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Preparation and characterization of *N*-(2-carboxybenzyl)chitosan as a potential pH-sensitive hydrogel for drug delivery

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Abstract—A novel water-soluble chitosan derivative [*N*-(2-carboxybenzyl)chitosan, CBCS] was synthesized. The chemical structure of CBCS was characterized by FTIR, ^{1}H NMR and UV spectroscopies. The degree of substitution (DS) of *N*-2-carboxybenzyl was determined by colloid titration. In different pH buffer solutions, the swelling characteristics of hydrogels based on CBCS (CBCSG) prepared by crosslinking with glutaraldehyde have been studied. Results showed that the swelling ratio (SR) of CBCSG decreased with an increase of the amount of glutaraldehyde, and that CBCSG swelled more significantly in alkaline solution than in acidic medium, showing the lowest SR at pH 5.0. The SR of CBCSG increased with the raising of the DS of the *N*-2-carboxybenzyl group in alkaline solution, but no significant change was observed in an acidic environment. CBCSG showed swelling reversibility when alternately soaked in pH 1.0 and 7.4 buffer solutions. Release profiles of fluorouracil (5-FU), a poorly water-soluble drug, from CBCSG were studied under both simulated gastric and intestinal pH conditions. The release was much quicker in pH 7.4 buffer than in pH 1.0 solution. Results indicated that CBCS could be a potential pH-sensitive carrier for colon-specific drug delivery system.

Keywords: Chitosan derivative; pH-Sensitive hydrogel; Fluorouracil; Drug delivery system

1. Introduction

Hydrogels, which are three-dimensional networks composed of a polymer backbone, water and crosslinking agents, can swell considerably in aqueous medium without dissolution. Hydrogels are gaining special interest as substances that exhibit phase transition (i.e., volume change) in response to changes in external conditions such as pH,[1] ionic strength[2] and temperature,[3] all of which are widely encountered in drug delivery systems.[4–6] In the design of oral colon-specific drug delivery system, pH-sensitive hydrogels have attracted increasing attention recently because they are useful techniques for colon-specific diseases by pH control based on pH change in human gastrointestinal tract.[5] Such drug delivery system could allow local treatment of a variety of colonic diseases such as Crohn's disease or ulcerative colitis. It also means that peptides and certain other labile drugs might be orally administrable if they were not released in the upper regions of the gastrointestinal tract.[6,7]

A variety of synthetic or natural polymers containing the weakly acidic or weakly basic groups have been employed as the pH-sensitive controlled release systems for drug delivery.[8] Many polysaccharides have been tried as colon-specific delivery systems, such as alginates,[9] pectins,[10] amylose,[11] chitosan,[12,13] and their complexes.[14] Among them, chitosan is one of the most commonly used.

Chitosan (CS) [poly-β-(1→4)-D-glucosamine], a natural polymer obtained by alkaline deacetylation of chitin, is nontoxic and biocompatible and can be completely digested by the colonic bacteria. These properties make chitosan a good candidate for the development of novel gastrointestinal drug delivery systems. Chitosan dissolves in acidic solutions since it has a number of amino groups, but it is insoluble under higher pH conditions due to the deprotonation of amines.[15] Chitosan is also of limited solubility in organic solvents. In order to

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overcome the above problems, some novel polyampholyte derivatives based on chitosan were prepared by introducing various groups to study their pH-sensitivity, tried as pH-sensitive carrier for colonic drug delivery system, for example, carboxymethyl chitosan,[12] N-alkylated chitosan[16] and maleilated chitosan.[17]

In this paper, the water-soluble derivative of chitosan, *N*-(2-carboxybenzyl)chitosan (CBCS) is synthesized and evaluated. The chemical structure of CBCS was characterized by FTIR, ^{1}H NMR and UV spectra. The DS of *N*-2-carboxybenzyl was determined by colloid titration. When introducing 2-carboxybenzyl groups onto the $-NH_2$ groups of chitosan, an amphoteric polyelectrolyte containing both cationic and anionic fixed charges was prepared. By varying the DS of the 2-carboxybenzyl group, we can obtain CBCS with various charge densities on the molecular chain, which provide a convenient way to investigate the swelling behavior of these polyampholyte hydrogels dependent on pH. Thus the pH sensitivity of CBCSGs based on CBCS with various DS values crosslinked by glutaraldehyde has been studied in this work. Additionally, the release profiles of a model drug (5-FU) from test hydrogels were studied in simulated gastric and intestinal media, respectively.

2. Experimental

2.1. Materials

Chitosan with a degree of deacetylation of 92.8% was supplied by Dalian Xindie Chitin Co., Ltd (China). 2-Carboxybenzaldehyde was purchased from Tokyo Kasei Kogyo Co., Ltd (Japan). Glutaraldehyde (25% aq solution) and sodium borohydride were from Shanghai Chemical Company, China. Potassium polyvinyl sulfate (PVSK), poly(diallyldimethylammonium) chloride (PDADMAC) and Toluidine Blue were of analytical grade for colloidal titration obtained from Wako Pure Chemical Industries, Ltd (Japan). 5-Fluorouracil (5-FU) was purchased from Sinopharm Chemical Reagent Co., Ltd (China). All other chemicals and reagents used were of analytical grade, and were used without further purification.

2.2. Synthesis of CBCS

Synthesis of CBCS was carried out as shown in Figure 1. Table 1 lists the ratios of reactants and conditions used for preparing each of the CBCS sample. The method given below is for CBCS5. Chitosan powder (2.0 g) was dissolved in 0.7% (w/v) aq HOAc (250 mL), to which a solution of 2-carboxybenzaldehyde (3.6 g) in abs EtOH (20 mL) was added dropwise over a 30-min period. After stirring at 50 °C in a water bath for 5 h, the mixture was cooled down to room temperature, and then an aq solution of $NaBH_4$ (1.2 g, 15 mL) was added dropwise to bring about reduction of the Schiff base. Stirring was continued at room temperature for 2 h. An adequate amount of MeOH was added into the reaction mixture, and the precipitate thus produced was filtered and washed with acetone. The solid obtained was soaked in acetone (100 mL) for 24 h, then filtered to obtain a pale-yellow product.

The partially derivatized product was dialyzed against deionized water for 3 days and precipitated by adding an adequate amount of acetone to obtain the purified CBCS, which was then dried under vacuum to constant weight for structure characterization.

2.3. Degree of substitution of *N*-2-carboxybenzyl groups

The DS of *N*-2-carboxybenzyl group was determined by colloid titration according to the method reported.[18]

A 0.02% (w/v) CBCS solution was prepared by dissolving appropriate amount of CBCS in 100 mL of distilled water. The CBCS solution (10 mL) was transferred into a conical flask, and 1 mL of 0.1 M NaOH was added to make the medium alkaline. PDADMAC standard solution (0.5 mM, 10 mL) was then added into the flask. To determine the titer V_1 (mL) of PVSK, the mixture was shaken for 2 min with one drop of 0.1% Toluidine Blue as an indicator, and 0.5 mM PVSK standard solution was added titrimetrically from a burette until the blue color of the indicator changed into reddish purple. At the same time flocculation or precipitation of the reacting complexes appeared abruptly. As a blank titration, 10 mL of distilled water was used in place of the CBCS solution. The other procedures were the same as the above, and the titer V_2 (mL) of PVSK was recorded. All the titration experiments were performed three times to calculate average values. The DS was expressed by the following equation: DS (mmol/g) $= c \cdot (V_2 - V_1)/100g$, where c is the concentration of the PVSK standard solution (mmol/L), and g is the quantity of CBCS sample (g).

2.4. Preparation of CBCSG and chitosan hydrogels (CSG)

CBCS solution (1.2%, w/v) was prepared in distilled water (15 mL). In the presence of different volume of crosslinking agent 1% (v/v) glutaraldehyde, the bubble-free solutions were poured into shallow dishes (with a diameter of 7.5 cm) with stirring for 2 min at room temperature and let to stand overnight for gel formation. The hydrogels (CBCSG) were rapidly frozen at −18 °C and then dried in a freeze-dryer (FD-1 freeze mobile, Boyikang Tech. Co., Beijing, China) to yield the xerogels.

Chitosan solution (1.2%, w/v) was prepared in 0.7% (w/v) aq HOAc (15 mL). Freeze-dried CSG were pre-