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Self-assembly of a swollen chitosan/chondroitin sulfate hydrogel by outward diffusion of the chondroitin sulfate chains

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Abstract

A hydrogel constituted of chitosan (CT) and chondroitin sulfate (CS) was synthesized. In previously reported works the stochiometric ratio has been used, but in this paper an excess of CS (40% CT and 60% CS) was used because the hydrogel could be applied as a CS carrier. The hydrogel properties were investigated by differential scanning calorimetry, wide-angle X-ray scattering (WAXS), scanning electron microscopy, and high-performance liquid chromatography. Changes in the pH of the gel-surrounding liquid had a considerable effect on both the release and the molecular reorganization of CS. Furthermore, the formed hydrogel exhibited interesting parameters for use in biotechnology, such as water affinity, thermal properties and morphology upon sequential pH variation. The protonation or deprotonation of the different groups that participate in the complex formation and the coiling or uncoiling of like or unlike chains concomitant to the release of CS are believed to be the main factors affecting the hydrogel properties. CS was released mainly at pH higher than 6.5, the value of pK_{aCT}, and the released CS maximum fraction was approximately 0.5. The WAXS data demonstrated that the CT/CS complex in the hydrogel presented macromolecular reorganization at pHs ranging from 6 to 12.

Keywords: Polyelectrolyte complex; Release of chondroitin sulfate; Chain reorganization

1. Introduction

Hydrogels are three-dimensional (3-D) hydrophilic polymer networks that can absorb a large amount of water when compared to other classes of materials [1,2]. As a result, they find plenty of specific applications, ranging from hygiene products [3], to soil conditioner [4], to biomedical [5] and pharmaceutical [6] devices. Hydrogel are the most similar materials to living tissues due to their large water content. Biocompatibility is another important feature that enables their use as biomaterials. Examples of applications are contact lenses, biosensor membranes, artificial heart coating, artificial skin, and drug delivery and release devices [1].

Hydrogels can be synthesized from either natural or synthetic constituents or their combination. Often, hydrogels synthesized from natural constituents are more biocompatible than those synthesized from synthetic constituents for a number of reasons. One such reason is the requirement of cross-linking agents for the formation of a 3-D synthetic network. The use of physical hydrogels (whose chemical cross-linking agents are not a concern because the polymer chains are not covalently bonded to each other) has increased rapidly [7]. An important strategy in the production of physical hydrogels is the formation of a polyelectrolyte complex by mixing anion- and cation-charged polymers in a common solvent. The unlikely charged polymers interact by electrostatic attraction, which often produces a consistent material. Chitosan (CT) and chondroitin sulfate (CS) simultaneously mixed in appropriate conditions form physical hydrogels. Because this system has been widely investigated, different technologies have

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been developed to this end and a number of commercial products are already available.

In view of both the academic and technological importance of CT/CS hydrogels, we have added their investigation to those hydrogels already synthesized by our research group [4,8–12], aiming at their application as drug release devices. As will be shown in this paper, an excess of CS (40% CT and 60% CS) was used because CS chains are released from the CT/CS hydrogel. This aspect differs from previously published works [13,14]. As well as the CS release being an interesting phenomenon, the chain reorientation of polymers during the CS release from hydrogel, resulting in a more structured material, was a very important finding and, to best the our knowledge, has not been previously published. The chain reorganization of the CT/CS hydrogel during CS release was investigated by differential scanning calorimetry (DSC), wide-angle X-ray scattering (WAXS), scanning electron microscopy (SEM) and the hydrogel zeta potential under several pH conditions.

2. Experimental

2.1. Materials

The CT used was 15% acetylated (9012-76-4, Acros Organics, Belgium), with an M_V of 4×10^5 g mol^{-1} according to the method proposed by Mao et al. [15]. The intrinsic viscosity of chitosans in 2% HAc/0.2 mol l^{-1} NaAc was measured using an Ubbelohde capillary viscometer (Model Cannon 100/E534) at 25.0 °C. Solution concentrations were adjusted based on the viscosity of the samples. The Mark–Houwink constants $K = 1.38 \times 10^{-5}$ and $a = 0.85$ were reported for chitosan with a DD value of 85%. CS, lot B5B234-B.01, kindly supplied by Solabia (Maringá, Brazil), presented an M_V of 2.2×10^4 g mol^{-1} according to the method proposed by Wasteson [16]. Viscometry was carried out in Ubbelohde capillary viscometer (Model Cannon 100/E534) at 25.0 °C. Solutions of chondroitin sulfate in 0.2 mol l^{-1} sodium chloride, containing 1–5 mg of polysaccharide per ml, were prepared. The K value was 5.0×10^{-6} and the a value was 1.14. All reagents and products were used as received, without previous purification.

2.2. Procedures

2.2.1. CT/CS hydrogel formation

Two aqueous solutions were prepared for the hydrogel synthesis. To prepare the first solution, 1 g of CT was dissolved in 80 ml of 0.57 mol l^{-1} HCl at 65 °C under constant stirring. For the second solution, the required amount of CS was dissolved in 20 ml distilled water to conc. 25 wt./vol.%. The CS solution was then poured into the CT solution under magnetic stirring at room temperature (ca. 25 °C). The pH of the CT/CS polyelectrolyte complex during its formation was 0.50. The suspension formed was stored for 24 h for sedimentation. The CT/CS polyelectrolyte complex was labeled CT/CS hydrogel. It was separated from the supernatant and purified by immersion in 500 ml of distilled water for 24 h. After immersion, the pH of the solution was adjusted to neutral by dropping a small volume of 1.0 mol l^{-1} aqueous NaOH. An aliquot of each supernatant was stored for posterior analysis. The mechanical strength of the obtained hydrogel was qualitatively evaluated by handling and showed a good consistency. Next, the swollen hydrogel was cut into small cubes (ca. 2 cm edge) and dried at room temperature for 48 h.

2.2.2. Preparation of buffer solutions with constant ionic strength

Buffer solutions, at a concentration of 50 mmol l^{-1} and with pHs ranging from 2 to 12, and were prepared following the United States Pharmacopeia–National Formulary (USP30-NF25 [17]). The amounts of feed solutions for the preparation of each buffer solution used in this work are described in Table 1.

2.2.3. Characterization of the CT/CS hydrogel in liquid medium state

2.2.3.1. Measurement of the degree of swelling. The degree of swelling was measured by using cube-shaped CT/CS hydrogel specimens. After drying at room temperature, the samples were weighed and soaked in 25 ml of the pH 2, 6 and 8 buffer solutions at constant ionic strength (Table 1) and 37 °C. The swollen hydrogel was weighed after the swelling equilibrium had been reached. The degree of swelling was calculated through the mass ratio of the swollen hydrogel at time t, m_t, to the dry hydrogel.

2.2.3.2. In vitro release of CS from the CT/CS hydrogel. The dry CT/CS hydrogel cubes were weighed and stored in 25 ml of the pH 2, 6 and 8 buffer solutions at constant ionic strength and 37 °C. Supernatant aliquots were collected at the desired immersion times. The amount of CS released was quantified in Star chromatographic workstation (Varian Inc. Scientific Instruments, USA) with a spectrophotometer detector based on model ProStar 350 diode array and operated by PolyView software and a PolySep-GFC-P 6000 chromatographic column, 300 × 7.8 mm (Phenomenex). Buffer solutions at pHs 2 (HCl/KCl), 6 and 8 (KH_2PO_4/NaOH) with a concentration of 50 mmol l^{-1} at a constant ionic strength of 0.1 mol l^{-1}, prepared with the quantities described in Table 1, were used as a mobile phase flowing at 0.5 ml min^{-1}. The eluted solution was monitored at $\lambda = 210$ nm, the reported CS maximum ultraviolet absorbance.

To quantify the amounts of CT and CS in the hydrogel, a known volume of supernatant was collected after the hydrogel had been precipitated and after adjusting the pH to 2. Furthermore, desired volumes of the supernatant were collected during hydrogel purification. All the collected aliquots were analyzed by high-performance liquid chromatography (HPLC). The amounts of both CS and CT incorporated into the hydrogel were calculated by com-

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