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TEMPO-oxidized Konjac glucomannan as appliance for the preparation of hard capsules

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ABSTRACT

TEMPO-oxidized Konjac glucomannan (OKGM) was developed as new material for preparing vegetarian hard capsules. OKGM of different degrees of oxidation: DO30%, DO50%, and DO80% were prepared to select optimum DO for capsule formation. FT-IR results proved that the primary alcohol groups on KGM were oxidized into carboxyl groups. XRD analysis suggested that TEMPO-oxidation decreased the crystallinity of KGM. DO80% was considered as the optimum candidate for capsule preparation owing to its superior solubility, transparency and reduced viscosity. The hydrophilicity of OKGM films, measured by contact angle measurement, increased with increasing DO. The elongation at break and tensile strength of the OKGM films enhanced with increasing DO. *In vitro* drug dissolution profile of OKGM capsules showed that the shell rupture time of DO80% capsule is about 5–10 min, and 80% of the drugs were released within 30–45 min. Thus DO80% OKGM was qualified to be used for gastric soluble hard capsules.

1. Introduction

Hard capsule as an important pharmaceutical excipient has many advantages such as easy swelling, taste masking and protecting drugs. Gelatin is the most commonly used food-based material for preparing hard capsules. The most important property of gelatin to make hard capsules is the reversible gel formation by hydrogen bonding at low temperature. The gel is dissolved by disrupting the hydrogen bonds above 35 °C (Tuleu et al., 2007). Because of this unique property, they can readily dissolve in biological fluids at body temperature. The liquid–gel transition is also convenient for the capsule manufacturing process. Moreover, gelatin has good film-forming properties, and it is surface active. All these advantages make gelatin an irreplaceable candidate for hard capsule preparation (Ku et al., 2010). However, gelatin capsules may become brittle after exposure to low humidity, and turn soft when stored at high temperatures (Chang, Raghavan, & Hussain, 1998). Besides, the amine groups of gelatin may react with drugs containing aldehyde groups (Ofner, Zhang, Jobeck, & Bowman, 2001). Moreover, as an animal-derived ingredient, the mad cow disease in the 1990s triggered scrutinizing the use of gelatin in pharmaceutical products. Religious, cultural and personal issues may affect patients' preference towards gelatin present in such products. So there is an urgent demand on developing plant-derived materials for preparing hard capsules.

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Hydroxypropyl methylcellulose (HPMC), a plant-derived material, has already been used to replace gelatin in hard capsules (Li, Martini, Ford, & Roberts, 2005). HPMC capsules have a low moisture content (4–6%) and may not become brittle when exposed to low humidity. HPMC capsules are chemically stable and do not interact with drugs (Jones, Basit, & Tuleu, 2012). Unlike a cold-set gel formed by gelatin, HPMC forms a gel by hydrophobic interaction at elevated temperatures. However, it was found that the HPMC capsules have a large gap (132.14 μm) between the body and cap. It was twice bigger than the gap of a gelatin capsule (66.86 μm). The large gap of the HPMC capsule may cause leakage of the encapsulated drug, leading to quality and safety problems (Ku et al., 2010). Starch polymers, the most abundant plant source in nature, were also utilized as capsule materials. Starch polymers have advantages such as low cost and good film-forming properties. However, starch cannot form a gel by itself. Hence, starch capsules are usually produced by blending starch with other gelling agents. Zhang et al. (2013a) reported preparation of starch hard capsules by blending hydroxypropyl starch with hydroxypropyl methylcellulose and carrageenan, Bae, Cha, Whiteside, and Park (2008) developed a starch capsule by adding κ-carrageenan and ι-carrageenan to promote gel formation. In addition, elongation at break is for a starch film smaller than for a gelatin film, implying that a starch hard capsule is more brittle than a gelatin capsule (Zhang et al., 2013b). Consequently, starch is mainly used for making soft capsules by extrusion together with large amounts of plasticizer and gelling agents (Zhang et al., 2013a). In some respects, it seems that the properties of vegetarian hard capsules based on HPMC and starch are inferior to those of gelatin capsules. Therefore, other vegetable sources for manufacturing hard capsules need to be explored.

Konjac glucomannan (KGM) is a natural polysaccharide stored in tubers of *Amorphophallus Konjac*. KGM is composed of β-D-glucose and β-D-mannose through β-1,4- or β-1,3-glycosidic linkages. A small portion of acetyl substituent is present at the C-6 position of the sugar residues (Zhang, Xie, & Gan, 2005). KGM is widely grown in Asia as a food source. It also has been used for weight control and intestinal health purposes (Chua, Baldwin, Hocking, & Chan, 2010). Recently, KGM has drawn much attention for being used in target delivery systems for controlled release of drugs (Alvarez-Mancenido et al., 2006; Rana et al., 2011; Wang & He, 2002). For example, KGM was used to prepare pH-sensitive (Du, Dai, Liu, & Dankovich, 2006) and light-responsive (Chen et al., 2014) microspheres for controlled release of drugs. Because KGM can be digested and fermented by colonic bacteria, it has been developed into a colon-specific carrier (Chen, Liu, & Zhuo, 2005) enabling sustained release of the drug. However, KGM has a high molecular weight (500–2000 kDa) and the maximum solubility in water is 1%, yielding a solution of high viscosity. These properties are not favorable for preparing hard capsules. By means of oxidation, the chemical structure of KGM can be optimized to increase the solubility and hydrophilicity, making it more suitable to manufacture hard capsules.

It has been well-established that nitroxyl radicals such as 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) can be applied as a mediator for efficient oxidation of natural polysaccharides (Denooy, Besemer, & Vanbekkum, 1994). TEMPO oxidation has a high selectivity for primary alcohol groups on C-6 position of sugar units, and the degree of oxidation (DO) can be accurately controlled (Li et al., 2010a). After TEMPO oxidation, the primary alcohol groups on KGM are converted into carboxyl groups, leading to an increased hydrophilicity and a decreased viscosity. In this way, the physical-chemical properties of oxidized KGM (OKGM) can be tuned to facilitate its application for capsule fabrication. We have discovered that OKGM can be dissolved in sufficiently high concentration and form a gel in a capsule mold by blending with a certain amount of carrageenan. Hard capsules can be prepared after the cooling and drying steps. Preliminary experiments showed that OKGM capsules have great potential as stomach-soluble capsules. Thus, OKGM polymers offer a new option of being suitable raw materials for the preparation of plant-derived hard capsules.

In this study, hard capsules made of OKGM polymers were developed. First, OKGM having different degrees of oxidation, *i.e.*, 30%, 50% and 80% (denoted DO30%, DO50%, and DO80%) were prepared to select the optimum DO for hard capsule formation. Then, the molecular weight, surface morphology, chemical structure and degree of crystallization of the various OKGM polymers were characterized. Next, the OKGM polymers were casted into films to determine their water wettability by contact angle measurement. Mechanical properties, *i.e.*, elongation at break and tensile strength, were compared between the different OKGM films. Finally, OKGM hard capsules were prepared. Erythromycin stearate was chosen as a model drug to monitor the *in vitro* dissolution of OKGM capsules. The results show the potentiality of OKGM for the preparation of hard capsules.

2. Materials and methods

2.1. Materials

Konjac glucomannan (purity 98%) was kindly offered by Yizhimoyu Co., Ltd (Hubei Province, China). β-Mannanase was purchased from Novozymes (Beijing, China). The enzymatic activity was 20 IU/mg. The oxidation catalyst 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) was purchased from Sigma-Aldrich, USA. Erythromycin stearate was purchased from Huagang Pharmaceutical Co., Ltd (Jilin Province, China). Carrageenan was purchased from Lvqi Food Colloid Co., Ltd (Fujian Province, China). All other chemicals were of analytical grade. Purified Milli-Q water was used throughout.

2.2. Preparation of TEMPO-oxidized Konjac glucomannan

Prior to TEMPO oxidation, Konjac glucomannan (KGM) polymers were partially hydrolyzed by β-mannanase to reduce the viscosity of their solutions. 1 mg β-mannanase was added into 4 L 8.1 g/L KGM solution. The enzymatic reaction was carried out at 50 °C for 10 min. The enzyme was inactivated by boiling in water bath for 15 min. After inactivation of the enzyme, the enzymatic hydrolyzed KGM (EKGM) was subjected to TEMPO-mediated oxidation, following the procedure developed by De nooy et al. (1994) and described in our previous work on TEMPO oxidation of starch (Li et al., 2010b). TEMPO-mediated oxidation resulted in OKGM that is selectively oxidized by sodium hypochlorite at the 6-position of the glucose or mannose units obtaining 95% selectivity at complete conversion of the primary alcohol groups. In this way, oxidized KGM (OKGM) of DO30%, DO50%, and DO80% were prepared. The DO was controlled by the amount of sodium hypochlorite added during oxidation. Oxidation was performed at a constant pH (pH 10.0) using 2.0 M NaOH in a pH-stat titrator.

2.3. Preparation of OKGM films

1% (w/v) KGM and OKGM (DO30%, DO50%, DO80%) polymer solution were prepared in distilled water. Solutions were then stirred for 4 h at room temperature. Glycerol (0.25% w/v) was added into OKGM and KGM solution to increase the mechanical strength of the films. 120 mL of OKGM and KGM solution was poured into a polyethylene film casting plate (120 mm × 80 mm) and air bubbles were removed by ultrasonication. The films were dried overnight at 60 °C in an oven. The dried films were peeled off from the plate and pre-conditioned for 48 h in a chamber of constant temperature (25 °C) and humidity (50% RH) to uniformized the moisture content