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Polymer–cysteamine conjugates: new mucoadhesive excipients for drug delivery?

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Abstract

In the present study, the features of two new thiolated polymers—the so-called thiomers—were investigated. Mediated by a carbodiimide cysteamine was covalently attached to sodium carboxymethylcellulose (Na-CMC) and neutralised polycarbophil (Na-PCP). Depending on the weight-ratio polymer to cysteamine during the coupling reaction, the resulting CMC–cysteamine conjugate and PCP–cysteamine conjugate showed in maximum 43 ± 15 and 138 ± 22 μmole thiol groups per g polymer (mean $\pm$ S.D.; $n = 3$), respectively, which were used for further characterisation. Tensile studies carried out with the CMC–cysteamine conjugate on freshly excised porcine intestinal mucosa displayed no significantly ($P < 0.01$) improved mucoadhesion, whereas, the mucoadhesive properties of the PCP–cysteamine conjugate were increased 2.5-fold compared with the unmodified polymer. The swelling behaviour of the CMC–cysteamine conjugate was uninfluenced by the covalent attachment of the sulfhydryl compound. In contrast the swelling behaviour of the PCP–cysteamine conjugate was improved significantly ($P < 0.01$) versus unmodified PCP. Furthermore, in aqueous solutions the disintegration time of tablets based on the CMC– and PCP–cysteamine conjugates was prolonged 1.5 and 3.2-fold, respectively, in comparison to tablets containing the corresponding unmodified polymers. According to these results, especially the PCP–cysteamine conjugate represents a promising new pharmaceutical excipient for various drug delivery systems.

Keywords: Sodium carboxymethylcellulose; Polycarbophil; Thiomers; Mucoadhesion

1. Introduction

In recent years the interest in bioadhesion has been inspired by the development of novel bioadhesive polymers for mucosal delivery (e.g. Alur et al., 1999; Lehr 2000). Bioadhesive, or more precise mucoadhesive drug delivery systems are aimed to adhere to various mucosal tissues (Lehr, 1996; Peppas and Buri, 1985). All traditionally used mucoadhesive polymers, e.g. poly(acrylates) or chitosan, are based on the formation of non-covalent bonds such as hydrogen bonds and ionic interactions with the mucus layer (Peppas and

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Mikos, 1990; Lehr et al., 1992). These polymers provide only a weak adhesion being in many cases insufficient to guarantee the localisation of a drug at a given target site. According to this, various attempts have been undertaken to improve the mucoadhesive properties of polymers. A promising new approach was the generation of thiolated polymers—or the so-called thiomers. Due to immobilised thiol groups these polymers are capable of forming covalent bonds with the mucus layer covering mucosal tissues, subsequently leading to improved mucoadhesive properties (Bernkop-Schnürch et al., 1999). The responsible mechanism for this effect is based on thiol/disulfide exchange reactions between the thiol groups of the polymer and the cysteine-rich subdomains of mucin glycoproteins (Gum et al., 1992). First success was achieved with polycarbophil–cysteine and chitosan–thioglycolic acid conjugates displaying a 2.75- and 10.3-fold, respectively, higher mucoadhesion on freshly excised porcine intestinal mucosa than the corresponding unmodified polymers (Bernkop-Schnürch et al., 1999; Kast and Bernkop-Schnürch, 2001). Due to these strongly improved mucoadhesive properties, thiomers seem to be advantageous over so far used polymers. The better adhesion to mucosal tissues should provide the localisation of the delivery system in specified regions, like the buccal, nasal or vaginal epithelium. Furthermore, a comparatively longer residence time at the site of drug absorption can be achieved. A limiting factor thereby seems to be the rapid turnover of the mucus (Woodley, 2001). But the intensified contact with the mucosal absorption membranes provided by thiomers should additionally guarantee an increased drug concentration gradient representing the driving force for a passive drug uptake (Luessen et al., 1999).

In order to make further progress in investigating the potential of thiolated polymers, it was the aim of this study to evaluate the properties of two recently generated thiomers: carboxymethylcellulose–cysteamine and polycarbophil–cysteamine (Bernkop-Schnürch et al., 2001) in a common dosage form. Among delivery systems tablets provide an accurate dosage and are easy to manufacture. Accordingly, the features of tablets based on CMC–cysteamine and PCP–cysteamine conjugates were evaluated focusing on swelling- and disintegration behaviour as well as on mucoadhesion. Obtained results should contribute to a better understanding of the function of thiomers in delivery systems.

2. Materials and methods

2.1. Materials

Sodium carboxymethylcellulose (average mol.wt. 1000 kDa) was purchased from Kwizda, Vienna, Austria. Polycarbophil (mol.wt. >700 kDa; Noveon AA1) was a gift of BF Goodrich, Bucksville, OH. Cysteamine hydrochloride and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride were obtained from Sigma, St. Louis, MO. All other reagents were of analytical grade and received from commercial sources.

2.2. Synthesis of the polymer–cysteamine conjugates

The covalent attachment of cysteamine to sodium carboxymethylcellulose (Na-CMC) and neutralised polycarbophil (Na-PCP), respectively, was achieved by the formation of amide bonds between the primary amino group of cysteamine and the carboxylic acid moieties of the polymers. One gram of Na-CMC and 250 mg of Na-PCP were each hydrated in 50 ml of demineralised water. The carboxylic acid moieties of the polymers were activated for 45 min by adding 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC) in a final concentration of 50 mM. The pH of the reaction mixture with Na-CMC was adjusted to 6–7 by the addition of 1 M HCl. In contrast, the pH of reaction mixtures containing PCP remained at 6 without adding NaOH or HCl. Cysteamine–hydrochloride was added to the activated Na-CMC and Na-PCP in different weight-ratios as listed in Table 1. The pH of the reaction mixtures was adjusted to 5 either with 1 M HCl or 1 M NaOH. Reaction mixtures were incubated for 3 h under stirring at room temperature. Resulting polymer–cysteamine

conjugates were isolated by dialysing at 10 °C in the dark against 0.2 mM HCl, two times against the same medium but also containing 1% NaCl to reduce ionic interactions between the polymer and the sulfhydryl compound and then again two times against 0.2 mM HCl. The acidic medium was necessary to quench oxidation reactions within the polymer conjugates. Samples being prepared and isolated in exactly the same way as polymer–cysteamine conjugates but without EDAC during the coupling reaction served as control for the following studies. Samples were lyophilised by drying frozen aqueous polymer solutions at −30 °C and 0.01 mbar (Christ Beta 1-8 K; Osterode am Harz, Germany). Polymer–cysteamine conjugates and controls were stored at 4 °C until further use.

2.3. Determination of the thiol group content

The amount of thiol groups on the polymer–cysteamine conjugate, i.e. degree of modification was determined by iodometric microtitration. Initially, 3.00 mg each of the polymer-conjugate and the control were hydrated in 1.00 ml of demineralised water. The pH was then adjusted to 2–3 by the addition of 100 μl of 1 M HCl. Afterwards 500 μl of aqueous starch solution (0.25%) was added. The samples were titrated with an aqueous iodine-solution (1 mM) until a permanent light blue colour maintained (Bernkop-Schnürch et al., 2000).

2.4. Evaluation of the swelling behaviour

The water absorbing capacity was determined by a gravimetric method as described previously by our research group (Kast and Bernkop-Schnürch, 2001). Briefly, 30 mg each of lyophilised polymer–cysteamine conjugates, controls and unmodified polymers 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 discs. Test discs were fixed on a needle and incubated in a 100 mM phosphate buffer solution pH 6.8 at 37 °C. At predetermined time intervals the swollen test discs on the needle were taken out of the incubation medium and the amount of water uptake was determined gravimetrically.

2.5. Disintegration studies

The disintegration behaviour of polymer tablets based on the lyophilised CMC–cysteamine and PCP–cysteamine conjugates was evaluated in comparison to tablets based on the corresponding unmodified polymers and the control-polymers. The stability of polymer tablets (30 mg; 5.00 mm in diameter) in 100 mM phosphate buffer pH 6.8 at 37 °C was analysed with a disintegration apparatus (Zerfallstester Type PZT E, Pharma Test, Hainburg, Germany) according to the European Pharmacopoeia with an oscillating frequency of 0.5 s^{-1}.

Table 1
Synthesis of polymer–cysteamine conjugates; carboxylic acid moieties of sodium carboxymethylcellulose (Na-CMC) and polycarbophil (PCP) were activated for 45 min with 1-ethyl-3(3-dimethylaminopropyl)carbodiimide hydrochloride (EDAC); after the addition of cysteamine, reaction mixtures were incubated for 3 h at room temperature under stirring

Polymer–cysteamine conjugate	Polymer/demin. water	EDAC (final conc.)	Added cysteamine–HCl	Thiol groups (μmol per g polymer); mean ± S.D., $n = 3$
CMC–cysteamine 4:1	1 g/50 ml	50 mM	0.25 g	43.4 ± 14.9
CMC–cysteamine 8:1	1 g/50 ml	50 mM	0.125 g	0
CMC–control	1 g/50 ml	–	0.125–0.25 g	0
PCP–cysteamine 8:1	0.25 g/50 ml	50 mM	0.03125 g	138.7 ± 22.6
PCP–cysteamine 12:1	0.25 g/50 ml	50 mM	0.02083 g	51.5 ± 9.0
PCP–control	0.25 g/50 ml	–	0.03125–0.02083 g	0

2.6. Tensile studies

Thirty milligrams each of lyophilised polymer–cysteamine conjugates, controls and unmodified polymers were compressed to test tablets as described above. The tablets were thereby attached to native porcine intestinal mucosa as described by our research group (Kast and Bernkop-Schnürch, 2001). After an incubation time of 30 min in 100 mM phosphate buffered saline (PBS) pH 6.8 at 25 °C, the mucosa was pulled from the tablet at a rate of 0.1 mm s^{-1}. The total work of adhesion (TWA) representing the area under the force/distance curve and the maximum detachment force (MDF) were calculated using the WINWEDGE software (TAL Technologies, Inc., Philadelphia, PA) in combination with EXCEL 97 (Microsoft, USA) (Bernkop-Schnürch et al., 1999).

2.7. Statistical data analysis

Statistical data analysis was performed using the t-test with $P < 0.01$ as the minimal level of significance unless indicated otherwise.

3. Results and discussion

3.1. Chemical properties of the polymer–cysteamine conjugates

Cysteamine was attached covalently to Na-CMC and Na-PCP under the formation of amid bonds as shown in Fig. 1. The carboxylic acid moieties of the polymers were activated by EDAC forming an *O*-acylurea derivative as intermediate product, which reacts with the primary amino groups of cysteamine. The amount of thereby covalently attached thiol groups is shown in Table 1. Although the reaction conditions were the same for PCP and CMC, PCP showed a higher degree of modification. The observation can be explained by the fact that PCP displays 2.6 fold more carboxylic acid groups than CMC which are necessary for the immobilisation of cysteamine. Additionally, the hydroxyl groups of CMC might interfere the coupling reaction to some extent.

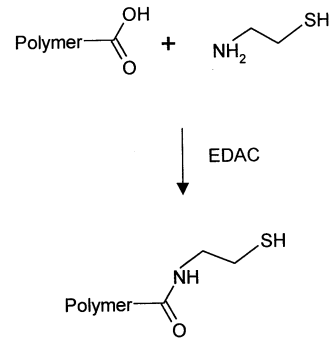

Fig. 1. Synthetic pathway of polymer–cysteamine conjugates; covalent attachment was achieved by the formation of amide bonds between the carboxylic acid groups of the polymer and the primary amino groups of cysteamine mediated by a carbodiimide (EDAC); polymer: sodium carboxymethylcellulose or polycarbophil.

These are likely reasons why CMC showed no coupling rate at a weight-ratio polymer to cysteamine 8:1, whereas, PCP displayed at the same ratio a coupling rate of 138.7 ± 22.6 μmol thiol groups per g polymer. According to these results, the type of polymer seems to have a great impact on the yield of covalently attached thiol groups.

The lyophilised polymer–cysteamine conjugates appeared as white, odourless powder of fibrous structure. They were easy swellable in aqueous solutions and formed transparent gels of high viscosity. The efficacy of the purification method for the resulting polymer–cysteamine conjugates could be verified by the controls. They were prepared in exactly the same way as the polymer conjugates but omitting EDAC during the coupling reaction and exhibited a negligible amount of thiol groups. Former analysis of these polymer-conjugates and controls using 2,4,6-trinitrobezene sulfonic acid (TNBS) to quantify primary amino groups showed none of them on conjugates and controls, as well. Hence, the unconjugated cysteamine was completely removed by dialysing (Bernkop-Schnürch et al., 2001). Furthermore an evidence for the formation of amide bonds between the polymer and cysteamine is thereby given, since the polymer conjugates exhibit numerous thiol groups but no primary amino groups.

3.2. Swelling behaviour

The swelling behaviour of mucoadhesive polymers has a great influence on their adhesive properties and cohesiveness (e.g. Mortazavi and Smart, 1993; Ch'ng et al., 1985). By absorbing, swelling, and capillary effects, mucoadhesive polymers are supposed to take water from the underlying tissue leading to a considerably strong adhesion (Duchêne and Ponchel, 1992). In order to evaluate this effect for the new polymers, water uptake studies were carried out with tablets based on the corresponding unmodified polymer, the polymer–cysteamine conjugates and controls. Thereby obtained results are shown in Figs. 2 and 3 Fig. 3. As shown in Fig. 2 the covalent attachment of cysteamine to Na-CMC had no influence on the swelling behaviour of the polymer. This observation is in good correlation with former studies on Na-CMC conjugates. The swelling behaviour of tablets based on CMC–cysteine conjugate had been evaluated with the result that the immobilisation of cysteine displayed no effect on the water uptake of the polymer (Bernkop-Schnürch et al., 2000). In contrast, the swelling behaviour of PCP was improved due to the attached cysteamine. The more thiol groups were introduced to the polymer, the higher was the water uptake rate. The swelling behaviour of the unmodified PCP and the control was very similar during the whole period of evaluation, whereas, the two conjugates showed differences in the water uptake already after 30 min.

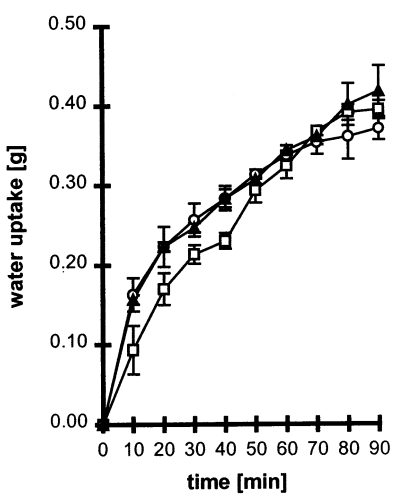

Fig. 2. Swelling behaviour of tablets (30 mg) based on unmodified CMC (□), CMC–cysteamine control (○) and CMC–cysteamine 4:1 (▲) in 100 mM phosphate buffer pH 6.8 at 37 °C. Indicated values are mean ± S.D. of at least three experiments.

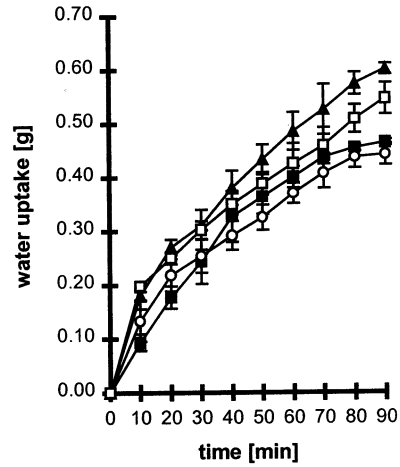

Fig. 3. Swelling behaviour of tablets (30 mg) based on unmodified PCP (■), PCP–cysteamine control (○), PCP–cysteamine conjugate 8:1 (▲) and PCP–cysteamine conjugate 12:1 (□) in 100 mM phosphate buffer pH 6.8 at 37 °C. Indicated values are mean ± S.D. of at least three experiments.

3.3. Disintegration studies of polymer–cysteamine conjugates

Disintegration studies were carried out to determine the influence of thiol groups on the cohesiveness of tablets based on polymer–cysteamine conjugates. Studies demonstrated an increased cohesiveness and stability of tablets consisting of thiolated polymers compared with unmodified Na-CMC and Na-PCP. Tablets comprising of unmodified polymers disintegrated relatively fast which was in good correlation with former studies carried out by Kaiho et al. (1996). In contrast, tablets based on polymer–cysteamine were comparatively more stable. The improved stability of these polymer–cysteamine conjugates can be explained by the formation of disulfide bonds within the thiolated polymers, providing an improved cohesiveness of the polymers. An evidence for the

formation of disulfide bonds within the conjugates was given recently (Bernkop-Schnürch et al., 2001). The formation of these bonds could be accelerated by raising the pH above 6 where the oxidation of free thiol groups is favoured. Hence, the oxidation of thiol groups led to a crosslinking within the polymer network and resulted into a stabilisation of the polymeric network. Results of disintegration studies are shown in Figs. 4 and 5. Comparing the disintegration time of PCP–cysteamine conjugate 12:1 and the one of CMC–cysteamine conjugate 8:1 reveals an obvious correlation between the amount of immobilised cysteamine to the polymer and the disintegration behaviour of tablets based on the thiolated polymer. Both polymers were modified to almost the same extent (see Table 1) and showed furthermore with 120 and 121 min, respectively, a very similar disintegration time. This observation might be explained by the fact that the thiol groups of the

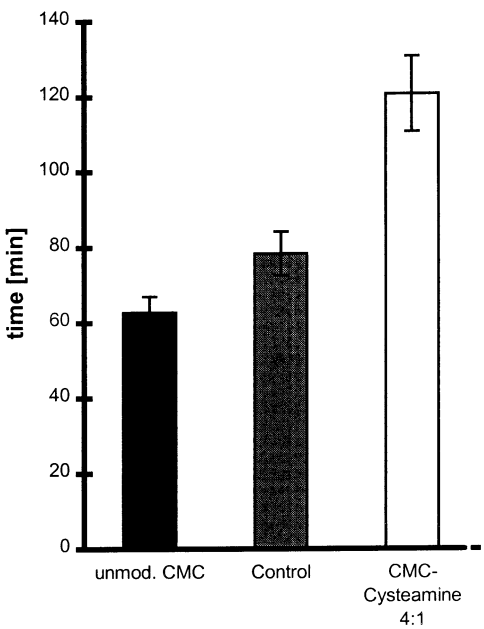

Fig. 4. Profile of the disintegration behaviour of tablets (30 mg; 5 mm i.d.) consisting of unmodified CMC compared with those consisting of CMC–cysteamine control and CMC–cysteamine conjugate 4:1, respectively. Disintegration studies were carried out with a disintegration test apparatus (Pharm. Eur.) in 100 mM phosphate buffer pH 6.8 at 37 °C. Indicated values are mean ± S.D. of at least four experiments.

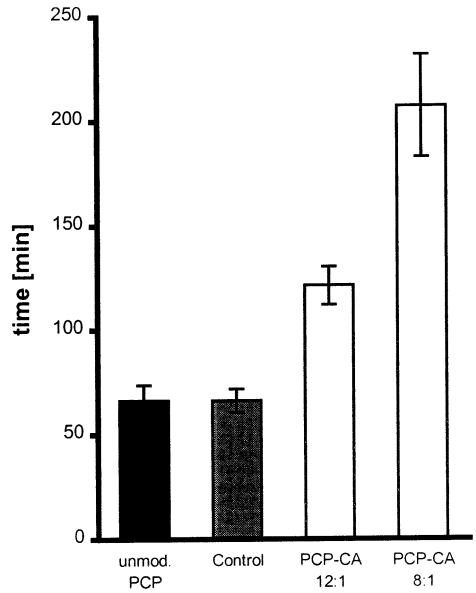

Fig. 5. Profile of the disintegration behaviour of tablets (30 mg; 5 mm i.d.) consisting of unmodified PCP compared with those consisting of PCP–cysteamine control, PCP–cysteamine conjugate 8:1 (PCP–CA 8:1), PCP–cysteamine conjugate 12:1 (PCP–CA 12:1), respectively. Disintegration studies were carried out with a disintegration test apparatus (Pharm. Eur.) in 100 mM phosphate buffer pH 6.8 at 37 °C. Indicated values are mean ± S.D. of at least four experiments.

different polymers are oxidised to disulfide bonds to the same extent. Hence the crosslinking within these two polymers results in a similar disintegration behaviour. On the one hand, crosslinking within the polymeric network might be an advantage for stabilising also microparticles based on these polymers. A higher stability of small particles would result in a prolonged drug release from these particles compared with unstabilised microparticles. Studies on pellets consisting of thiolated PCP showed a prolonged disintegration time in comparison to pellets based on unmodified Na-PCP (data not shown). On the other hand, the improved stability of thiomer tablets also seems to be of high relevance concerning buccal or oral drug delivery. Buccal tablets based on thiolated polymers, for instance, should increase the patient compliance. Due to their slow disintegration the tablet is stable over hours and can be removed easily without eroding. Drug delivery systems

based on mucoadhesive polymers providing such a high stability might strongly reduce a presystemic metabolism in the small intestine of rapidly degraded drugs like (poly)peptides (Woodley 1994; Luessen et al., 1995; Bernkop-Schnürch 1999). A fast disintegration of the delivery system would lead to a larger surface area exposed to degrading enzymes.

3.4. Mucoadhesion studies

3.4.1. CMC–cysteamine conjugates

Tensile studies with tablets based on thiolated CMC showed no significant improvement in the mucoadhesive properties. This observation can be explained on the one hand by the amount of attached thiol groups which was relatively low compared with the PCP–cysteamine conjugates. On the other hand, Snyder et al. (1981) showed that disulfide bond formation between two molecules takes place more rapidly if the thiol groups of both reactants are surrounded by opposite charges. Therefore, the negative charged carboxylic acid moieties of the polymer are essential for a rapid disulfide bond formation between the thiolated polymer and the thiol groups of mucus glycoproteins being surrounded by positive charges in form of arginine and lysine. By immobilisation of cysteamine to CMC many negative charges from the polymer will be lost. As a result disulfide bond formation of the cysteamine substructure and the thiol groups of the mucus layer seems to be a very slow process. This observation might explain why the mucoadhesion of CMC could not be improved significantly by the introduction of cysteamine.

3.4.2. PCP–cysteamine conjugates

Tensile tests with tablets of PCP–cysteamine conjugates demonstrated a clear correlation between the amount of polymer-linked cysteamine and the adhesive properties of the polymer. Results of adhesion studies are shown in Fig. 6. The total work of adhesion (TWA) of PCP–cysteamine conjugate 12:1 increased thereby 1.7-fold compared with the unmodified PCP and 1.4-fold compared with the control. The PCP–cysteamine conjugate 8:1 showed a strongly improved mucoadhesion by a 2.5-fold higher TWA compared with the unmodified polymer and a 2-fold higher mucoadhesion compared with the control. The maximum detachment force (MDF; data not shown) was in accordance with the corresponding TWA. The improved swelling behaviour of PCP–cysteamine conjugates seems thereby to have an influence on the improved mucoadhesive properties. The more thiol groups were attached to the polymer the more increased the water uptake and the mucoadhesion, respectively. In contrast, the swelling behaviour of the cationic polymer chitosan, for instance, was not improved significantly by the introduction of thioglycolic acid, whereas, the mucoadhesive properties were improved 3.5-fold compared with the control and 10-fold to the unmodified polymer (Kast and Bernkop-Schnürch, 2001).

Compared with the structurally similar polycarbophil–cysteine conjugates, PCP–cysteamine conjugates showed a lower mucoadhesion on porcine intestinal mucosa (Bernkop-Schnürch et

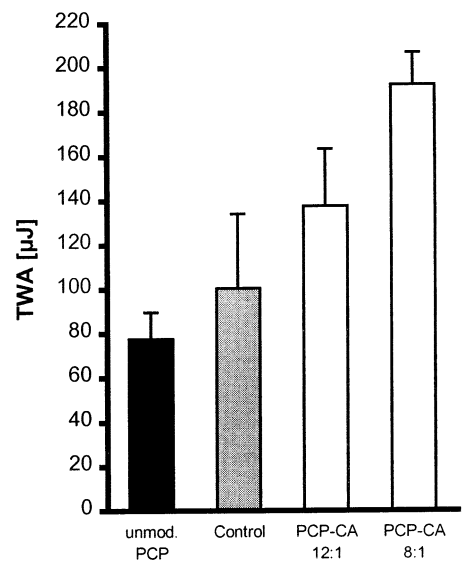

Fig. 6. Comparison of the adhesive properties of the unmodified PCP, PCP–cysteamine control, PCP–cysteamine conjugate 8:1 (PCP–CA 8:1) and PCP–cysteamine conjugate 12:1 (PCP–CA 12:1), respectively. Represented values are means ($\pm$ S.D.; $n = 3–5$) of the total work of adhesion (TWA) determined in tensile studies at pH 6.8 with tablets of indicated test material on porcine intestinal mucosa.

al., 1999). The TWA of PCP–cysteine conjugates increased due to the amount of cysteine but reached a plateau phase at a coupling rate of 5 μmol thiol groups per g polymer. A further increase of cysteine resulted in a decrease of the TWA. Even with a very low coupling rate of cysteine to PCP, the TWA was higher than the one of PCP–cysteamine conjugates. In contrast to cysteine, cysteamine does not exhibit a carboxylic acid group, which is also able to bind via hydrogen bridges to the mucus layer. These bonds may increase the mucoadhesion of PCP–cysteine conjugates. By the introduction of cysteamine to the polymer the hydrophilic properties of polymer conjugates are decreased. Therefore, the formation of hydrogen bonds between the thiol group bearing ligand and the mucosal tissue can be excluded.

4. Conclusions

The thiolation of Na-CMC and Na-PCP, respectively, described here leads to conjugates with modified features compared with the unmodified polymers. These studies indicate that polymer–cysteamine conjugates are capable of forming inter- and/or intramolecular disulfide bonds. Due to this effect, the mechanical stability of tablets based on such thiomers could be significantly improved. Based on this feature and the mucoadhesive properties of the conjugates, CMC–cysteamine and especially PCP–cysteamine could be potential candidates for various mucoadhesive applications.

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