High efficiency and low cost preparation of size controlled starch nanoparticles through ultrasonic treatment and precipitation

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ABSTRACT

The purpose of this work was to develop an approach to produce size controlled starch nanoparticles (SNPs), via precipitation with high efficiency and low cost. High concentration starch aqueous pastes (up to 5 wt.%) were treated by ultrasound. Viscosity measurements and size exclusion chromatography characterization revealed that, after 30 min ultrasonic treatment, viscosity of the starch pastes decreased two orders of magnitude and the weight average molecular weight of the starch decreased from 8.4×10^7 to 2.7×10^6 g/mol. Dynamic light scattering measurements and scanning electron microscopy observations showed that the SNPs prepared from the starch pastes with ultrasonic treatments were smaller (~75 nm) and more uniform. Moreover, SNPs could be obtained using less non-solvents. X-ray diffraction results indicated that effect of the ultrasonic treatment on crystalline structure of the SNPs was negligible. Ultrasound can be utilized to prepare smaller SNPs through nanoprecipitation with higher efficiency and lower cost.

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1. Introduction

Starch nanoparticles (SNPs) have attracted considerable attention in recent years because of their great potential for applications in the food industry (Kim, Park, & Lim, 2015). Since starch-based nanoparticles could act as stabilizers for oil-water emulsions (Li, Sun, & Yang, 2012; Tan et al., 2014), the edibility and biodegradability of SNPs enable them to be used as particulate emulsifier in food and pharmaceuticals. SNPs could also be used as nanocapsules of food ingredients (Fathi, Martin, & McClements, 2014). It was reported that the nanoparticles of starch-β-carotene composites improved the dispersibility of β-carotene in water and enhanced the stability of β-carotene against chemical oxidation (Kim & Huber, 2016). At the nano-scale, which is frequently defined as the range of 10–1000 nm (Mohanraj & Chen, 2007; Rao & Geckeler, 2011), properties of particles can be significantly different from the original bulk material (Hoover, Hughes, Chung, & Liu, 2010; Le Corre, Bras, & Dufresne, 2010). Size and size distribution of SNPs are of paramount importance for the applications mentioned above, and preparation of size controlled SNPs with high efficiency and low cost is the basis of their applications.

Various methods have been attempted to prepare starch or starch derivative nanoparticles (Kim et al., 2015). Among the numerous manufacturing methods, nanoprecipitation is a very simple and convenient method for production of nanoparticles with desired sizes (Perevyazko et al., 2012). The precipitation process involves a successive addition of a dilute starch solution into a non-solvent or inversely. Previous works showed that concentration of starch solution, volume ratio of solvent to non-solvent and types of solvent and non-solvent influenced the size of precipitated SNPs (Chin, Pang, & Tay, 2011; Dong, Chang, Wang, Tong, & Zhou, 2015; Tan et al., 2009; Wu et al., 2015). In order to synthesize smaller SNPs through precipitation, highly diluted starch solution and larger volume of non-solvents have to be used because high starch concentration leads to the formation of viscous solution. High viscosity of starch solution hampers diffusion of starch solution toward non-solvent which results in larger particles. However, using highly diluted starch solutions and larger volume of non-solvents to prepare SNPs will inevitably decrease production efficiency and increase cost.

Ultrasonic treatment is a method which can be used to physically modify polymers, such as starch. Some advantages of this method are that it is simple, effective and environmentally friendly. It was demonstrated that ultrasonic treatment of chitosan and starch aqueous solutions is an efficient procedure to reduce the molecular weight of these polysaccharides due to the intense mechanical effect associated with cavitation (Czechowska-Biskup, Rokita, Lotfy, Ulanski, & Rosiak, 2005). Cavitation is the collapse of microbubbles that burst and propagate as a sound wave passes

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through a solution (Langton, 1969; Price & Smith, 1993). The shear forces created due to the collapse of the bubbles may break covalent bonds in polymeric materials (Farzi, Saffari, Emam-Djomeh, & Mohammadifar, 2011; Zhang et al., 2013). It was reported that viscosity of starch solution and molecular weight of starch were reduced after ultrasonic treatment (Iida, Tuziuti, Yasui, Towata, & Kozuka, 2008).

In this paper, a facile approach to prepare size-controlled SNPs using high concentration starch aqueous pastes and less nonsolvent via nanoprecipitation was presented. In order to develop a high efficiency and low cost manufacturing method of SNPs, the starch aqueous pastes with higher concentration were treated by ultrasound, aiming to reduce viscosity of the starch pastes without decreasing concentration, so that the high concentration starch aqueous pastes could be used for nanoprecipitation with less nonsolvent. Through examining the size, morphology and structure of the SNPs obtained by using ultrasonic treated starch aqueous pastes, effects of ultrasonic treatment of starch aqueous pastes on the morphological and structural characteristics of SNPs were investigated.

2. Materials and methods

2.1. Materials

The native potato starch (containing about 20% amylose) was purchased from HuahaiShunda Grain & Oil Seasoning Co. Ltd. (Cangzhou, China). Absolute ethanol was purchased from Beijing Chemical Works (Beijing, China). Isoamylase from *Pseudomonas sp.* (E-ISAMY) was from Sigma–Aldrich (Castle Hill, NSW, Australia). Dimethyl sulfoxide (DMSO, GR grade for analysis) was purchased from Merck Co. Inc. (Kilsyth, VIC, Australia). All other chemicals were reagent grade and used as received.

2.2. Ultrasonic treatment of starch paste

Starch aqueous paste with a certain concentration (3 wt.% or 5 wt.%) was prepared by dispersing starch granules in distilled water, and heating in a water bath at 100 °C for 60 min under continuous stirring of 160 rpm to completely gelatinize starch granules. After the temperature of the obtained starch paste was cooled to 20 °C, 60g of the starch paste, held in a plastic container with height of 68 mm and diameter of 54 mm, was treated by ultrasound for a certain time using a 22 kHz ultrasound generator (HN-1000Y, Shanghai Hanno Instrument Corp., Shanghai, China) equipped with a tapered horn tip (10 mm end diameter). The power output of 100 W and 2/4 s on/off pulses were applied.

2.3. Viscosity measurement

Apparent viscosity of the starch aqueous pastes after the ultrasonic treatments was measured at 20 °C using a viscometer (RVDV-III, Brookfield Engineering Laboratories, Middleboro, USA) at the spindle rotation of 90 rpm.

2.4. Preparation of SNPs

SNPs were prepared by adding the starch aqueous paste dropwise into absolute ethanol which was continually agitated with ultrasound. The volume ratio of starch paste to non-solvent (absolute ethanol), S/NS, was 1/2, 1/5, and 1/10, respectively. The resulting mixture was then centrifuged at 4000g for 5 min. The precipitated SNPs were obtained by removing the supernatant and rinsed 1 time by centrifugation with absolute ethanol. The final product was freeze dried.

2.5. Size-exclusion chromatography

Size-exclusion chromatography (SEC) was used to analyze molecular size distribution of the potato starch before and after the ultrasonic treatments. Freeze dried SNPs obtained from the starch aqueous pastes with different ultrasonic treating times were dissolved in DMSO containing 0.5 % (w/w) LiBr at a concentration of 2 g/l in thermomixer at 80 °C for 24 h during which the thermomixer was inverted by hand occasionally. The prepared solutions were centrifuged at 4000g for 10 min and the supernatants were transferred to SEC vials for analysis. Starch molecules were eluted using DMSO/LiBr solution as the mobile phase and were separated using porous chromatogram column as the stationary phase.

Freeze dried SNPs obtained from the starch aqueous pastes with different ultrasonic treating times were de-branched using isoamylase, following the method described by Hasjim, Lavau, Gidley, and Gilbert (2010) with modifications. Starch precipitate (∼4 mg, dry weight) was dissolved in 0.9 ml water and then mixed with 2.5 µl isoamylase, 0.1 ml acetate buffer solution (0.1 M, pH 3.5), and 5 µl sodium azide solution (0.04 g/ml). The mixture was incubated at 37 °C for 3 h. The de-branched starch suspension was then heated in a water bath at 80 °C for 2 h after being neutralized with 0.1 M NaOH, and then freeze-dried. The obtained freeze dried samples after de-branched were dissolved in DMSO containing 0.5 % (w/w) LiBr at a concentration of 4 g/l for subsequent analysis by SEC.

The weight distributions of the whole (fully branched) and debranched starch molecules were analyzed using the SEC system (Agilent 1260 series, Agilent Technologies, Waldbronn, Germany) which consisted of an isocratic pump, auto sampler without temperature regulation, an online degasser, a refractive index detector (Optilab T-Rex, Wyatt Technology Corp., America) and a multiangle laser light scattering (MALLS) detector (Wyatt DAWN Heleos II, Wyatt Technology Corp., America). Astra 6 software was used for data processing. The samples were injected into a GRAM precolumn with a flow rate of 0.3 ml/min for starch solution and 0.6 ml/min for de-branched starch solution. The separation was carried out with GRAM columns (Polymer Standard Service (PPS), Mainz, Germany) placed in a column oven at 80 °C. The column dimension of pre-column was 8 × 50 mm (internal diameter × length). The dimension of column used for de-branched starch was 8 × 300 mm and the type was GRAM 100 and GRAM 1000. The dimension of column used for fully branched starch was 8 × 300 mm and the type was GRAM 30 and GRAM 3000. A series pullulan standards (PSS, Mainz, Germany) with molecular weights from 342 to 2.35×10^6 were used to generate a universal calibration curve and determine the hydrodynamic volume V_h or hydrodynamic radius R_h ($V_h = 4/3 \cdot \pi R_h^3$) (Vilaplana & Gilbert, 2010) from the elution volume. The Mark–Houwink parameters for pullulan in this eluent at 80 °C measured by PSS were $K = 2.424 \times 10^{-4}$ dL/g and $\alpha = 0.68$ (Cave, Seabrook, Gidley, & Gilbert, 2009; Wang et al., 2015). The specific RI (refractive index) increment value, dn/dc, measured by PSS was 0.071 ml/g.

2.6. Characterization of SNPs

The mean size of the obtained SNPs was measured by dynamic light scattering (DLS) using a Malvern Zetasizer Nano-ZS90 (Malvern Instruments Ltd., UK). The measurements were performed using the samples prepared by dispersing the SNPs in deionized water at a concentration of about 0.1 mg/ml. The mean size and polydispersity index (PDI) were reported.

The morphologies of the SNPs were observed using a scanning electron microscope (ZEISS, MERLIN Compact, Germany). Powder