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Research paper

Thiolated hydroxyethylcellulose: Synthesis and in vitro evaluation

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ABSTRACT

In recent years, thiomers have received considerable interest due to advantageous characteristics, such as improved mucoadhesive and permeation enhancing properties. Thiolated polymers, however, are characterized by an ionic charge which represents for various applications a great limitation. The aim of this study was therefore to synthesize a novel thiolated polymer not exhibiting ionizable groups. Hydroxyethylcellulose (HEC) was chosen as polymer backbone. The chemical modification was achieved by the replacement of hydroxyl groups on the carbohydrate structure with thiol moieties, using thiourea as thiolating reagent. The resulting thiolated hydroxyethylcellulose (HEC-SH) was characterized in vitro regarding its gelling properties, swelling behaviour, mucoadhesion on freshly excised porcine intestinal mucosa and permeation enhancing effect across rat intestinal mucosa. The new thiomer displayed up to 131.58 ± 11.17 μmol thiol groups per gram polymer, which are responsible for the observed in situ gelling capacity. The swelling behaviour and the mucoadhesive properties of tablets based on HEC-SH were 1.5-fold and 4-fold improved compared with unmodified HEC, respectively. The permeation enhancing effect of 0.5% (m/v) HEC-SH on rhodamine 123 (Rho-123) transport was 1.9-fold improved compared with buffer only. According to these results, HEC-SH seems to represent a promising tool for the development of in situ gelling, mucoadhesive delivery systems with permeation enhancing properties.

1. Introduction

Among mucoadhesive oral drug delivery systems, those based on thiomers are some of the most extensively studied during the last years [1,2]. Due to the capability of thiol groups to form disulfide bonds with glycoproteins covering mucosal membranes [3,4], thiomers can improve various intrinsic polymeric properties such as mucoadhesion, enzymatic and efflux pump inhibition, mucosal permeation enhancement, controlled release and swelling capacity. This guarantees the advantage of prolonged drug delivery, localization of therapy and a targeting to specific tissues [5,6]. Moreover, the enzymatic degradation of perorally administrated (poly)peptide drugs can be avoided. A drawback of thiolated polymers for certain applications, however, is their ionic character [7,8]. Positively or negatively charged functional groups on the molecular structure of a polymer represent target moieties for the introduction of sulphydryl ligands and therefore play a key role in thiomers synthesis. However, the ionic character of thiomers causes limitations like the incompatibility with ionic drugs of opposite charge, an unintended pH-dependent drug release or a poor cross-linking within the thiomer due to ionic repulsion. Furthermore, relatively high quantities of expensive functional group activators are required for synthesis. It would be instead necessary to develop a polymer that is compatible with ionic drugs, reactive in a pH-independent manner, possibly produced under affordable cost and that can maintain all the benefits typical for thiomers. The aim of this study was to develop a non-ionisable thiomer that overcomes the drawbacks of well-established ionic thiolated polymers. Hydroxyethylcellulose (HEC), which was chosen for this purpose, is a water-soluble cellulose ether made by swelling cellulose with NaOH and treating with ethylene oxide. It offers numerous advantages rendering it a good candidate to this purpose. Beyond its chemical stability, biodegradability and biocompatibility, it exhibits properties such as a wide range of viscosity grades [9]. Moreover, HEC is characterized by a chemical heterogeneity and substitution pattern in comparison, for example, with cellulose, which is expected to improve properties of polymer tablets [10,11]. Replacement on primary hydroxyl groups of the hydroxyethylenic chains as well as on secondary hydroxyl groups on the glucose units shall be achieved by bromination followed by substitution with thiol groups utilizing thiourea. The obtained polymer shall then be characterized regarding its viscoelastic and mucoadhesive properties, permeation enhancement, swelling behaviour and disintegration capability.

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2. Material and methods

2.1. Materials

Hydroxyethylcellulose (HEC, ~145 mPa s 1% H_20), LiBr, *N*-bromosuccinimide (NBS), triphenylphosphine (Ph_3P), thiourea ($CS(NH_2)_2$), *N,N*-dimethylacetamide (DMA), cellulose membrane tubings with a molecular mass cut-off of 12 kDa, 5,5′-dithiobis(nitrobenzoic acid) (Ellman's reagent), 2,4,6-trinitrobenzenesulfonic acid (TNBS), L-glutathione reduced form (GSH), and 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (Hepes) were purchased from Sigma–Aldrich (Vienna, Austria). Molecular sieves 4 Å were obtained from Roth®.

Cell culture medium was prepared by using MEM powder 9.66 g/L (modified with Earle's salts, phenol red, 19 amino acids and the non-essential amino acids L-ala, L-asn, L-asp, L-glu, L-gly, L-pro and L-ser), sodium bicarbonate 2.2 g/L, L-glutamine 2 mM, penicillin/streptomycin solution (100 units penicillin and 0.1 mg of streptomycin per liter medium) and 20% fetal calf serum (FCS). All other reagents were of analytical grade and received from commercial sources.

2.2. Modification of hydroxyethylcellulose

To the purpose, HEC was chosen as basic hydrophilic polymer for the replacement of its primary and secondary hydroxyl groups with bromine moieties, which were subsequently replaced by thiol groups. The particular grade of HEC was chosen as polymer backbone for its similarity in terms of molecular weight, viscosity and structure, to previously selected polymers for thiomers synthesis. Thiolated hydroxyethylcellulose (HEC-SH) was synthesized via bromo-hydroxyethylcellulose derivative, an intermediate product prepared as already described [12]. HEC (molecular mass: ~250 kDa; Sigma–Aldrich) was dried in a desiccator under diminished pressure before use. DMA was dried with CaH_2, distilled under diminished pressure and stored over molecular sieve (Roth type 4 Å). Lithium bromide (anhydrous) was dried at 180 °C under reduced pressure. All dissolution and bromination experiments were carried out under Argon. In a typical reaction, 0.1 g of dried hydroxyethylcellulose was dissolved in 10 mL of DMA, and the mixture was heated for 1 h at 160 °C with stirring. The temperature was lowered till 90 °C and 2.2 g of LiBr was added. This LiBr-organic solvent system was found to be suitable for the homogeneous bromination of HEC. The mixture was kept for a further 1 h at this temperature while stirring and then lowered to 60 °C. A clear solution was obtained within 12 h. After cooling the mixture with ice-water, 10 mL of 2% (m/v) NBS and 10 mL of 3% (m/v) Ph_3P (both in DMA solution) were added. The solution was kept at 70 °C for 2 h while stirring. NBS and Ph_3P used in equimolar amount are recommended reagents for the replacement of primary and secondary hydroxyl groups in carbohydrates with bromine [13]. As shown in Fig. 1, the reaction is considered to proceed through the formation of alkoxyphosphonium salts followed by attack of the ylide ions on to the phosphonium ester bonds [14]. After the reaction, the mixture was poured into 400 mL of acetone, dialyzed against water and dried.

After preparing bromo-hydroxyethylcellulose, the replacement of bromine with a thiol group was realised by treating the product with thiourea ($CS(NH_2)_2$), commonly employed to convert alkyl ylides to thiols [15]. In detail, the obtained product (100 mg) was dissolved in a solution of ($CS(NH_2)_2$) (40 mg) in ethanol (10 mL) [16]. The mixture was stirred for 16 h at 80 °C and 2 mL of 3 M NaOH solution was added. After stirring for 5 min at room temperature, the mixture was neutralized with 3 mL of 3 M H_2SO_4 solution. The final product was precipitated in acetone, dialyzed against water, dried at −77 °C (Virtis Bench top freeze-drier, Bartelt, Graz, Austria) and stored at 4 °C until use.

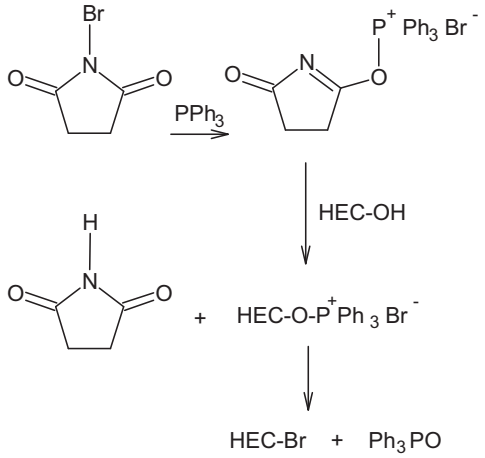

Fig. 1. Bromination of HEC.

2.3. Determination of the thiol group content

The amount of immobilised thiol groups on polymer backbone was determined spectrophotometrically with Ellman's reagent according to a method described previously [17]. Disulfide content of samples was measured after reduction with $NaBH_4$ [18].

2.4. Rheological measurements

Oscillatory shear experiments were performed on a thermostatically controlled plate–plate combination viscosimeter (Haake MARS Rheometer, 379–0200, Thermo Electron GmBH, Karlsruhe, Germany; Rotor: C35/1°, $D = 35$ mm) [19]. Preliminary strain sweep measurements were taken to determine the linear viscoelastic region for all samples and were taken at deformation range of 0.5–500 Pa and at a constant frequency of 1 Hz. HEC-SH and unmodified hydroxyethylcellulose serving as control were dissolved in phosphate buffer pH 7 in a final concentration of 1.5% (m/v). In order to determine the increase in viscosity by the formation of disulfide bonds within the thiolated polymer as a function of time, all samples were incubated at 37 °C for 30 min. The effect of oxidizing agent H_2O_2 was investigated at the final concentration of 0.01% (v/v). At predetermined time points, aliquots (1.5 mL) were transferred on the plate of the rheometer, and the samples were investigated over a 0.1- to 10-Hz frequency range at a constant temperature of 37 ± 1.0 °C. The deformation in the frequency sweep tests was kept constant at 1.0 Pa. The parameters obtained were the complex modulus G^* and the phase angle δ. The elastic modulus G', the viscous modulus G'', and the dynamic viscosity η' and loss tangent ($\tan\delta$), a parameter that represents the ratio between the viscous and elastic properties of the polymer, were also calculated.

2.5. In vitro permeation studies across freshly excised rat intestinal mucosa

For permeation studies, the lower part of small intestine of non-fasting male Sprague–Dawley rats weighing between 300 and 400 g was immediately removed after sacrificing the rats. The excised intestine was cut into strips, rinsed free of luminal contents and mounted in Ussing type chambers (0.64 cm^2 surface area) without stripping off the underlying muscle layer. The preheated transport medium containing 250 mM NaCl, 2.6 mM $MgSO_4$,

10 mM KCl, 40 mM glucose and 50 mM $NaHCO_3$ was buffered with 40 mM Bis-Tris pH 6.8 and was added to the apical and the basolateral site (1 mL). In order to ensure oxygenation and agitation, a mixture of 95% O_2 and 5% CO_2 was bubbled through each compartment. The Ussing type chambers were then placed in a water bath at 37 °C. After a 20-min equilibration period, the marker Rho-123 in a final concentration of 0.001% (m/v) was added to the apical chamber for absorptive transport (AP to BL).

The absorptive transport of Rho-123 was investigated in the absence and presence of 0.5% (m/v) thiolated polymer and 0.5% (m/v) unmodified HEC, respectively, both dissolved in the transport medium at pH 6.8. This concentration was chosen for comparison reasons with previously performed thiomers investigations. After 0, 30, 60, 90, 120, 150 and 180 min, 100 μL was taken out from the acceptor chambers and replaced by the same amount of fresh transport medium. The amount of permeated Rho-123 was determined by fluorimetric detection (Fluostar Galaxy) at 485 nm (extinction) and 520 nm (emission) and thus by interpolation from an according standard curve.

Previously performed histological investigations on rat intestinal mucosa demonstrated that after permeation studies, the mucus is still present and that the viability of the intestinal membrane is guaranteed [20].

2.6. Mucoadhesion studies on the rotating cylinder

Tablets of 30 mg weight based on unmodified hydroxyethylcellulose composition and HEC-SH 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 which had a thickness of 1.5 mm.

In order to evaluate the mucoadhesive properties of thiolated HEC and unmodified HEC, mucoadhesion studies were performed on the rotating cylinder [21]. 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 1 L of 100 mM phosphate buffer pH 6.8 at 37 ± 0.5 °C (according to the USP). The fully immersed cylinder was agitated at 100 rev./min. The detachment of the test tablets was determined visually.

2.7. Determination of the swelling behaviour

The water-absorbing capacity of HEC-SH as well as HEC tablets was determined by a gravimetric method as described previously [22]. In brief, tablets were fixed on a needle and incubated in 100 mM phosphate buffer solution pH 6.8 at 37 ± 0.5 °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 from the following equation:

$$\text{uptaken water (mg)} = \text{tablet mass } t - \text{tablet mass } t_0$$

where t represents predetermined time intervals and t_0 represents time zero

2.8. Disintegration test of HEC-SH tablets

The stability of tablets in 100 mM phosphate buffer pH 5, 6.8 and 7.2 was analysed with a disintegration test apparatus according to the European Pharmacopeia at 37 °C. The oscillating frequency was adjusted to 0.5 cycles/s (30 cycles per min, Ph. Eur.).

2.9. MTT test

To assess cell viability after incubation with HEC-SH, MTT assay was performed. This is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. MTT solution was prepared by dissolving MTT in serum-free MEM without phenol red at a concentration of 0.5 mg/mL, filtered through 0.20-μm filter and stored at 2–8 °C.

Caco-2 cells were plated into 12-well plates at a density of 5×10^4 cells/mL. After 24 h of preincubation (37 °C, 5% CO_2, 95% relative humidity), cell layers were washed with warm MEM without phenol red and then incubated with 50 μL of HEC or with HEC-SH both in MEM solution (0.5% m/v). Plates incubated with warm medium were used as control. Experiments were performed in triplicate. The plate was incubated at 37 °C, 5% CO_2, 95% relative humidity for 2 and 24 h. After having aspired the medium with test solution, cells were washed once with PBS to remove traces of the polymer. Subsequently, 1 mL MTT solution was added to each well. After 2 h of further incubation, the medium with unreacted dye was aspired. The converted dye was solubilized with 1 mL DMSO by pipetting several times. The dye solution was transferred into tubes and centrifuged at 13.000 rpm for 2 min prior to measurements of OD 570 nm with background subtraction of OD 650 nm. Cells having being incubated with unmodified HEC and with 5% (v/v) Triton X-100 served as negative and positive controls, respectively. Percentage of cell viability was calculated by comparison with 0% viability (positive control) and 100% viability (negative control) of untreated cells.

2.10. 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. Synthesis of thiolated hydroxyethylcellulose (HEC-SH)

The synthesis of thiolated hydroxyethylcellulose (HEC-SH) has been achieved via the replacement of hydroxyl groups on the carbohydrate structure by thiol moieties as illustrated in Fig. 2. This

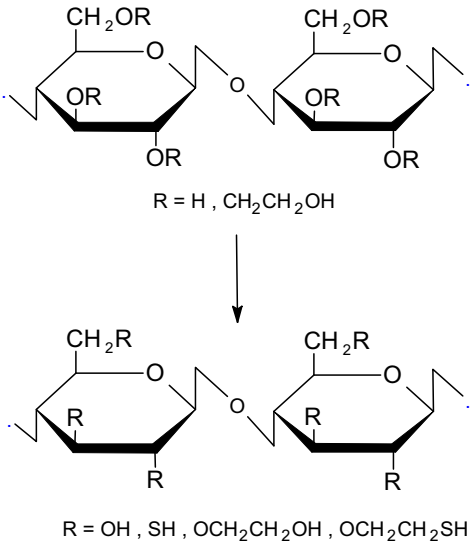

Fig. 2. Synthetic pathway of thiolated hydroxyethylcellulose (HEC-SH).

modification proceeded through the formation of the intermediate product bromo-hydroxyethylcellulose, prepared by dissolving the polymer in LiBr-DMA solvent system. In this first step of the synthetic pathway, 0.1 g of HEC was dissolved in 10 mL of this solvent system although the solution was highly viscous and tended to gelificate at room temperature due to the presence of a high concentration of LiBr. After adding reagents NBS and Ph_3P however, the mixture returned to a liquid stable state loosing its high viscosity. The product precipitated in acetone appeared as fine structured, odourless and orange-yellow, most probably because of the presence of bromine on carbohydrate backbone. The replacement of hydroxyl group by bromine is considered to take place through a nucleophilic substitution [23]. Since the primary hydroxyl groups show highest reactivity to nucleophilic substitution in comparison with secondary hydroxyl groups, such replacement was expected mainly on hydroxyl groups at terminal position of hydroxyethylenic chains. FT-IR analysis was performed with the lyophilized product, but the recognition of bromine stretching peak on the spectra was not possible, due to its interference with other signals (data not shown). The substitution of brome atoms with thiol moieties was achieved in ethanolic solution using thiourea and then confirmed by Ellman's test.

The amount of thiol groups immobilized on 1 g of hydroxyethylcellulose was quantified to be 131.58 ± 11.17 μmol, whereas the number of disulfide bonds was not significant. As already shown by our research group, thiomers are subject to oxidation of thiol groups to disulfide bonds. In case of HEC-SH, the use of Argon during the synthesis ensures the absence of oxygen and might explain the negligible disulfide bonds formation. Furthermore, as increasing concentrations of thiourea had no influence on the amount of immobilized thiol groups, it can be assumed that the thiolation reaction strictly depends on the extent of bromine attached to the carbohydrate. The content of free thiol groups is similar to the one obtained for a previously developed thiomer, polycarbophil-cysteamine (PCP-cysteamine) [24]. The lyophilized thiolated hydroxyethylcellulose (HEC-SH) appeared as pale yellow, odourless fibrous structured polymer being soluble at physiological pH. Solubility of 1% (w/v) HEC-SH was determined in water and DMA by incubating the polymer solution at room temperature for 30 min and subsequent centrifugation of samples. HEC-SH was found to be completely dissolved in DMA and highly hydrated in water. From 0.1 g of HEC, 0.064 g of HEC-SH was obtained. The amount of thiol groups for polymer repeating unit was determined to be 0.15.

3.2. Rheological studies

Rheological measurements were taken within the linear viscoelasticity region, and experimental parameters such as G', G'', η' and tan δ were measured. The viscosity of thiolated HEC gels (1.5% w/v) was determined for 6 h. Unmodified HEC served as control.

Results showed that after having added 0.01% (v/v) H_2O_2 to samples, the viscosity of unmodified HEC remained constant over a time period of 6 h, whereas it seems to increase 5.5-fold in case of the HEC-SH compared with the same solution at $t = 0.08$. As depicted in Table 1, at the end of the experiment the η' of HEC-SH with 0.01% H_2O_2 was 1306 ± 7.5 mPa s.

The phase shift can be confirmed by the loss tangent tan δ, which describes the ratio between the elastic and the viscous modulus. Values of tan δ decreasing to lower than one indicate that a sol–gel transition occurs [25]. In the case of HEC-SH, tan δ was calculated to be 0.004 ± 0.22 after 6 h. As already reported, thiomers undergo the sol–gel transition via oxidation of immobilized thiol groups to disulfide bonds [26]. The formation of inter- and/or intramolecular disulfide bonds can be indirectly quantified by the decrease in the amount of thiol moieties, which takes place at pH lower than the p*K*a value of the thiol group [1].

Table 1
Loss tangent (tan δ) and dynamic viscosity η' (mPa s) measured at a frequency of 1 Hz of 1.5% (m/v) solution of HEC-SH and unmodified HEC at pH 7 over a time period of 6 h. Indicated values are mean ($n = 3$, ±SD).

Polymer	Time (h)	tan δ	η' (mPa s)
SH-HEC	0.8	3.020 ± 0.85	235.1 ± 10.5
	1	0.020 ± 0.42	1022.3 ± 13.4
	2	0.010 ± 0.91	1168.6 ± 14.5
	4	0.008 ± 1.28	1275.0 ± 12.0
	6	0.004 ± 0.22	1306.3 ± 7.5
HEC	0		134.7 ± 6.8
	6		137.4 ± 1.5

Chitosan–TGA, for example, has shown a decrease in thiol groups content of about 90% in the presence of the oxidizing agent $NaIO_4$ [27]. The increase in the dynamic viscosity of HEC-SH observed after 5 min from the experiment might be explained by the proximity of the hydroxyethylenic chains, which lead to the oxidation of their terminal thiol groups. Further evidence that such oxidation is responsible for the gelation process is given by unmodified HEC which miss the thiol bearing side chains and therefore does not show any increase in viscosity. Within this study, moreover, the elastic modulus G' of HEC-SH gels at $t = 6$ h was found to be independence from the applied frequencies (Fig. 3) and 110-fold higher at 1-Hz frequency compared with $t = 0.08$ h. This behaviour implies the cross-linking of the gel which is typical for a system that is only physically entangled [28]. Mechanical spectra of 1.5% (m/v) HEC/H_2O_2 served as control (Fig. 4).

The described properties of the thiomer to form a gel could make it a promising tool for the development of vaginal, nasal and ocular drug delivery systems, besides its oral application.

3.3. Permeation studies

As already reported, thiolated polymers are known to exhibit a strong permeation enhancing effect for the paracellular uptake of drugs [29]. The influence of unmodified HEC as well as thiolated HEC on permeation of Rho-123 across freshly excised rat intestinal

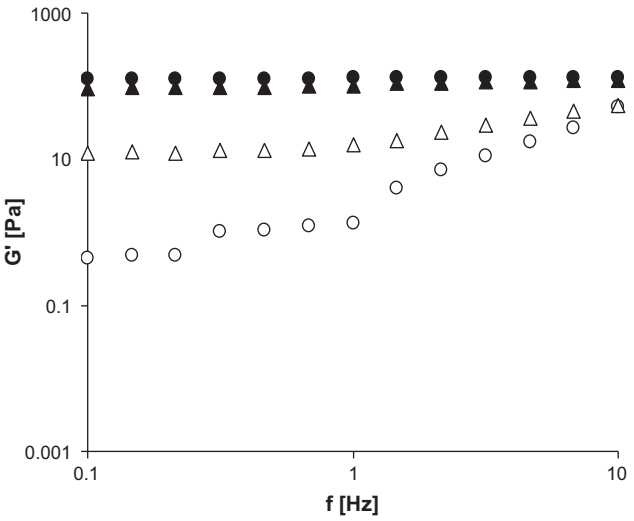

Fig. 3. Example of 'typical' mechanical spectra of 1.5% (m/v) HEC-SH/H_2O_2; elastic modulus G' after 0.08 (○), 1 (Δ), 2 (▲) and 6 (●) h of gelation. G' is plotted as a function of oscillating frequency.

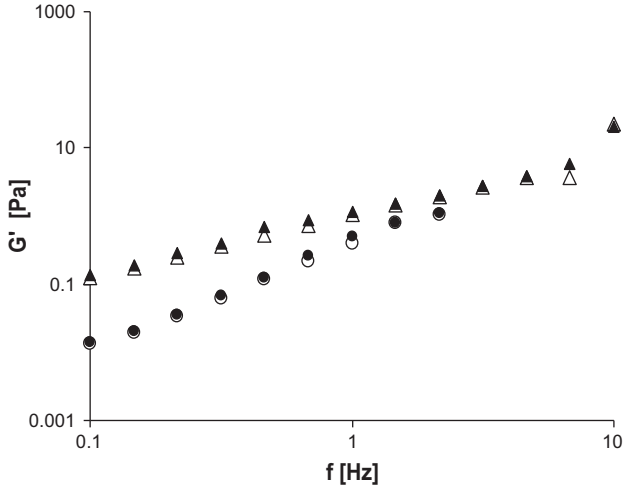

Fig. 4. Example of 'typical' mechanical spectra of 1.5% (m/v) HEC/H_2O_2; elastic modulus G' after 0.08 (○), 1 (Δ), 2 (▲) and 6 (●) h of gelation. G' is plotted as a function of oscillating frequency.

mucosa was therefore investigated in Ussing type chambers. Results of the study are illustrated in Fig. 5 and demonstrate a significant permeation enhancing effect of HEC-SH compared with control. In fact, due to the addition of 0.5% (m/v) HEC-SH, corresponding to 0.65 μM thiol groups/mL, Rho-123 uptake was 1.9-fold increased in comparison with buffer only. Such improvement in marker uptake is most probably based on an opening of tight junctions, already observed for previously investigated thiomers [20]. A covalent interaction via disulfide bonds with the enzyme tyrosine phosphatase, regulating the opening and the closing process of tight junctions, was postulated to be responsible for such permeation enhancing effect [30].

3.4. Disintegration studies

Disintegration studies were carried out with tablets of thiolated HEC and unmodified polymer. Although the mechanical stress to that formulations is exposed in the Ph. Eur.-test apparatus does 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. As shown in Fig. 6, the disintegration studies revealed a much higher stability of tablets based on thiolated polymers in comparison with the tablets based on unmodified polymer. In particular, the matrix tablets containing HEC-SH were stable for almost 2 h at pH 7.2. It was observed that the higher the pH of buffer solution, the more HEC-SH tablets were stable. The pH-dependent character of HEC-SH, in contrast to the pH independence of HEC backbone, is probably due to the presence of thiol groups whose reactivity is related to their p*K*a value and therefore to the pH of the surrounding media. Disintegration behaviour of tablets can be explained by the formation of stabilizing disulfide bonds within the polymeric network, which is favoured at higher pH values [31]. The prolonged stability of matrix tablets based on thiomers seems to be of high practical relevance compared with well-establish polymeric carrier systems. A rapid disintegration of the delivery system would lead to drug release to non-target sites. The increased cohesion is substantial, and therefore this thiomer seems to represent useful novel excipients in order to increase the stability of matrix tablets in oral drug delivering.

3.5. MTT test

Since HEC-SH is a new polymer, its possible toxic effect was investigated on Caco-2 cell monolayers. Cell lines different from the Caco-2 cells were not used because the polymer itself has a molecular weight, which makes it not absorbable by the intestinal epithelium. The viability of Caco-2 cells after 2 h of exposure to the thiomer was assessed with MTT assay quantifying the metabolic activity of mitochondria of cells. A quantitative determination of viable cells after incubation period of 2 h showed 98.6 ± 2.8% cell viability for the investigated concentration. This indicates that HEC-SH is not harmful for cells. Unmodified HEC used as negative control was found to be non-cytotoxic. The cytotoxicity of HEC-SH was determined after 24 h of incubation time as well. The decrease in the viability of cell was not dramatically altered, and 64.8 ± 5.4% of cells were viable after 24 h of the experiment. In case of HEC-SH, a wide range of concentrations was not used in cytotoxicity assay as concentrations higher than 0.5% (m/v) led to a too high viscosity of the sample. Moreover, the chosen concentration permits to compare cytotoxicity of HEC-SH to that of well-established thiomers.

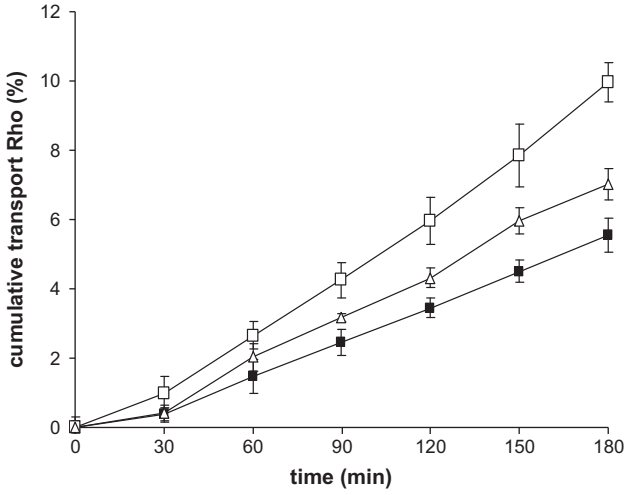

Fig. 5. Permeation studies on freshly excised rat intestinal mucosa mounted in Ussing type chambers. Effect of 0.5% (m/v) HEC (white triangles) and 0.5% (m/v) HEC-SH (white squares) in comparison with Rho-123 control (black squares). Indicated values are mean (±SD) of at least three experiments.

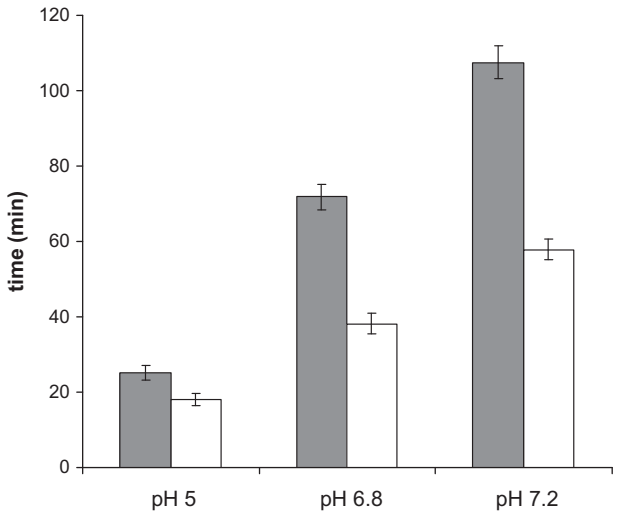

Fig. 6. Comparison of the disintegration behaviour of matrix tablets containing HEC-SH (grey bars) and unmodified polymer (white bars). Indicated values are mean (±SD) of at least three experiments.

3.6. Mucoadhesion studies

In order to investigate the mucoadhesive properties of thiolated hydroxyethylcellulose and to analyse its cohesiveness, mucoadhesion studies were performed with the dissolution apparatus according to the USP in combination with a standard steel rotating cylinder and freshly excised porcine mucosa. This test has been established being much closer to the in vivo situation then a simple tensile study otherwise used for this purpose [21]. Results are reported in Fig. 7. It was observed that tablets based on HEC-SH exhibit improved mucoadhesive properties in comparison with the corresponding unmodified HEC. In particular, a clear correlation between the presence of immobilized thiol groups and the mucoadhesiveness of the polymer was proven. In fact, tablets based on HEC-SH remained for a time of 2 h steadily attached to the mucosa, and the contact time to mucosal tissue was 4-fold increased compared with adhesion time of unmodified HEC. Furthermore, neither erosion nor disintegration could be observed for HEC-SH tablets. In contrast to so far developed thiolated polymers that show more pronounced mucoadhesive properties, HEC-SH is missing the ability to form ionic interactions with mucus and its constituents, for instance, between the polymer itself and the anionic moieties of mucins. The missing of additional ionic interactions such as for chitosan–TBA [32], which exhibit positive charges strongly interacting with negative substructures of the mucus, can explain the weaker mucoadhesion of HEC-SH compared with this thiomer.

3.7. Swelling behaviour

Since the swelling behaviour of mucoadhesive polymers has a great influence on their adhesive and cohesiveness properties [33], tablets water uptake was evaluated with thiolated and unmodified HEC. The results of swelling studies are presented in Fig. 8. The swelling behaviour observed for unmodified and HEC-SH indicates the rate at which these matrices absorb water. As shown in the graph, thiomer underwent hydration as soon as the tablets came in contact with the test medium and its hydration rapidly continued for one hour. Afterwards, water uptake reached a plateau. On the contrary, unmodified HEC showed less pronounced hydration within the first hour of immersion in the buffer followed by a steady rate of water absorption. At the end of the experiment, water uptake of tablets based on HEC-SH was 1.5-fold higher than

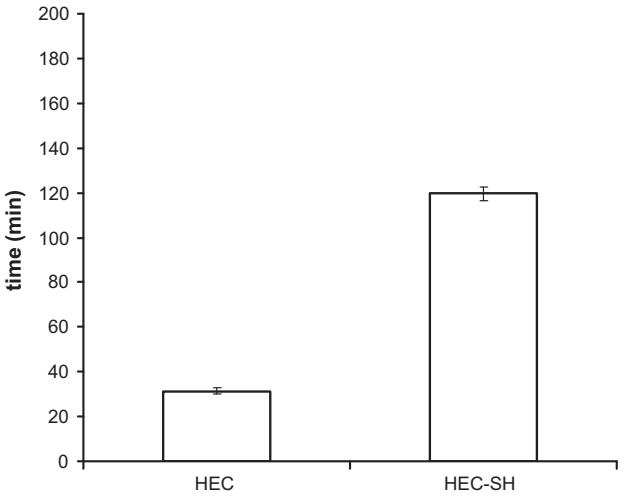

Fig. 7. Comparison of the adhesion time of HEC-SH and unmodified HEC on porcine intestinal mucosa. The indicated time of adhesion represents the mean (±SD) of at least three experiments.

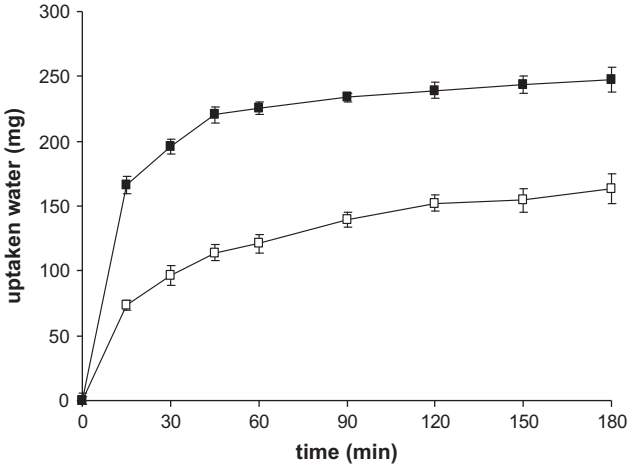

Fig. 8. Swelling behaviour of tablets based on HEC-SH (black squares) and unmodified HEC (white squares) in 100 mM phosphate buffer pH 6.8 at 37 °C; indicated values are mean (±SD) of at least three experiments.

that of control tablets. The different swelling capacities of the two polymers seem to be due to the presence of thiol groups on HEC structure, which are responsible for the formation of intrachain disulfide bonds. This modification allows the polymeric matrix to form a network which is able to successfully absorb and retain water. Furthermore, as the immobilization of thiol groups on HEC could determine an improved swelling behaviour in aqueous solutions, it might be confirmed that the observed mucoadhesive properties are related to such water-absorbing and swelling capacity.

4. Conclusions

To date, thiomers have been synthesized by chemical modification of polymers that present positively or negatively charged functional groups on their backbone. This ionic character represents a limit to the versatility of these delivery systems. Within this work therefore, a new non-ionic thiolated polymer namely thiolated hydroxyethylcellulose (HEC-SH) has been synthesized and characterized in vitro. Although free of ionic charges, the new polymer might guarantee in situ gelling properties, that is the capability to increase its viscosity once having being brought to the application site, as well as swelling and mucoadhesive properties in the range of previously investigated thiomers. Moreover, the permeation enhancing effect of HEC-SH on Rho-123 seems to be improved compared with control. However, an enhancement of the immobilized thiol groups on the carbohydrate structure would be suggested to improve the thiomer's features. Since hydroxyethylcellulose was never used as a backbone for the synthesis of a thiomer, its potential cytotoxic effect was investigated by MTT test on Caco-2 cell lines, and the result proved that HEC-SH is not harmful for cells. In conclusion, this new thiolated polysaccharide showed promising properties and combines the beneficial aspects of well-established thiomers overcoming one of their major drawbacks namely ionic charges. HEC-SH is therefore a potential candidate for the development of drug delivery systems.

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