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Preparation and characterization of size-controlled starch nanoparticles based on short linear chains from debranched waxy corn starch

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ABSTRACT

Starch nanoparticles (SNPs) with controllable particle sizes were prepared by nanoprecipitation using short linear chains from debranched waxy corn starch. The morphology, size distribution, degree of polymerization, crystal structure, and thermal properties of SNPs prepared with different volume ratio of starch solution to absolute ethanol were investigated by transmission electron microscopy, dynamic light scattering, high-performance size-exclusion chromatography, X-ray diffraction, differential scanning calorimetry, thermogravimetric analysis, and Fourier transform infrared spectroscopy analysis. When the volume ratio of starch solution to absolute ethanol was 10/40 mL/mL, the SNPs had the smallest particle size (20–100 nm) and degree of polymerization. All SNPs displayed a typical V-type crystalline structure and had a high relative crystallinity (43.2–49.5%). Compared with native waxy corn starch, the melting temperature of SNPs was higher and the temperature range was broader.

1. Introduction

Starch nanoparticles (SNPs) have attracted much attention because of their unique properties that are significantly different from their bulk materials. Starch is an abundant, inexpensive, nontoxic, naturally renewable, and biodegradable biopolymer. There is a growing interest in using starch as a precursor material in the synthesis of starch-based nanoparticles for various biomedical and industry applications such as drug delivery carriers (Rodrigues & Emeje, 2012; Simi & Abraham, 2007), implant materials (Thielemans, Belgacem, & Dufresne, 2006), and biodegradable edible film (González & Alvarez Igarzabal, 2015; Shi, Wang, Li, & Adhikari, 2013).

Several researchers have explored a variety of synthetic methods to prepare starch nanocrystals and nanoparticles, such as acid hydrolysis, enzymatic hydrolysis and recrystallization, high-pressure homogenization and miniemulsion cross-linking, microemulsion and anti-solvent precipitation (Chin, Azman, & Pang, 2014; Gavory et al., 2011; Kim, Park, & Lim, 2015; Le Corre, & Angellier–Coussy, 2014; Shi, Li, Wang, Li, & Adhikari, 2011; Zhou, Luo, & Fu, 2014). The methods of preparing nanoparticles should be cost-effective and easy to scale-up production. Compared with other synthetic methods, nanoprecipitation is a promising method as there is no need for specialized equipment and complex operating conditions, the associated costs are reasonably low, and the risk of sample contamination is often significantly reduced. The nanoparticles prepared by nanoprecipitation have been widely used in a wide variety of industries such as in the production and development of food ingredients, pharmaceuticals, health care products, and coloring substances (Joye & McClements, 2013). In recent years, few studies have reported the preparation of SNPs by nanoprecipitation. Chin, Pang, and Tay (2011) reported SNPs ranging between 300 and 400 nm in size were prepared by nanoprecipitation from native sago starch. Ma, Jian, Chang, & Yu (2008) reported that SNPs prepared by delivering ethanol as the precipitant into corn starch-paste solution dropwise possessed sizes in the range from 50 to 300 nm. Wu et al. (2016) reported that SNPs ranged in size from about 200 to 500 nm were prepared with corn starch dimethylsulfoxide solution through nanoprecipitation. Kim and Lim (2009) reported the preparation of SNPs by n-butanol precipitation of amylomaize starch aqueous dimethyl sulfoxide solution and successive enzymatic hydrolysis. However, the SNPs prepared by methods described in the literature still have some

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drawbacks such as relative large particle size and low yield. Ku et al. (2014) stated that the property of the nanoparticle depended on its size and shape. Therefore, controlling the size of particles and their uniformity in distribution during generation of nanoparticles is very important.

At present, most starch-based nanoparticles are prepared using native starch as precursor material. Natural starch usually consists of amylose and amylopectin. Amylopectin is a branched polysaccharide composed of hundreds of α-l, 4-glucan chains (short linear chains), which are interlinked by α-l, 6 linkages. Amylopectin is built with three types of chains: short chains, consisting of both outer (A) or inner (B) chains with a mean degree of polymerization (DP) ranging between 14 and 18, inner (B) long chains with DP 45–55, and a few B-chains with DP above 60 (Buléon, Colonna, Planchot, & Ball, 1998). The average chain length of most amylopectins is 17–26 and depends on the type of crystallinity of the granular starch. (Pérez & Bertoft, 2010). Isoamylase and pullulanase are classically used to debranch amylopectin for analytical or preparative purposes (Buléon et al., 1998; Pérez & Bertoft, 2010). Starch-based nanoparticles (having a resistant starch fraction) have been obtained previously by several authors using debranching procedure followed by various treatments with isoamylase from maltodextrins (Pohu, Planchot, Putaux, Colonna, & Buleon, 2004), from waxy starches (Cai & Shi, 2010, Cai, Shi, Rong & Hsiao, 2010; Zhao & Lin, 2009), with pullulanase from waxy starches (Shi & Gao, 2011; Sun, Li, Dai, Ji, & Xiong, 2014). Li et al. (2016) reported size-controlled SNPs obtained by self-assembly with different surfactant. To our knowledge, there are no reports, as yet, on the preparation of SNPs based on short linear chains through nanoprecipitation.

Therefore, the aim of this study was to develop SNPs with controllable particle sizes using short linear chains by nanoprecipitation. The morphological characteristics, size distribution, DP, crystal structure, and thermal characteristic of SNPs prepared from different volume ratios of aqueous starch solution to absolute ethanol were investigated.

2. Materials and methods

2.1. Materials

Waxy corn starch (approximately 2% amylose and 98% amylopectin) was obtained from Tianjin Tingfung Starch Development Limited Company (Tianjin, China). Disodium hydrogen phosphate (Na_2HPO_4), citric acid ($C_6H_8O_7$) and absolute ethanol were purchased from Sinopharm Chemical Reagent Limited Company (Beijing, China). Pullulanase (E.C.3.2.1.41, 6.17×10^{-4} kat/g) was supplied by Novozymes Investment Limited Company (Beijing, China). Pullulan standards (molar mass 342, 1320, 6200, 10600, 21700 g/mol) were purchased from Sigma Chemical Company, (St Louis, Missouri, USA).

2.2. Sample preparation

The short linear chains were obtained according to Sun, Li, et al. (2014) with some modifications. Waxy corn starch (WCS) (100 g/L in pH 4.8 phosphate/citrate buffer solutions (0.2 mol/L sodium hydrogen phosphate and 0.1 mol/L citrate) were gelatinized in boiling water and stirred vigorously for 30 min. The temperature of cooked waxy corn starch was cooled to 58 °C and pullulanase (3.09×10^{-6} kat/g of dry starch) was added. After an 8 h incubation period, the samples were centrifuged at 2000 g for 5 min to remove the undebranched molecules. The enzymolysis time of 8 h was selected based on the results of preliminary examinations in order to obtain small nanoparticles. The debranched starches were treated with pullulanase again and their DP did not change, indicating the debranched starches were linear chains. After discarding the sediment, the supernatant was heated at 100 °C for 10 min to inactivate the enzyme, and then centrifuged at 2000 g for 2 min to remove the inactivated enzyme, followed by cooling to room temperature. Then, the debranched starch (short linear chains) was precipitated using excess absolute alcohol and washed 3 times with distilled water until neutral and then freeze dried.

Short linear glucan solution (10 g/L) was cooked at 100 °C for 30 min. Then, to avoid the starch recrystallization, a fixed quantity of absolute ethanol (10, 15, 20, 30, 40, 50 mL) was instantly added drop-wise into 10 mL of cooked short linear glucan solution, which was continually stirred using a magnetic stirrer for 1 h at a constant rate of 600 rpm. Samples of nanoparticles were obtained by centrifugation, rinsed with absolute ethanol three times to remove excess water, and then freeze dried. SNP 1:1, 2:3, 1:2, 1:3, 1:4, and 1:5 represent SNPs were prepared at 10/10, 10/15, 10/20, 10/30, 10/40, 10/50 mL/mL (starch solution/absolute ethanol) ratio, respectively.

The yield was calculated by dividing the weight of freeze-dried precipitate by the initial dry weight of waxy corn starch. The SNPs prepared by nanoprecipitation had a high yield. The final yield of the SNP 1:3, SNP 1:4 and SNP 1:5 were 76.4, 82.6, and 78.3%, respectively.

2.3. Transmission electron microscopy (TEM)

Transmission electron micrographs of the SNPs were taken with a Hitachi 7650 TEM (Hitachi, Tokyo, Japan) with an acceleration voltage of 80 kV. The SNPs were deposited on a carbon-coated grid without any treatment. The mean diameter of SNPs obtained from TEM images were measured using Image J 1.45 software, accurately by choosing at least 100 particles from different TEM micrographs.

2.4. Dynamic light scattering (DLS)

The average size, size distribution and polydispersity of the nanoparticles were estimated by dynamic light scattering (DLS) using a Malvern Zetasizer Nano (Malvern, Worcestershire, UK) equipped with a He–Ne laser (0.4 mW; 633 nm) and a temperature-controlled cell holder. The intensity of the scattered light was detected at 90° to the incident beam. The measurements were performed in samples diluted in deionized water and analyzed at 25 °C (Pignatello et al., 2006). The diagrams of particle size distributions were obtained by using the Zetasizer software v 7.11 provided with the apparatus, which estimated the proportions of size as a function of the light intensity observed with the photodetector. The mean intensity weighted diameter and polydispersity index (PDI) was recorded as the average of three mean measurements.

2.5. High-performance size-exclusion chromatography (HPSEC)

The DP of SNPs were analyzed using a high-performance size-exclusion chromatograph (HPSEC) equipped with a differential refractive index (DRI) detector. HPSEC was carried out according to Jiang, Campbell, Blanco, and Jane (2010) with some modifications. The SNPs were dissolved in 900 mL/L dimethyl sulfoxide by heating for 30 min in boiling water bath with constant magnetic stirring. An aliquot (50 μL) of the dispersion was filtered through a nylon membrane (0.25 μm pore size). The HPSEC-DRI system consisted of a pump (LC-20AT), an injection valve (SIL-20A), and a DRI detector. PL-aquagel-OH 30 analytical columns with a guard column were used to analyze the DP of the samples. The temperature of the columns (PL-aquagel-OH 30) was maintained at 35 °C. Distilled-