European Journal of Pharmaceutics and Biopharmaceutics 76 (2010) 421–427

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Thiolated hydroxyethylcellulose: Synthesis and in vitro evaluation

Federica Sarti [a], Alexander Staaf [b], Duangkamon Sakloetsakun [a], Andreas Bernkop-Schnürch [a,*]

[a] Department of Pharmaceutical Technology, University of Innsbruck, Innsbruck, Austria
[b] Department of Pharmaceutical Chemistry, University of Innsbruck, Innsbruck, Austria

ARTICLE INFO

Article history:
Received 18 January 2010
Accepted in revised form 23 August 2010
Available online 15 September 2010

Keywords:
Thiomers
HEC
In situ gelling
Mucoadhesive polymer
Disulfide bonds

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.

* Corresponding author. Department of Pharmaceutical Technology, Institute of Pharmacy, University of Innsbruck, Innrain 52, Josef Möller Haus, A-6020 Innsbruck, Austria. Tel.: +43 512 507 5371; fax: +43 512 507 2933.
E-mail address: andreas.bernkop@uibk.ac.at (A. Bernkop-Schnürch).

0939-6411/$ - see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2010.08.008

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.

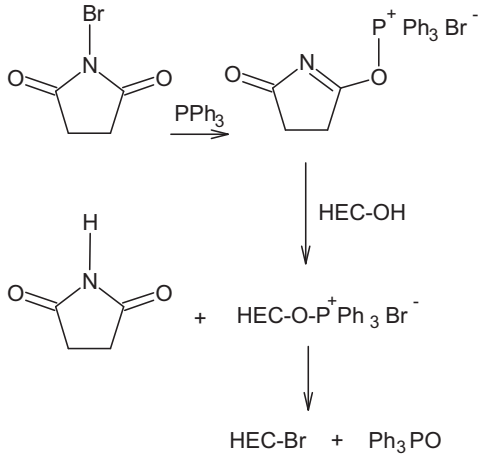

Fig. 1. Bromination of HEC.

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 $MgSO_4$,