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Development of carboxymethyl chitosan hydrogel beads in alcohol-aqueous binary solvent for nutrient delivery applications

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ABSTRACT

Carboxymethyl chitosan (CMCS) hydrogel beads were normally prepared from a composite formulation with other polymers, such as alginate. In present study, a novel method was developed to prepare CMCS hydrogel beads in alcohol-aqueous binary solvents. The morphology and shape of the beads were highly dependent on alcohol concentration. The most spherical hydrogel beads were obtained with 3% calcium and 30% alcohol concentration. The chemical crosslinking agent, glutaraldehyde, was needed to maintain the hydrogel integrity and morphology upon drying. Vitamin D3, a model nutrient, was encapsulated into the beads and 96.9% encapsulation efficiency was obtained. The effects of freeze-drying and room temperature drying were studied on the swelling behaviors and release properties in simulated gastrointestinal conditions. Possible mechanisms for CMCS hydrogel beads formation in binary solvents were discussed. The CMCS hydrogel beads prepared in 30% alcohol-aqueous binary solvent may be a promising delivery system for hydrophobic nutrients or drugs.

1. Introduction

Hydrogels are the networks of hydrophilic polymeric chains that can hold a significant amount of water, from 10% to thousand times of their dry weight (Hoffman, 2002). Hydrogel may be physically crosslinked between polymeric polyelectrolytes and multivalent ions or chemically crosslinked by crosslinking agents, such as glutaraldehyde. Hydrogels fabricated from natural polymers are the biomaterials of particular interest in food science and biomedical applications, such as drug and nutrient carriers, encapsulation of cells, and tissue engineering matrices.

Chitosan (CS) is the *N*-deacetylated product of chitin, a natural biopolymer from exoskeleton of crustacean, insects, and fungi. CS and its derivatives have been extensively studied for their drug delivery applications in various forms (Dash, Chiellini, Ottenbrite, & Chiellini, 2011), including nano-/microparticles (Dass, Contreras, Dunstan, & Choong, 2007; Luo, Zhang, Cheng, & Wang, 2010), nanofibers (Uygun, Kiristi, Oksuz, Manolache, & Ulusoy, 2011), films (Kithva, Grondahl, Martin, & Trau, 2010), membranes (Liu et al., 2012), and hydrogel beads (Torelli-Souza, Cavalcante Bastos, Nunes, Camara, & Amorim, 2012). The major concern of its applications is the solubility problem that CS only dissolves in acidic medium with pH lower than 6. CS hydrogel beads have been prepared by electrostatic crosslinking between CS and tripolyphosphate (TPP). The challenge of CS/TPP hydrogel beads for oral drug delivery is that it may dissociate quickly under gastric conditions with low pH, therefore chemically crosslinking and/or secondary coating were generally applied to achieve controlled release of encapsulated drugs (Durkut, Elçin, & Elçin, 2006; Jain, Jain, Gupta, & Ahirwar, 2007).

Carboxymethyl chitosan (CMCS) is one of the most investigated water-soluble derivatives of CS for biomedical applications (Mouryaa, Inamdara, & Tiwari, 2010). The drug delivery systems prepared from CMCS-based formulations have received increasing attention in recent years (Chen, Tian, & Du, 2004; El-Sherbiny, 2010; Luo, Teng, & Wang, 2012; Snima, Jayakumar, Unnikrishnan, Nair, & Lakshmanan, 2012). Because of the carboxymethylation, CMCS possesses negative charges when dissolved in water, the CMCS hydrogels are generally prepared by physically crosslinking with calcium and/or polyelectrolyte biopolymers, or by chemically crosslinked with chemical agents. CMCS hydrogels were generally formed through cylindrical mold (Chen, Wu, et al., 2004) or cast drying (Chen et al., 2004; Guo & Gao, 2007), in the form of a piece of gel. However, preparation and application of CMCS hydrogel in the form of beads are rarely reported. Unlike hydrogel formed in certain mold, the hydrogel beads are normally formed simultaneously via crosslinking between biopolymer molecules and crosslinker agents, no further cutting or shaping procedure is required. The

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chain rigidity and inefficient chain entanglement of CMCS in aqueous solution have been reported during the preparation of CMCS nano fiber (Du & Hsieh, 2008). A study on the CMCS-alginate hydrogel beads pointed out that the beads were formed when CMCS was crosslinked with calcium in the presence of alginate, otherwise an irregular shape of gel would form (Lin, Liang, Chung, Chen, & Sung, 2005). Although the CMCS-calcium hydrogel beads can be prepared when the CMCS with low molecular weight (2.5×10^4 Da) was used, the loading capacity and encapsulation efficiency of these beads were low unless they were further coated with CS (Liu, Jiao, & Zhang, 2007). This report indicated that CMCS with low molecular weight has a shorter chain and less charged groups, which may be easier to be entangled.

The objective of the present study is to develop a novel method to prepare hydrogel beads using medium molecular weight CMCS. The alcohol-aqueous binary solvents were chosen in order to reduce the chain rigidity and increase the chain entanglement of CMCS. The shape of the beads was further fixed by adding glutaraldehyde as a chemical crosslinking agent. Possible mechanisms of the formation of CMCS hydrogel beads in alcohol-aqueous binary solvents were also discussed. The application of prepared CMCS hydrogel beads as an encapsulant was explored for Vitamin D3 (VD3) and release properties were characterized.

2. Materials and methods

2.1. Materials

CS with deacetylation degree of 77% with medium molecular weight was purchased from Sigma–Aldrich. Calcium chloride, 25% Glutaraldehyde (GA), VD3, and other reagents were of analytical grade and purchased from Sigma–Aldrich.

2.2. Preparation of CMCS from CS

CMCS was prepared according to a previously published literature (Chen & Park, 2003). Sodium hydroxide (27.2 g) was first dissolved in 40 ml water, and to which 160 ml of isopropanol was added. Then, 20 g CS was dispersed into the solution and kept at 50 °C for 1 h under mild stirring. After that, 30 g of monochloroacetic acid dissolved in 40 ml of isopropanol was added to the mixture dropwise and reacted at 50 °C for 4 h. The reaction solution was then filtered, and washed with 80% alcohol until the filtrate solution was neutral. The resulting solid was dried overnight in an oven at 60 °C to obtain CMCS.

2.3. Characterization of prepared CMCS

The molecular weight of CMCS was determined by viscosity measurement (Brookfield) and calculated according to classic Mark–Houwink equation $[\eta] = 7.92 \times 10^{-5}$ M (Sun, Zhou, Mao, & Zhu, 2007). The degree substitution (DS) was determined by potentiometric titration (Vaghani, Patel, & Satish, 2012). Briefly, 0.1 g of CMCS was dissolved in 100 ml of 0.05 M HCl with mild stirring and the pH was adjusted to 2.0 by 0.1 M NaOH. The CMCS solution was then titrated with 0.1 M NaOH by 0.5 ml increment up to 26 ml, until the final pH of CMCS solution reached 12.0. The conductivity was monitored by using a Conductivity Meter (AP85 Series, Accumet). The titration curve was plotted to calculate DS shown in Eq. (1) as follows. The chemical structures of CS and CMCS were monitored by Fourier transform infrared spectroscopy (FT-IR, Jasco 4100 series with an attenuated total reflection cell) (Jasco Inc., Easton, MO, USA). Dried powder of sample was placed onto ATR crystal directly. The spectra were acquired at 750–4000 cm^{-1} wave numbers with a 4 cm^{-1} resolution.

$$DS = \frac{(V_2 - V_1)}{(V_3 - V_2)} \times DD \quad (1)$$

where DS is the degree of substitution of CMCS and DD is the degree of deacetylation of original CS. V_1, V_2, V_3 were the consumed volume of NaOH in different linear section of the titration curve, as designated at Fig. 2.

2.4. Zeta potential of CMCS solution in binary system

The zeta potential of CMCS in different binary solution was also investigated. CMCS (3%) was dissolved in distilled water as stock solution. Then, 200 μl of CMCS stock solution was diluted by 1.8 ml of water and alcohol to obtain different CMCS binary solutions, ranging from 10% to 90% alcohol concentrations. The freshly prepared samples were subjected to zeta potential measurement using a laser Doppler velocimetry (Zetasizer Nano ZS90, Malvern, U.K.). All measurements were performed in triplicate.

2.5. Preparation of crosslinked CMCS hydrogel beads

The CMCS hydrogel beads in this study were prepared by a reported method (Lin et al., 2005) with modifications. The 3% calcium solution was dissolved in different alcohol-aqueous binary solutions, with alcohol concentration ranging from 0% to 90%. Then, 2 ml of 3% CMCS solution was added dropwise into 10 ml of the above calcium binary solution through a pipette tip (1000 μl) with gently stirring. Hydrogel beads were formed instantaneously. The beads were cured in binary solution for 1 h, followed by the addition of 20 μl of 25% glutaraldehyde. The final concentration of glutaraldehyde in crosslinking solution was 0.05%, and the beads were crosslinked for another 1 h. The formed beads were then rinsed with 10 ml distilled water four times to remove unreacted calcium chloride and glutaraldehyde, and subsequently dried under room temperature for 48 h. The freeze-drying method was also studied to explore the effect of drying process on prepared CMCS hydrogel beads. Briefly, the freshly prepared beads were collected and pre-dried under −80 °C overnight and then freeze-dried (Labconco) for 24 h.

2.6. Morphological observation

The surface morphology of prepared hydrogel beads were examined by a scanning electron microscopy (SEM, Hitachi SU-70, Pleasanton, CA, USA). Samples were dried and mounted on metal stub and then coated with gold using gold sputter coater machine

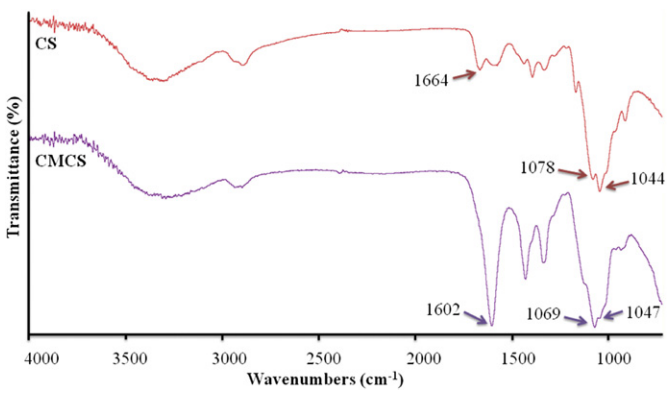

Fig. 1. FT-IR spectra of CS and CMCS.