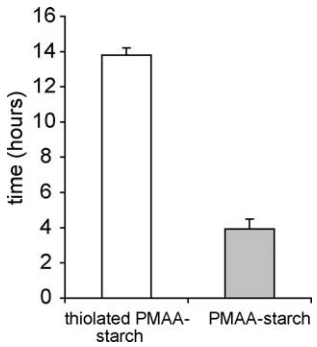

Fig. 5. Comparison of the adhesion-time of test tablets containing the thiolated PMAA–starch composition and the unmodified PMAA–starch composition on freshly excised porcine mucosa according to the rotating cylinder method. Indicated values are means ± SD of at least three experiments.

performed utilising two different test methods. Tensile studies, showed a 3-fold improvement in the TWA due to the immobilisation of thiol groups on PMAA. The MDF was thereby in good accordance with the TWA. The result shown in Fig. 4 confirms the theory that the mucoadhesive properties of polymers can be significantly improved by the covalent attachment of thiol groups. This result is in good agreement with various earlier studies performed with both cationic and anionic thiolated polymers and their corresponding unmodified versions [9–11]. The TWA values of various mucoadhesive polymers are summarised in Table 1. In comparison to other mucoadhesive polymers tested under the same conditions, the thiolated PMAA composition exhibits a TWA, which is in the range of that of polycarbophil having been the most adhesive polymer before the development of thiolated polymers. The rotating cylinder method, on the other hand, has the advantage that the cohesiveness and swelling behaviour of the mucoadhesive delivery system is comparatively more reflected in the obtained results than is the case for tensile studies. Nevertheless, as shown in Fig. 5, the results obtained by this method were in good correlation with the results of tensile studies.

Because of these strong mucoadhesive properties and a swelling only above gastric pH values, the thiolated PMAA–starch composition represents a promising new excipient, which might provide a prolonged residence time in the gut.

Table 1
Comparison of the mucoadhesive properties of various polymers determined via tensile studies performed under the same test conditions

Polymer	Total work of adhesion (µJ); means ± SD ($n = 3–8$)	Reference
Thiolated polycarbophil	280 ± 68	[11]
Thiolated chitosan	234 ± 0	[9]
Thiolated sodium carboxymethyl-cellulose	157 ± 6	[12]
Polycarbophil	110 ± 28	[11]
Sodium carboxymethylcellulose	108 ± 17	[12]
Thiolated sodium alginate	102 ± 36	[10]
Thiolated PMAA–starch	90.2 ± 15.2	Described herein
PMAA–starch	27.5 ± 2.9	Described herein
Sodium alginate	26 ± 1	[10]
Chitosan HCl	23 ± 10	[9]

3.6. Drug delivery systems based on the thiolated PMAA–starch composition

Recently, our research group could demonstrate no drug release in gastric fluid but a controlled drug release in intestinal fluid for various drugs as well as a model peptide from tablets comprising a PMAA–starch composition [8]. A similar effect can be expected for the thiolated version of the PMAA–starch composition. Apart from tablets, micro- and nanospheres in particular seem to be promising formulations for prolonging the intestinal residence time of the delivery system. The co-precipitation of thiolated PMAA with starch in aqueous acidic solutions, where the immobilised sulfhydryl groups are not subject to oxidation, might provide the basis for a production method of such particulate delivery systems.

Interesting drug candidates are orally given therapeutic agents such as various antibiotics [15], cyclosporin A [16] and riboflavin [17], which are poorly taken up from their absorption window in the small intestine. A prolongation of the small intestinal residence time would strongly improve their bioavailability. Akiyama and Nagahara, for instance, demonstrated a significantly improved absorption of riboflavin in human volunteers by oral administration of mucoadhesive microspheres versus non-adhesive microspheres [17]. Moreover, in the case of

peptide drugs, an intimate contact between the delivery system and the small intestinal mucosa might be highly advantageous. The adhesion of the delivery system on the mucosa prevents pre-systemic metabolism of the therapeutic agent by luminally secreted proteases. Takeuchi et al., for instance, demonstrated a significantly improved bioavailability of orally administered calcitonin incorporated in liposomes, only when the delivery system was coated with a mucoadhesive polymer [18]. Moreover, due to the use of chitosan-coated liposomes the bioavailability of orally administered insulin was also strongly improved [19]. Similar effects can be expected for thiolated PMAA–starch compositions but have to be verified by ongoing in vivo studies.

In addition, thiolated polymers were shown to exhibit enzyme inhibitory properties, which should provide a protective effect towards an enzymatic attack for peptides incorporated in a polymeric network [20,21]. This additional advantage of thiolated PMAA in combination with its high mucoadhesive and cohesive properties should render it a promising new excipient in non-invasive peptide drug delivery.

4. Conclusion

In this study, a novel polymeric drug carrier matrix system based on a PMAA–starch composition has been generated, which does not swell in the acidic milieu of the stomach. Due to the immobilisation of thiol groups on PMAA the mucoadhesive and cohesive properties of the polymeric system were significantly improved. These features render the system for prolonging the residence time of orally given drug delivery systems in the small intestine.

Acknowledgements

This work was supported by Grant No. P13820-MOB from the Fonds zur Förderung der wissenschaftlichen Forschung (FWF) to A. Bernkop-Schnürch. The authors wish to thank Mr Ströbel and co-workers from the slaughterhouse Totzenbach for the supply of porcine intestinal mucosa.

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