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Polysaccharide-based nanocapsules for controlled release of indomethacin

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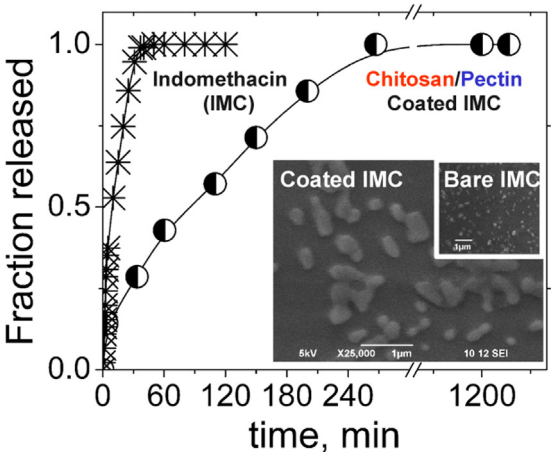
HIGHLIGHTS

- Indomethacin nanoparticles were encapsulated with chitosan and pectin.
- Polysaccharides were self-assembled through layer-by-layer procedure.
- Polysaccharide coating was used for controlled release of indomethacin.
- Coating thickness and release rate of the drug were determined.

GRAPHICAL ABSTRACT



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ABSTRACT

Indomethacin (IMC) nanoparticles are encapsulated through layer-by-layer self-assembly of natural polysaccharides chitosan (CHI) and pectin (PEC). The adsorption of both polymers is realized in aqueous solutions containing 10^{-2} M NaCl and pH 4.5, where the drug particles are practically insoluble. The stoichiometry of charges and the thickness of the polymer coating on the IMC particles, as well as the stability of their suspensions, are identified using electric light scattering. We found linear increase in the coating thickness with the number of deposited layers. Correspondingly, the electric light scattering is found to increase with increasing number of the layers, showing small excess of positive charges when the coating is terminated with the more highly charged CHI. The release rate of IMC from the nanocapsules is monitored with UV-absorbance in buffer at close to physiological pH 7, when the solubility of IMC increases several times. We found that the encapsulation of the IMC nanoparticles prolong the release time of the drug to about 5 h. The released amount of IMC is also found to depend on the concentration of NaCl in the dipping polysaccharide solutions.

1. Introduction

Design of systems for controlled drug delivery play an important role in the modern science and medicine. Controlling the deliv-

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ery rate will increase drug effectiveness and reduce adverse side effects. One specific task consists in designing stimuli-responsive nano-sized drug delivery systems, which protect their content from external influences and release the drug only in response to certain environmental conditions [1]. Layer-by-layer deposition of charged polymers on colloidal particles is now viewed as a promising candidate for producing of coatings with controlled release capability. The method allows the polymers to self-assemble on the particle surface with a coating thickness that depends on the intrinsic properties of the polyelectrolytes as well as on the experimental conditions [2].

Generally, the time for the drug release can be controlled by the capsule thickness, which is usually adjusted by changing the layer number, pH or salt concentration during the process of drug encapsulation [3]. In particular, the coatings from polyelectrolytes with pH dependent charge density are considered inherently responsive since changes in pH or salt concentration of the surrounding media influence their interactions [4]. Polysaccharides offer excellent possibility for drug coating and pH controlled release properties. These natural polymers are commonly used for applications in biomedical and environmental fields because they possess good biocompatibility, biodegradability and low toxicity [5].

Chitosan (CHI), a cationic polysaccharide derived from chitin and comprising of β-(1-4)-glucosamine and *N*-acetyl-D-glucosamine monomeric units, is one of the most studied biomaterials for pharmaceutical and biomedical applications [6]. CHI is very difficult to dissolve in water, alkaline solutions or organic solvents due to the formation of intermolecular hydrogen bonds of its molecules. However, it is soluble in dilute acid solutions, which is mainly due to the protonation of its amino groups in the aqueous acid solution (pK_a value of the glucosamine segments is 6.3–7 [7,8]). Pectin (PEC) is an anionic polysaccharide, consisting predominantly of linear chains of (1,4)-α-D-galacturonic acid residues and their methyl esters, interrupted by (1,2)-α-L-rhamnopyranosyl units [8]. It is widely used in food industry and also has potential for biomedical applications [6]. PEC is water soluble, with pK_a value of the galacturonosyl segments in the range of 3.5–4.5 [9]

CHI is soluble in acidic pH of the stomach (pH 1.4), which would make pure CHI coating lose its advantage in controlling the site and rate of release of an encapsulated drug. PEC is also unable to effectively carry encapsulated drug to pass through the stomach and small intestine due to its high solubility, which causes pure PEC formulations to dissolve quickly. However, the electrostatic cross-linking between CHI and PEC will prevent the dissolution of a CHI/PEC coating in stomach, ensuring the sustained release of the drugs reaching the colon (pH 7.4). The formation of polyelectrolyte complexes between CHI and PEC occurs most extensively in the pH range between the pK_a's of the two polymers. Besides pH, which is the most important factor affecting the formation of CHI/PEC coating and its properties, the salt concentration is also important.

Indomethacin (IMC) is a non-steroidal anti-inflammatory drug that is widely used in treatment of trauma and rheumatoid arthritis. It is an acidic hydrophobic drug (pK_a 4.5) that is poorly soluble in water and acidic pH, but is rapidly soluble in alkaline solutions. Due to poor dissolution in the stomach, IMC may show low oral bioavailability or may cause side effects because of a prolongated contact with the mucosa. Encapsulation of the IMC particles with polysaccharides provides a way to control the drug release from such formulations. Ye et al. has investigated the controlled release of IMC microcrystals (sized 5–10 μm) encapsulated with CHI and alginate [10]. They found a significant decrease of the IMC release time with increasing the deposition temperature from 20 to 60 °C. This was attributed to the increase in the coating thickness and formation of more perfect coating on the IMC microcrystals. Since assembling on nanometer templates is particularly important for in vivo applications, suspension of water-insoluble IMC nanoparticles (ca. 200 nm) has been recently produced by sonication of micron range crystals of the drug and the suspension is stabilized against aggregation by coating the IMC particles with CHI/PEC multilayer films [11]. The coating was found to reduce the release time of the drug as compared to the time for the bare IMC nanoparticles. In both studies, the layer-by-layer technique has been applied in water at pH 4, while the IMC release was studied at close to the physiological pH 7.

In the present work, we apply encapsulation of nano-sized IMC particles with CHI and PEC at pH 4.5 in the presence of 10^{-2} M NaCl. At pH 4.5, CHI is fully dissociated in a solution, whereas the PEC is dissociated ca. 80% [12]. At this pH, Coimbra et al. found the formation of CHI/PEC complexes with very similar compositions of pectin and chitosan [6]. In comparison to encapsulation in the absence of salt, the coating of IMC particles in the presence of NaCl will increase the thickness of the CHI/PEC coating after deposition of less number of layers ensuring same (or less) release rate of the drug. Encapsulation of the IMC nanoparticles in the presence of salt also helps stabilization of the suspension against aggregation at close to physiological conditions. The assembly of the polysaccharide coating was studied in situ by electric light scattering method and electrophoresis. Scanning Electron Microscopy confirmed the formation of thicker coating after deposition of only four polyelectrolyte layers as compared to the coating obtained in the absence of salt, and UV-spectroscopy showed a reduced release of IMC molecules from the CHI/PEC capsules at close to the physiological conditions.

2. Experimental

2.1. Materials

Chitosan of medium molecular weight 50–190 kDa and degree of deacetylation ≥75% was obtained from Sigma-Aldrich. Citrus pectin with molecular weight 50–100 kDa and degree of esterification 33% was obtained from Herbstreith & Fox KG. Both polymers were used without further purification. CHI solution of concentration 1 g/l was prepared by dissolution in 5×10^{-3} M hydrochloric acid solution and PEC of concentration 1 g/l was dissolved in double-distilled water. The stock polymer solutions were filtered through a 0.45 μm filter to remove possible aggregates.

Indomethacin was obtained from Sigma-Aldrich and finely ground into microcrystals with a mortar and pestle. A certain amount (0.05 g) of IMC was dispersed in 50 ml of water and ultrasonication was applied to the suspension at 0 °C (Sonopuls Ultrasonic Homogenizer HD 2200). After 30 min of sonication, the IMC suspension was centrifugated at 4500 rpm for 20 min to remove the settled (big) particles [11]. The supernatant, containing mainly small IMC particles, was diluted to concentration of ca. 0.1 g/l and its pH was adjusted to 4.5 by adding HCl. This procedure gives particles that can be approximated with elliptical disks (Fig. 1A). By electron microscopy, the average dimensions of the major and minor axes of the particles are determined to be a = 200 ± 70 nm and b = 140 ± 50 nm (axial ratio 1.4) [11].

2.2. Preparation of CHI/PEC coating on IMC nanoparticles

The ionic strength of the solutions was adjusted by means of NaCl to be 10^{-2} M (in some cases to 10^{-4} or 10^{-1} M) and pH 4.5 was maintained by the addition of HCl. The first layer was deposited by adding the IMC suspension to a solution of CHI with concentration 10^{-1} g/l and stirring for 20 min. The excess polymer was removed by centrifugation of the suspension at 13 500 rpm for 60 min. The settled particles were redispersed by sonication for 2 min in a solu-

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