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Konjac glucomannan microspheres for low-cost desalting of protein solution

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ABSTRACT

In this study, low-cost konjac glucomannan (KGM) microspheres used for desalting were developed by an inverse dispersion method. High concentration of KGM was pretreated to reduce viscosity by acid hydrolysis method under the condition of high temperature and pressure. The selectivity of the obtained cross-linked KGM gels with different degree of crosslinking was studied by constructing calibration curves (K_{av}) of standard molecular weight substances. The stability of the gels was investigated, which showed that the KGM microspheres are tolerant to a wide range of pH and stable in all commonly used aqueous buffers, and insensitive to autoclaving as well. Furthermore, protein-desalting performances of GM-1250, a cross-linked KGM microsphere, were evaluated with two proteins, bovine serum albumin and filamentous hemagglutinin, which turned out that GM-1250 is comparable to the widely used commercial product – Sephadex G25 Fine. From economic considerations, KGM gel is a prospective alternative for dextran gels in protein desalting process.

1. Introduction

Konjac glucomannan (KGM) is the main component of konjac which has long been used in China, Japan, and South East Asia as a food source and a traditional medicine (Chua, Baldwin, Hocking, & Chan, 2010). Recently KGM has been marketed for its potential use in the treatment of obesity (Kraemer et al., 2007), obesity-related dyslipidemia (Vasques et al., 2008), and diabetes (Vuksan et al., 2000, 2001) since it is a good dietary fiber. KGM has also shown promise in controlled drug delivery systems (Chen, Liu, & Zhuo, 2005; Du et al., 2004; Yu & Mao, 2008), plasma substitutes (Li et al., 2010), and in the potential of antimicrobial materials (Xu et al., 2008). These studies have proved pivotal to the increasing demand for KGM, hence prompting studies on industrial applications should be conducted in order to increase its market potential.

As one kind of polysaccharides, KGM has good hydrophilicity and biocompatibility. It is composed of a linear chain of β-1,4-linked D-glucose and D-mannose residues in a molar ratio of 1:1.6, lightly branched through β-1,6-glucosyl units (Maeda, Shimahara, & Sugiyama, 1980; Shimahara, Suzuki, Sugiyama, & Nisizawa, 1975). This structure is very similar to dextran which has been widely used as separation media (Stone & Carta, 2007; Tao & Carta, 2008; Thommes, 1999) especially for desalting and small molecules, but the price of KGM is much lower than that of dextran (about 10%). According to its structure and economic considerations, KGM microspheres may also be a good choice for bio-separation.

A patent on KGM chromatography media appeared in 1987 in Japan. The starting material was an ester KGM dissolved in a solvent, which was then suspended in an aqueous solution. By evaporation of the solvent from the droplets, solidified particles were formed (Yoshiaki, 1987). In 1993, another patent application was filed describing a kind of cross-linked KGM particles which could be used for biomacromolecule separation (Xiao & Wang, 1993). However, few reports are available on solving the problem of low solubility and on the preparation of microspheres for desalting.

Protein desalting is the process of removing dissolved salts and minerals from aqueous solution. The main technologies used in desalting are dialysis, diafiltration, and gel filtration. Gel filtration provides certain advantages over dialysis (Shimoni, Reuveni, & Cais, 1993). In fact, dialysis is generally a slow technique that requires large volumes of buffer and carries the risk that material and target protein activity will be lost during the process (Berry & Kauvar, 1993; Vanderkaay & Vanhaastert, 1995). As for gel

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filtration, sample volumes up to 30% of the total volume of the desalting column can still be conducted. The high speed and capacity of the separation allows even relatively large sample volumes to be carried out rapidly and efficiently in the group separation process (Porath, 1981). In order to achieve a good effect of separation, several characteristics of gel filtration medium are very important, such as pore size and matrix rigidity. The matrix should also have an appropriate molecular exclusion limit with a dense structure, from which the large molecular weight solute can be excluded. The matrix should have an appropriate molecular exclusion limit with a dense structure, from which the large molecular weight solute can be excluded. Sephadex-G25, a dextran-based commercial medium has been widely used for desalting protein and DNA preparations (Blondeau, 1985). However, dextran, which is a bacterial-derived polysaccharide generally produced by enzymes from certain stains of *Leuconostoc* or *Streptococcus* (Levesque & Shoichet, 2006), has relatively high-cost. The price of dextran powder is about $80 per kilogram while the price of crosslinked dextran hydrogel beads jumped more than 25 times, selling at a whopping $2100 a kilo. On the other hand, the fine flour of konjac is very low cost (Beneke, Viljoen, & Hamman, 2009; Zhou et al., 2012), with a price of $12.2 a kilo. Thus, it is potential for KGM microspheres becoming a low-cost and effective materials for industrial application.

In the present work, we have attempted to fabricate a low-cost KGM medium for desalting and small molecules separation. But owing to the extreme high molecular weight and viscosity of KGM, it is difficult to prepare KGM solutions with high concentration. A gel is formed in the presence of $\geq$1% KGM concentration (w/w), which has little fluidity (Alonso-Sande, Teijeiro-Osorio, Remunan-Lopez, & Alonso, 2009; Kobayashi, Tsujihata, Hibi, & Tsukamoto, 2002), making a challenge for KGM microsphere preparation. Although various chemical modifications such as carboxylmethylation can be processed to increase its solubility (Kobayashi, Tsujihata, Hibi, & Tsukamoto, 2002), it is not practical to obtain KGM solution with concentration high enough to prepare the microspheres. In this study, we circumvented this disadvantage by cutting down the chain length to decrease its molecular weight. Acid hydrolysis under the condition of high temperature and pressure was used to cleave the original long chain. Moreover, we applied online viscosity testing to determine the degree of hydrolysis and control the molecular weight. The KGM microspheres were synthesized by inverse suspension method, and the structure was characterized by SEM image analyzer. We evaluated the molecular weight fractionation range, the stability, and desalting effect of KGM particles. The performance of KGM microspheres was also compared with that of the commercial product Sephadex G25.

2. Materials and methods

2.1. Materials

The fine flour of konjac (the content of KGM was approximately 65%) was purchased from Hu-Bei Konson Konjac Gum Co. Ltd. (China). Span80 used as emulsifier in the oil phase was purchased from Farco Chemical Supplies (China). Liquid paraffin and petroleum ether were chosen as oil phase and were supplied by Sinopharm and Tianjin Jin Dong Tian Zheng Precision Chemical Reagent Factory (China), respectively. Epichlorohydrin used as the cross-linking agent was purchased from Xilong Co. Ltd. (China). Sephadex G25 and gel filtration LMW calibration kit were products from GE Healthcare. Other reagents were of analytical purity and were purchased from Beijing Chemical Reagent Company (China).

2.2. Degradation of KGM

The KGM was swelled in 0.5 M hydrochloric acid solution (4/3, w/w), and then the jelly was heated at 115 °C for 55 min, followed by adding 45% sodium hydroxide containing a small percentage of sodium borohydride with vigorous stirring. Finally, the obtained solution was filtered and the filtrate was collected. The concentration of KGM in the filtrate was about 12%, and the viscosity was 362 mPa s at 35 °C.

2.3. Determination of molecular weight

20 g deionized water was added into 3.0 g degraded KGM solution prepared in 2.2. The pH was adjusted to 7.0 with HCl, and the concentration of KGM in the solution was adjusted to 1% (w/w). The samples were determined by multi-angle laser light scattering, MALLS (Wyatt Technology Corporation, WTC) combined with gel permeation chromatography (GPC). The operation was under the following conditions: Agilent 1100 high performance liquid chromatography instrument, TSK-GEL G5000 HHR column (7.8 mm × 300 mm), inject volume 100 μL, mobile phase 50 mM sodium phosphate and 100 mM sodium sulfate, pH 7.0. The velocity of flow was 0.5 mL/min, and operation time was 40 min. Samples were filtered by 0.2 μm membrane before injecting into the HPLC column. Astra software was utilized for data acquisition and analysis.

2.4. Preparation of KGM gel

A 2 L three-necked reaction flask equipped with a semicircular paddle stirrer was filled with 1.2 L oil phase comprised of 1 L liquid paraffin, 0.2 L petroleum ether, and 3.5% (w/w) of Span80. The reaction flask was placed in a water-bath at 60 °C and the oil was stirred for 30 min until well-mixed. 12% KGM solution (400.0 g) was added into the flask and the mixture was stirred for 1 h. Epichlorohydrin, the cross-linking agent, was added dropwise into the reaction flask in about 30 min. The reaction was then lasted for 8 h. Finally, the formed microspheres were washed with 95% ethanol, ligroin, and deionized water successively. KGM microspheres with 50% cross-linker (200.0 g) were named as GM-1250, while microspheres with 25% cross-linker (100.0 g) were named as GM-1225.

2.5. Characterization of the KGM gel

2.5.1. SEM observation of KGM microspheres

The shape and surface feature of KGM microspheres after drying were observed by a JEM-6700F scanning electron microscopy (JEOL, Japan). The sample was placed on a metal stub and coated with platinum under vacuum by an ion sputter (JFC-1600, JEOL).

2.5.2. Particle size distribution

The particle size distribution of KGM microspheres was measured by a laser diffractometry using Ls230 Coulter (Coulter Co., USA).

2.6. Chromatographic procedure

2.6.1. Column packing procedure for KGM gel

The KGM gel and Sephadex G25 used in the chromatographic experiments were packed, respectively, according to the following procedure. The column (XK16/30 column, GE Healthcare) was mounted vertically, and then 20 mL well-mixed gel slurry (80% (v/v) KGM gel in deionized water) was poured into the column. When the gel had settled down (after 2 h), the column was washed with deionized water at a flow rate of 1.0 mL/min for 1 h. And then