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Matrix tablets based on thiolated poly(acrylic acid): pH-dependent variation in disintegration and mucoadhesion

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Abstract

This study examined the influence of the pH on the mucoadhesive and cohesive properties of polyarcylic acid (PAA) and thiolated PAA. The pH of PAA (molecular mass: 450 kDa) and of a corresponding PAA-cysteine conjugate was adjusted to 3, 4, 5, 6, 7 and 8. The amount of immobilised thiol groups and disulfide bonds was determined via Ellman's reagent. Tablets were compressed out of each pH-batch of both thiolated and unmodified PAA and the swelling behaviour, the disintegration time and the mucoadhesiveness were evaluated. The amount of thiol/disulfide groups per gram thiolated PAA of pH 3 and pH 8 was determined to be 332 ± 94 μmol and 162 ± 46 μmol, respectively. The thiolated PAA tablets displayed a minimum four-fold higher water uptake compared to unmodified PAA tablets. A faster and higher water uptake of both polymer types was observed above pH 5. Thiolated polymer tablets showed a 3–20-fold more prolonged disintegration time than unmodified PAA tablets. The cohesiveness of PAA-cysteine conjugate increased at higher pH, whereas the unmodified PAA behaved inversely. A 3–7-fold stronger mucoadhesiveness was observed for the PAA-cysteine conjugate tablets compared to unmodified PAA tablets. For both thiolated and unmodified polymer the mucoadhesiveness was 2–4-fold enhanced below pH 5. The difference in mucoadhesion between the two polymer types was most pronounced at these lower pH values. In this study substantial information regarding the pH-dependence of mucoadhesion and cohesion of unmodified polyacrylates and of thiolated polyacrylates is provided, representing helpful basic information for an ameliorated deployment of these polymers.

Keywords: Polyacrylic acid; Thiomers; pH-influence; Mucoadhesion; Disintegration

1. Introduction

In recent years, thiolated polymers or thiomers have been introduced into pharmaceutical literature, representing a new class of mucoadhesive hydrophilic macromolecules. They are characterised by the immobilisation of thiol moieties on the backbone of well-established hydrophilic polymers such as polyacrylates, cellulose derivatives and chitosans. In contrast to conventional mucoadhesive polymers being able to interact with the mucus layer only due to comparatively weak non-covalent bonds, thiomers are believed to be able to form covalent bonds with cysteine-rich subdomains of glycoproteins (Leitner et al., 2003a). As a consequence thiolated polymers

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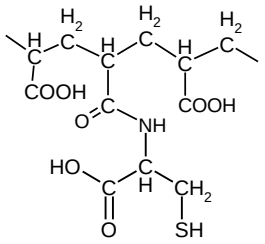

Fig. 1. Schematic presentation of the chemical substructure of polyacrylic acid cysteine conjugate.

display strongly improved mucoadhesive properties (Marschütz and Bernkop-Schnürch, 2002). Moreover, high cohesiveness of drug carrier systems based on thiomers can be provided due to the formation of intra- and intermolecular disulfide bonds within the polymeric network (Bernkop-Schnürch et al., 2000). Further advantages are their enzyme inhibitory properties (Bernkop-Schnürch et al., 2001b) and their permeation enhancing effect (Clausen and Bernkop-Schnürch, 2000). In combination all these features render thiolated polymers promising excipients for the non-invasive administration of hydrophilic macromolecules. Recently, a proof of concept has been given in vivo for the oral delivery of insulin (Marschütz et al., 2000), calcitonin (Guggi et al., 2003a,b) and low molecular weight heparin (Kast et al., 2003).

Although results obtained by the use of thiomers are very promising, further improvements in their mucoadhesive and cohesive properties seem to be feasible. A crucial role for the design of more potent thiomers would appear to be the activity of the thiol group itself, which is strongly pH-dependent. However, the influence of pH on the mucoadhesive and cohesive properties of thiomers has yet to be studied, although an understanding of the influence of this parameter might strongly contribute to further improvements.

The aim of this study was to evaluate the influence of pH on the cohesive and mucoadhesive properties of a representative model thiomer which had been compressed into tablets. Non-crosslinked polyacrylic acid-cysteine conjugate of 450 kDa (PAA-450-cysteine; see Fig. 1) was chosen, as it was shown to be a valuable vehicle for the non-invasive delivery of hydrophilic macromolecules (Caliceti et al., 2003). Among anionic polymers this thiomer was shown to exhibit the highest so far determined mucoadhesive properties and excellent cohesiveness (Leitner et al., 2003b). As the degree of modification of the polymer was raised, the mucoadhesive and cohesive features were enhanced (Marschütz and Bernkop-Schnürch, 2002).

In this study the pH of a PAA-450-cysteine conjugate was adjusted to six different levels and the amount of thiol and disulfide groups on each polymer was determined. The swelling and disintegration behaviour of tablets of the six different polymers was determined as was the decrease in free thiol groups after incubation of tablets in aqueous solutions. Furthermore, tensile studies and mucoadhesion studies with the rotating cylinder method were performed.

2. Materials and methods

2.1. Preparation and purification of thiolated PAA and controls

PAA-cysteine conjugate with a molecular mass of approximately 450 kDa was kindly supplied by MucoBiomer (Leobendorf, Austria). The polymer was hydrated in demineralised water (1% solution) and dialysed six times at 10 °C in the dark against 1 mM HCl. After dialysis the hydrated polymer was divided in six aliquots, that were adjusted to pHs 3, 4, 5, 6, 7 and 8 with 5 M NaOH. In order to avoid an oxidation of the thiol groups during this process the pH was adjusted as quickly as possible on crushed ice utilising automatic temperature compensation equipment (ATI-Orion 420A, Boston, MA). Thereafter, samples were immediately frozen at −80 °C. Frozen polymer solutions were lyophilised at −30 °C and 0.01 mbar (Christ Beta 1-8K; Osteorode am Harz, Germany). Control polymer (PAA 450 kDa; Sigma-Aldrich, Steinheim, Germany) without immobilised cysteine was treated and purified in the same way. All conjugates and controls were stored at 4 °C until use.

2.2. Quantification of the thiol/disulfide content

The total amount of sulfhydryl groups immobilised on the polymer is represented by the summation of

Table 1
Amount of thiol/disulfide groups per gram PAA-cysteine conjugate adjusted before lyophilisation to pH levels

PAA_{450}-cysteine conjugate	Nomenclature for resulting tablets	Thiol groups (μmol/g polymer ± S.D.)	Disulfide groups (μmol/g polymer)*
PAA_{450}-cys pH 3	Thiomer pH 3 tablets	332 ± 31	94
PAA_{450}-cys pH 4	Thiomer pH 4 tablets	293 ± 22	83
PAA_{450}-cys pH 5	Thiomer pH 5 tablets	263 ± 26	75
PAA_{450}-cys pH 6	Thiomer pH 6 tablets	241 ± 11	68
PAA_{450}-cys pH 7	Thiomer pH 7 tablets	179 ± 27	51
PAA_{450}-cys pH 8	Thiomer pH 8 tablets	163 ± 39	46

* Indicated values for thiol groups are means of at least three determinations.

free thiol groups and of oxidised thiol groups available in form of disulfide bonds.

The amount of free thiol groups of all polymers, as listed in Table 1, was determined photometrically using Ellman's reagent (DTNB, 5,5′-dithiobis(2-nitrobenzoic acid), Sigma, St. Louis, MO) as described previously (Marschütz and Bernkop-Schnürch, 2002).

To determine the total amount of bound cysteine 0.5 mg of conjugate adjusted to pH 3 before lyophilisation, was hydrated in 1 ml of 0.05 M phosphate buffer pH 8.0 for 30 min. Then, 0.6 ml of a freshly prepared 3% solution of sodium-borohydride were added to the hydrated polymer in order to reduce all disulfide bonds to free thiol groups. The mixture was incubated for 2 h in an oscillating waterbath at 37 ± 0.5 °C. Thereafter, 0.5 ml of 1 M HCl was added in order to destroy the remaining sodium-borohydride within 10 min. After the addition of 0.1 ml acetone the mixture was agitated for another 5 min. The solution was neutralised by the addition of 1 ml 1 M phosphate buffer pH 8.5 and 0.2 ml of 0.5% (w/v) DTNB dissolved in 0.5 M phosphate buffer pH 8.0 was added. After incubation for 15 min at room temperature an aliquot of 200 μl was transferred to a 96-well microtitration plate and the absorbance was measured at 450 nm with a microtitration-plate reader (Anthos Reader 2001, Salzburg, Austria). The quantity of bound cysteine was calculated using a standard curve obtained by the sulfhydryl group determination of a series of solutions containing increasing concentrations of cysteine hydrochloride (Sigma-Aldrich, Steinheim, Germany). The amount of disulfide bonds can be calculated by subtracting the quantity of free thiol groups from the total number of sulphydryl moieties present on the polymer.

2.3. Tablet manufacture

Thiolated polymers, as listed in Table 1, and the corresponding unmodified polymers were compressed into flat-faced tablets of 30 mg (5.0 mm diameter) (Hanseaten Type El, Hamburg, Germany). The compaction force was kept constant during the preparation of all tablets. The resulting tablets were designated thiomer or unmodified PAA pHs 3, 4, 5, 6, 7 or 8 tablets, respectively, according to Table 1.

2.4. Investigation of the swelling properties of tablets

The capacity of the tablets to absorb water was determined gravimetrically as described previously (Kast and Bernkop-Schnürch, 2001). In brief, tablets were fixed on a needle and incubated in 20 ml of 100 mM phosphate buffer pH 6.8 on an oscillating waterbath at 37 °C. At predetermined time points the hydrated test discs on the needle were taken out of the incubation medium and the mass of swollen tablets was determined.

2.5. Evaluation of the disintegration behaviour

The stability of thiolated polymer tablets and of control tablets was analysed in 100 mM phosphate buffer pH 6.8 with the disintegration test apparatus according to the European Pharmacopoeia at an oscillating frequency of 0.5 s^{-1} and 37 °C.

2.6. Oxidation of thiol groups

The oxidation of thiol groups within the different PAA-cysteine conjugate tablets was determined as a function of time. Tablets were incubated in 20 ml of

100 mM phosphate buffer pH 6.8 at 37 °C under continuous agitation. At predetermined time points tablets were taken out of the incubation medium and frozen at −80 °C in order to stop any further oxidation of the thiol moieties. The frozen tablets were lyophilized and pulverised in a mortar. Thereafter the amount of reduced sulfhydryl groups was determined with Ellman's reagent. The degree of oxidation was directly proportional to the decrease of reduced thiol moieties.

2.7. Mucoadhesion studies in vitro

2.7.1. Tensile studies

Tensiometer studies were carried out on native porcine intestinal mucosa with thiolated polymer tablets and control tablets in 100 mM phosphate buffer pH 6.8 as described previously (Kast and Bernkop-Schnürch, 2001). The total work of adhesion (TWA) representing the area under the force/distance curve was determined using the WINWEDGE software (TAL Technologies, Inc., Philadelphia, PA) in combination with EXCEL 5.0 (Microsoft).

2.7.2. Evaluation of the mucoadhesive properties with the rotating cylinder method

Mucoadhesion studies were also performed on native porcine intestinal mucosa attached to the rotating cylinder, which was agitated with 125 rpm in 100 mM phosphate buffer pH 6.8 at 37 ± 0.5 °C in order to evaluate with a second test system the binding of the tablets to the mucosa (Bernkop-Schnürch and Steininger, 2000). The detachment, disintegration and/or erosion of test tablets was determined visually over a 24 h time period.

2.8. Statistical data analysis

Statistical data analysis was performed using the Mann–Whitney Test with $p < 0.05$ as the minimal level of significance. Calculations were done using the software Xlstat version 5.0 (b8.3).

3. Results and discussion

3.1. Characterisation of the thiolated PAA

The lyophilised polymer-cysteine conjugates appeared as white, odourless powder of fibrous structure and were easy hydratable in aqueous solutions. The amount of thiol groups for each batch adjusted before lyophilisation to different pH levels was quantified via Ellman's reagent. The results of this test, shown in Table 1, demonstrate that raising the pH from 3 to 8 causes a decrease in thiol groups from 332 ± 31 μmol/g to 162 ± 40 μmol/g (mean ± S.D., $n = 3$), respectively. As the amount of thiol groups being oxidised during the adjustment of the pH was negligible (data not shown), this decrease in thiol groups can be explained by an increase in the molecular weight of the polymer due to the formation of sodium carboxylate substructures. Furthermore, in this study an approach for the determination of disulfide bonds beside thiol groups on polymers was performed for the first time. Results of this study shown in Table 1 provide evidence for the same trend as observed for thiol groups. The higher the pH of the polymer, the lower is the amount of disulfide bonds. Also in this case an increase in the molecular mass of the polymer due to the formation of sodium carboxylate substructures seems to be responsible for this effect.

3.2. Investigation of the swelling properties of tablets

The swelling behaviour of tablets based on mucoadhesive polymers has a great impact on their adhesive properties, stability and release of incorporated drugs (Mortazavi and Smart, 1993). The strong adhesion of many hydrophilic polymers to the mucosa is at least partially based on their capability of water uptake, where absorption, swelling and capillary effects lead to a water flux from the underlying tissue to the polymer (Duchene and Ponchel, 1992). The extent and rate of this water flux is affected by the degree of crosslinking and chain length of the mucoadhesive macromolecules (Smart, 1999). However, such 'adhesion by hydration' is not an unlimited process: an excessive water uptake causes a leakage in cohesiveness of dosage forms based on the majority of so far established mucoadhesive polymers, transforming such formulations into an over-hydrated slippery mucilage (Lehr, 1996).

Consequently, a strong relationship between swelling behaviour, mucoadhesive properties and cohesiveness of mucoadhesive formulations is provided. As the mucoadhesive and cohesive features of thiolated polymers are directly dependent on redox reactions of the thiol groups, being strongly pH-

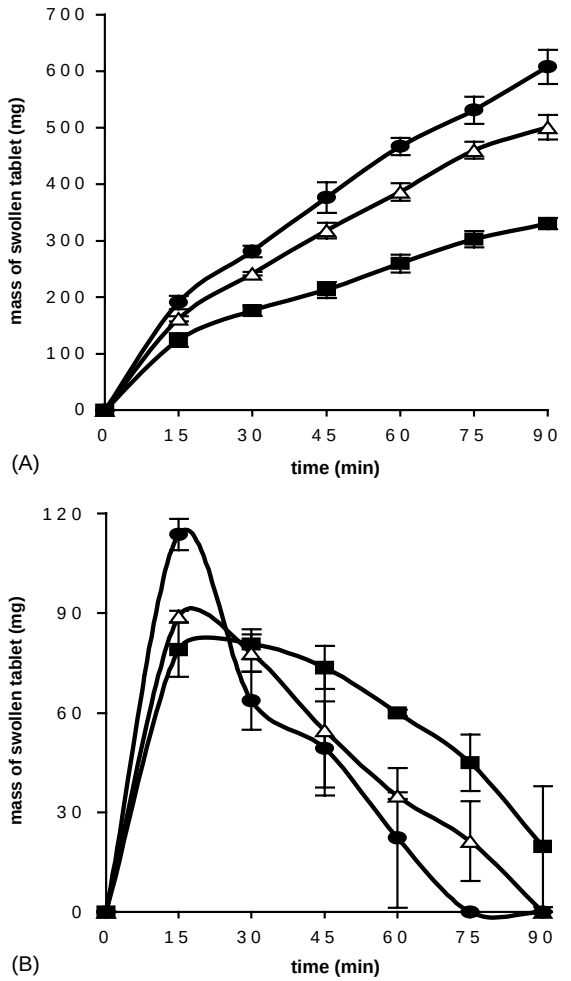

Fig. 2. (A) Swelling behaviour of thiomer pH 4 tablets (■), thiomer pH 5 tablets (△) and thiomer pH 6 tablets (●) in 100 mM phosphate buffer pH 6.8 at 37 ± 0.5 °C. (B) Swelling behaviour of unmodified PAA pH 4 tablets (■), unmodified PAA pH 5 tablets (△) and unmodified PAA pH 6 tablets (●) under the same conditions. Indicated values are means (±S.D.) of at least three experiments.

dependent, the influence of the pH on the water uptake of the PAA-cysteine conjugate was investigated. Fig. 2A displays the water uptake of thiomer pHs 4, 5 and 6 tablets, respectively, whereas Fig. 2B shows the results of the same study with tablets of the corresponding unmodified PAA. The swelling behaviour of the PAA-cysteine conjugate tablets differs totally from that of the unmodified PAA tablets. The weight of the thiomer tablets increases continuously over the

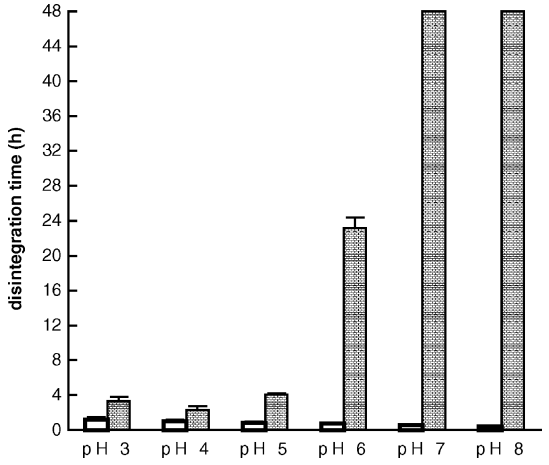

Fig. 3. Comparison of the disintegration behaviour of thiomer tablets (grey bars) or unmodified PAA tablets (white bars); studies were carried out with a disintegration test apparatus in 100 mM phosphate buffer pH 6.8 at 37 °C. Indicated values are means (±S.D.) of at least three experiments.

90 min of the experiment, while the control tablets, after an initial augmentation, progressively loose their weight until they are completely dissolved and/or eroded. However, for both polymer types the swelling process seems to be strongly pH-dependent. Thiomer pH 4 tablets showed an 11-fold weight gain during 90 min, whereas a 20-fold weight gain was observed for thiomer pH 6 tablets. Accordingly, the higher pH leads to a faster water uptake, which can be explained by a better hydratability due to a comparatively higher quantity of ionic substructures within less acidic polymers. A similar pH-dependent behaviour can be recognised for the unmodified PAA tablets as well, but here the cohesiveness of the compressed polymer is insufficient to guarantee the stability of the system.

3.3. Evaluation of the disintegration behaviour

Disintegration studies were carried out with tablets of PAA-cysteine conjugate and unmodified PAA. Although the mechanical stress to which formulations are exposed in the Ph.Eur.-test apparatus do not strictly relate to in vivo conditions, the disintegration behaviour of polymers compressed into tablets evaluated in this way is a good indicator for their cohesive features. The results of this study are displayed in Fig. 3. All tablets comprising unmodified PAA dis-

integrated within 80 min, furthermore, the higher the pH of the tablets was, the lower the stability. Results correlated well with the swelling behaviour of the tablets shown in Fig. 2, demonstrating that a more rapid swelling leads consequently to lower cohesive properties. In contrast, PAA-cysteine conjugate tablets were significantly more stable with a further increase in stability at higher pH values. This can be explained by the formation of stabilizing disulfide bonds within the polymeric network, which is favoured at higher pH values (Snyder, 1987), and contributes much more to stability than a limited swelling. Accordingly, the much higher cohesiveness observed for thiomer pHs 6, 7 and 8 tablets is unequivocally due to the formation of intra- as well as intermolecular disulfide bonds within the polymeric network, guaranteeing the stability of the matrix systems in spite of the very large weight increase. Consequently, a higher stability and a faster drug release due to a faster water absorption of thiolated polymer based drug carrier systems can be achieved by adjusting the polymer solution to a higher pH before lyophilisation, while an acidic pH of polymer should guarantee a slower water uptake leading to a sustained release of an incorporated drug.

3.4. Oxidation of thiol groups

In order to characterise the oxidation process of thiol groups to disulfide bonds within matrix tablets, the degree of oxidation was quantified as function of time in aqueous solutions. The data of this study are resumed in Fig. 4A and B. Previous investigations demonstrated for tablets based on thiomers a rapid formation of disulfide bonds within the first hour of incubation, being followed by a comparatively lower oxidation process within the next hours (Bernkop-Schnürch et al., 2001a). Although this course was seen within this study as well, a tremendous difference between the thiomer tablets listed in Table 1 was observed. The amount of reduced thiol groups within thiomer pH 3 tablets was of 80.6 ± 3.2% of the initial quantity, whereas only 7.8 ± 1.8% (means ± S.D., $n = 4$) reduced thiol groups could be detected within thiomer pH 8 tablets after 5 h of incubation. This observation provides additional evidence for the previously adduced explanation of the higher cohesiveness of tablets being based on PAA-cysteine conjugates adjusted to neutral or slightly basic pH values.

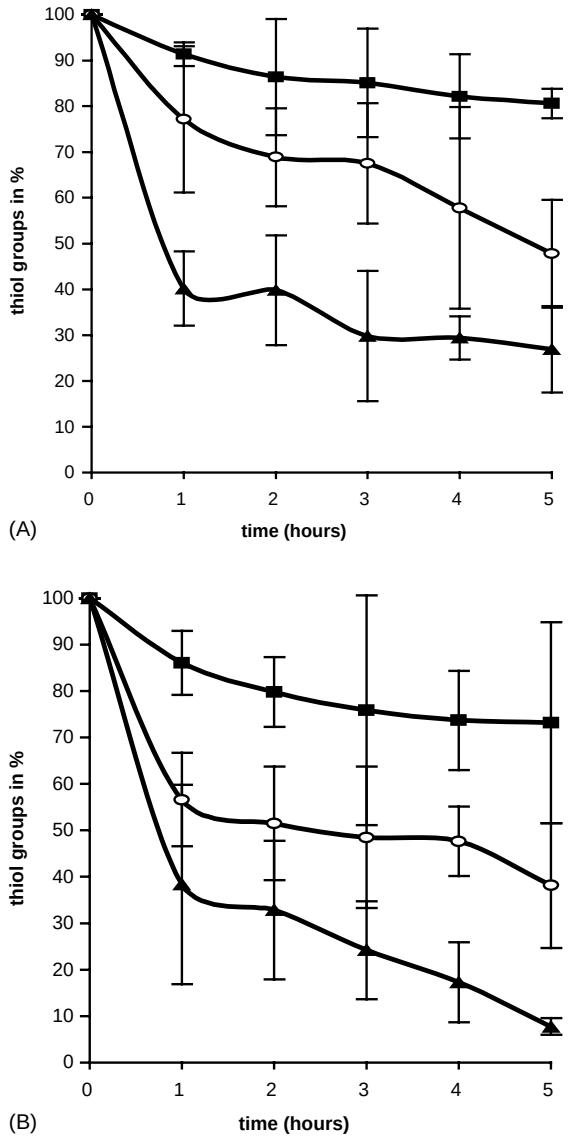

Fig. 4. (A) Decrease in free thiol groups within thiomer pH 3 tablets (■), thiomer pH 5 tablets (○) and thiomer pH 7 tablets (▲) in 100 mM phosphate buffer pH 6.8 at 37 °C. (B) Decrease in free thiol groups of thiomer pH 4 tablets (■), thiomer pH 6 tablets (○) and thiomer pH 8 tablets (▲) under the same conditions. Indicated values are the means (±S.D.) of at least three experiments.

3.5. Mucoadhesion studies in vitro

Two different experimental set-ups were utilised to evaluate the influence of the pH on the mucoadhesive properties of the PAA-cysteine conjugate.

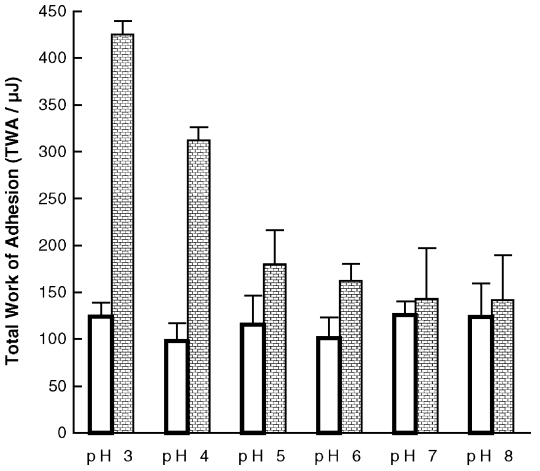

Fig. 5. Influence of the pH on the mucoadhesive properties of thiomer tablets (grey bars) and of the corresponding unmodified PAA tablets (white bars). Represented are the values (±S.D., $n = 4$–8) of the total work of adhesion, which was determined via tensile studies.

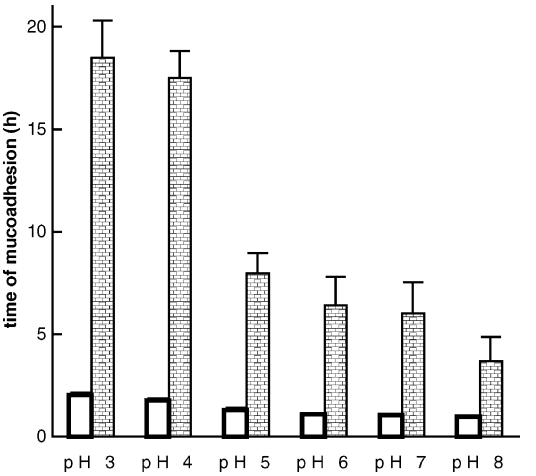

Fig. 6. Comparison of the adhesion time of thiomer tablets (grey bars) and of unmodified PAA tablets (white bars) on freshly excised porcine mucosa according to the rotating cylinder method. Indicated values are means (±S.D.) of at least four experiments.

Results of tensile studies shown in Fig. 5 demonstrated that the pH change has a great impact on the mucoadhesive properties of thiomer tablets. The total work of adhesion (TWA) of thiomer pH 3 tablets, determined to be $425 \pm 15\,\mu J$ (mean ± S.D., $n = 5$), was more than two-fold higher, in comparison to the TWA of thiomer pHs 8, 7, 6 and 5 tablets. Although a trend, showing a continuous increase in TWA values from thiomer pH 8 tablets to thiomer pH 3 tablets, was documented, a significant increment in TWA was recorded only for formulations based on pHs 3 and 4 batches. All other TWA values did not differ significantly from each other.

Results of mucoadhesion studies performed with the rotating cylinder method are shown in Fig. 6. Since this test system takes also the cohesiveness of the polymers into account, it is supposed to be closer to in vivo conditions than simple tensile studies described above. The adhesion time of thiomer pH 3 tablets was of 18.5 ± 1.8 h, and was greater than four-fold the adhesion time of thiomer pH 8 tablets. These results confirm the trend of tensile studies, showing that the lower the pH of the conjugate was, the significantly higher were its adhesive features. In both studies the difference in the mucoadhesive properties between thiolated and unmodified PAA became more pronounced the lower the pH of the tested polymers was. For tablets being based on PAA-cysteine conjugate neither erosion nor disintegration could be observed during incubation. In particular thiomer pHs 3, 4 and 5 tablets remained attached to the mucosa for a significantly longer time than they were stable when exposed to the much higher mechanical stress in the disintegration apparatus.

The increase in the mucoadhesiveness of tablets based on thiolated polymer adjusted at lower pH values might be mainly explained by the combination of three effects:

(I) The increase in polymer weight in consequence of a partial neutralisation leads to a decrease of the quantity of thiol moieties per gram polymer as shown in Table 1. Hence, a comparatively lower amount of thiol groups is available for the formation of disulfide bonds with mucus glycoproteins.

(II) A much higher weight increase of tablets comprising higher pH values occurs due to a faster and higher (two-fold) water uptake. Therefore, a higher strength of adhesion would be required to guarantee the same capability of these tablets to stick to the mucosa.

(III) A premature oxidation of thiol functions before coming into contact with the mucus layer can be prevented by a low pH within the matrix system, as the reactivity of thiol functions is limited at lower pH values. Only sulfhydryl structures coming into direct contact with the mucus gel layer will be activated as a consequence of a pH shift to pH 5–7 (Khanvilkar et al., 2001).

Consequently, choosing lower pH values may cause a longer residence time of drug carrier systems based on PAA-cysteine conjugate at the absorption site. Moreover, results demonstrate that also mucoadhesiveness of unmodified polyacrylates can be raised by lower pH values within the polymer. Among polyacrylates the majority of anionic mucoadhesive polymers such as Na-carboxymethylcellulose or Na-alginate have as main substructure carboxylic functions, which renders their pH-dependent behaviour very similar. It can therefore be assumed, that a significantly stronger adhesive capability of anionic polymers in general should be achieved by adjusting their pH to lower levels.

4. Conclusion

This study examined the influence of the pH on the mucoadhesive and cohesive properties of polyacrylic acid (PAA) and thiolated PAA. The pH of thiolated PAA-cysteine conjugate providing the highest mucoadhesiveness could be identified to be pH 3. At higher pH values a manifestly lower adhesiveness of the thiomer must be taken into account. However, a higher cohesion and water uptake of thiolated PAA can be obtained by choosing a higher pH.

The findings of this study contribute important basic information to the knowledge in the field of thiolated polymers, which will be valuable for an improvement of non-invasive delivery systems for hydrophilic macromolecules based on such polymers.

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