Preparation and characterization of starch nanoparticles by desolvation method

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ABSTRACT

Nanoparticles prepared from biocompatible and biodegradable materials have attracted considerable attention in food and pharmaceutical science. Starch is one of abundant biopolymer. In this study, starch nanoparticles were produced by desolvation method and the influence of various parameters during desolvation was studied in terms of change in size, zeta potential and morphology of starch nanoparticle. Starch nanoparticles were produced through precipitation of starch in non-solvent (ethanol, methanol or acetone) with a constant flow rate (1 ml/min). Particle size and zeta potential of nanoparticles were determined by Dynamic Light Scattering and morphology of particles was characterized by Scanning Electron Microscopy (SEM). Amylose/amylopectin ratio was varied by mixing Hylon VII (National Starch 70% amylose starch) and Amioca (National Starch 98% amylopectin starch) to obtain various amylopectin/amylose ratios (100%, 90%, 80%, 70%, 60%, 50% and 30% amylopectin). Starch nanoparticles produced from those starch mixtures in ethanol and larger nanoparticles were obtained from higher amylopectin starch. It was also found that temperature has significant effect on producing very small particles with a diameter of 78 nm by raising temperature of starch solution. Zeta potential measurements showed that the absolute zeta potential values also increased considerably when the temperature of the solvent was higher (40 °C and 60 °C). However, other parameters showed no significant change in surface charge of particles found between -7 to -15 mV. The stability of starch nanoparticles was improved using 1% surfactant, Sodium dodecyl sulfate (SDS), and particles remained stable for 3 days. By increasing the stirring speed of ethanol from 200 rpm to 800 rpm in controlled environment, progressive increase in particle size was achieved and larger particles were observed in SEM. The results indicated that the type of non-solvent (ethanol, methanol, acetone) and solvent/non-solvent ratio were the determinent factors for nanoparticle formation. Starch nanoparticles were closer to being spherical for all circumstances except freeze dried nanoparticles. After freeze drying process contributed to the formation of fibrous structures while vacuum drying method helped to maintain spherical shape of nanoparticles. Other parameters including concentration of NaCl, ultrasound treatment during nanoprecipitation, and pH of starch solution showed no impact on particle size, zeta potantial or morphology of particles.

Keywords: Starch nanoparticles, desolvation, biocompatible nanoparticles

1 INTRODUCTION

Starch is one of th most abundant biopolymer in nature and major ingredient in food and pharmaceutical industries [1]. Increasing interest to nanoparticles prepared from biopolymers led to generate nanoparticles from biopolymers such as starch. Nanoprecipitation is one of the ways to generate nanoparticles. This technique allows producing particles in nanoscale with a narrow size distribution. Nanoprecipitation method was discovered and patented by Fessi and co-workers [2]. This method is also called as solvent displacement, solvent diffusion, solvent shifting, or flash nanoprecipitation (with small modification of nanoprecipitation). Nanoprecipitation is commonly used for precipitation of polymers such as poly(ε-caprolactone), polylactide, poly(lactide-co-glycolide) or poly(hydroxyl-butyrate) due to simplicity, rapidity and reproducibility of the process. In addition to these polymers, other natural polysaccharides (chitosan, dextran, starch etc.) are considered alternative sources of nanotechnology applications [3]. The nanoprecipitation process does not allow predicting particle size or shape alone. The formation of particles can be controlled and optimized by different parameters such as mixing rate, polymer concentration, solvent/non-solvent ratio or type of non-solvent [4]. In this study, starch nanoparticles were prepared from corn starch by nanoprecipitation method and the influence of various parameters during desolvation was studied in terms of change in size, zeta potential and morphology of starch nanoparticle.

2 MATERIALS & METHOD

Native corn starch was purchased from Sigma-Aldrich (St. Louis, MO, USA). Amioca starch containing 99% amylopectin and Hylon VII and unmodified high amylose corn starch (approximately 70% amylose) were obtained from Ingredion (Bridgewater, NJ, USA). Approved Crosslinking agent phosphorous oxychloride (POCl3), sodium hydroxide (NaOH), and sodium chloride (NaCl)

were provided from Sigma-Aldrich (St. Louis, MO, USA). Urea was purchased from Fisher Scientific (Pittsburgh, PA, USA). Ethanol, methanol, acetone, and hydrochloric acid (HCl) were obtained from Life Sciences Storeroom (Urbana, IL, USA). All chemicals were reagent grade.

The alkaline solution was prepared dissolving NaOH (0.8 wt %) and urea (1 wt %) in cold deionized water under magnetic stirring. 0.5 g of native corn starch (1 wt%) was dispersed in alkaline solution and the solution was heated to 68 °C for 15 hours. The use of urea facilitates the solubilization of starch by breaking the inter-molecular hydrogen bonds in the starch caused by the self-association of its monomeric units. The solution was cooled down to room temperature and the pH of starch solution was found 12.6.

The starch nanoparticles were prepared by nanoprecipitation method. The starch solution was filtered with 0.45 µm polyvinylidene difluoride (PVDF) syringe filters prior to use. 0.5 ml of starch solution was dripped into 5 ml ethanol stirring at 350 rpm and flow rate of starch solution was maintained constant at a rate of 1 ml/min with a syringe pump. The suspension was centrifuged at 8000 rpm for 10 min and washed twice with ethanol. The starch nanoparticles were dispersed in 2 ml deionized water prior to particle size and zeta potential analyses. The samples were dried in vacuum oven for 2 hours or freeze dried before Scanning Electron Microscopy.

2.1 Particle size and zeta potential analysis

The average particle size and zeta potential value of particles were measured by dynamic light scattering using Brookhaven ZetaPALS Zeta Potential and Particle Size Analyzer (Brookhaven Instruments Corporation, Holtsville, NY, USA). The particle size distribution data was analyzed by dedicated software to the instrument. Prior to the measurement, the sample at 1 mg/ml concentration was prepared by suspending starch nanoparticles in deionized water. The particle size analysis was perfomed in a quartz cuvette and measured with scattering angle 90 °. The pH of each sample were measured prior to zeta potential analysis and analysis was conducted using a disposable cuvette. Measurements were perfomed in a quartz cuvette. All the measurements were performed at 25 °C.

2.2 Scanning Electron Microscopy (SEM)

It is important to microscopically examine the morphological changes of starch nanoparticles which are produced at different conditions by varying parameters. Morphological analysis was carried out by Hitachi S-4700 High Resolution Scanning Electron Microscope (Hitachi High Technologies America, Inc., Dallas, TX, USA). The starch nanoparticles were gently deposited on an aluminum stub and coated with gold/palladium for 40 seconds by Emitech K575 Sputter Coater (Quorum Technologies Ltd, Company West Sussex, RH19 2HL, UK) under high vacuum. The coated sample was observed under accelerating voltage of 10 kV.

3 RESULT

The measurement of particle size distribution of starch nanoparticles was carried out by dynamic light scattering and presented in Figure 3.1 The starch nanoparticles showed bimodal size distribution where the scale of smaller and larger particles were 45-65 nm and 185-265 nm respectively.

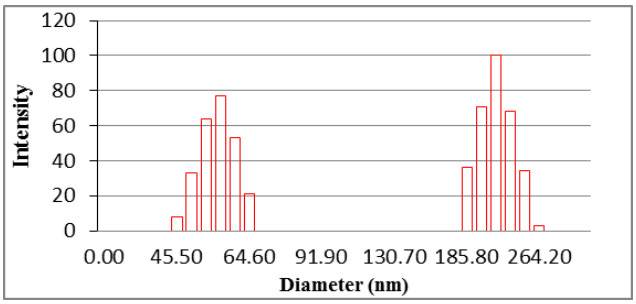

Figure 3.1. The particle size distribution of starch nanoparticles in intensity mode

The effect of temperature on nanoparticle size was investigated by maintaining the temperature of starch solution at 8, 22, 40 and 60 °C ± 2 °C whereas temperature of ethanol was constant at 8 °C ± 2 °C during performing nanoprecipitation. As shown in Figure 3.2, the smallest nanoparticle was measured as 78.7 nm where the temperature of starch solution and ethanol was 60 ± 2 °C and 8 ± 2 °C respectively. The Flory Huggins theory can be used to describe interaction of solvent/non-solvent/polymer systems through the formation of nanoparticles. The interaction parameter is the quantification of solvent-solvent interaction or the interaction of polymer chains with solvents and related to Hildebrand solubility parameter and temperature.

$$\chi_{sp} = \frac{V_r}{RT}(\delta_s - \delta_p)^2 \qquad (1)$$

Vr is the reference volume; δs and δp represent the solubility parameters of solvent and polymer respectively. R is universal gas constant and T refers to temperature (Kelvin). When the difference in the solubility parameters between the starch and the ethanol increases, the degree of the enthalpic contribution increases and the free energy of mixing becomes less negative and eventually separation is favored. This represents the driving force for the precipitation process and the formation of the nanoparticles. As seen in Equation 1, χ is depended to temperature and increasing the temperature decreases the interaction

parameter indicating that smaller particles are produced at higher temperatures.

Zeta potential measurements showed that the absolute zeta potential values also considerably increased to when the temperature of the solvent was higher (40 °C and 60 °C). Zeta potential of starch nanoparticles was measured -23 mV for starch nanoparticles produced from starch solution at 60 °C.

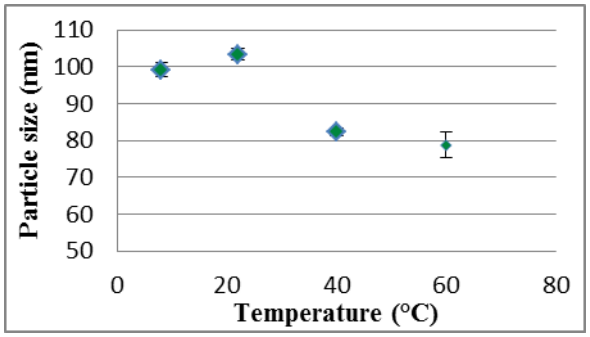

Figure 3.2 The effect of temperature on particle size of starch nanoparticles

The other important parameter is agitation speed of ethanol in nanoprecipitation process. The starch nanoparticles were prepared by addition of starch solution into ethanol stirring with a magnetic stirrer working at different speeds (200, 400, 600 and 800 rpm) while other parameters were kept constant as described above. As it is shown in the particle size data (Figure 3.3), particle size depends on the agitation speed of ethanol during precipitation process and increasing stirring speed led to the formation of larger nanoparticles. This could be explained by formation of nanoparticle concentrated regions due to centrifugal forces which induced the aggregation of nanoparticles as a consequence [5].

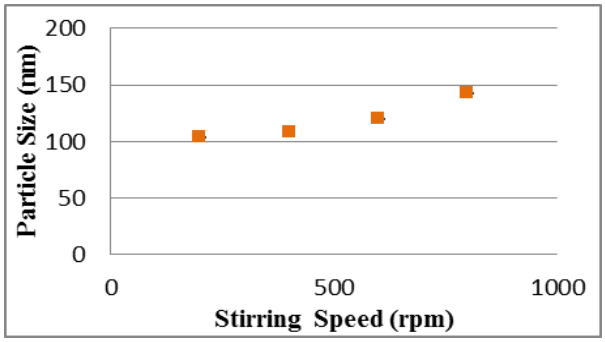

Figure 3.3 The effect of stirring rate of ethanol on size of nanoparticles during nanoprecipitation

The SEM images of starch nanoparticles formed under differet stirring speeds show that smaller particle formation occurred at high stirring rate as well as very large particles (Figure 3.4) due to aggregation of small nanoparticles acused by high centrifugal forces. This result is inconsistent with our particle size measurements which showed increasing trend in particle size with increase in stirring speed of ethanol.

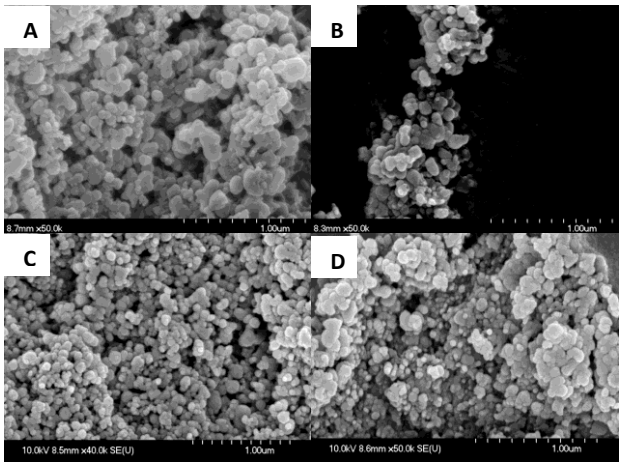

Figure 3.4 SEM micrographs of starch nanoparticles prepared by precipitating starch solution (pH 12.6) in ethanol (10:1) with a flow rate of 1 ml/min under constant stirring at a) 200 rpm, b) 400 rpm, c) 600 rpm and d) 800 rpm

Starch mainly comprises of two molecules, amylose and amylopectin having different molecular weights. Due to the existance of two distinctly different molecules of varying molecular weights, it is useful to investigate the influence of amylose and amylopectin on the formation of particle size. The varying amylopectin content of starch mixtures (99, 90, 80, 70, 50, and 30%) were prepared by mixing the required amount of Hylon VII (30% amylopectine), native corn starch (70% amylopectin) and Amioca starch (99% amylopectin). The starch solutions were prepared from these blends as previously described and nanoparticles were produced with same method mentioned before. The influence of amylopectin content of starch blends is shown in Figure 3.5. Larger starch nanoparticles were generated with starch blends with higher amylopectin content which could be attributed to higher molecular weight of amylopectin molecule. The previous studies reported that the molecular weight of polymer has a significant impact on controlling the particle size [6]. The high molecular weight apparently promotes the formation of larger particles.

To evaluate the influence of type of non-solvent, three non-solvents (ethanol, methanol and acetone) were used as desolvating agent. In three different desolvating agents, methanol exhibited a great influence on formation of small starch nanoparticles below 80 nm. Increasing ethanol:starch solution ratio decreases the particle size. Contrary to the results of ethanol and methanol, larger nanoparticles were obtained using acetone as a desolvating

agent depending on the non-solvent/solvent ratio and particle size increased to 144 nm when the non-solvent/solvent ratio was 30:1.

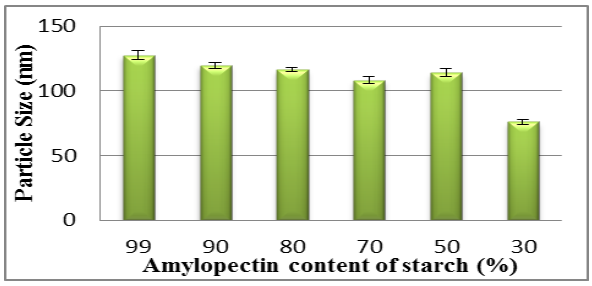

Figure 3.5 Particle size results of nanoparticles produced from different starch mixtures containing different amylopectin proportions

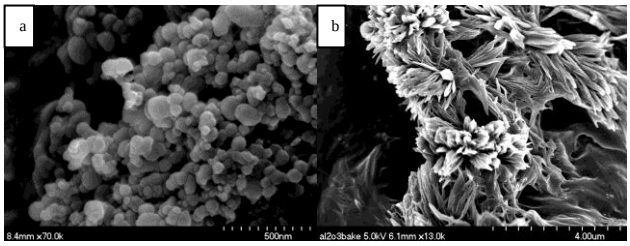

Figure 3.6 SEM images of a) vacuum-dried starch nanoparticles, b) formation of fibrous structure after freeze drying of starch nanoparticles

SEM images of freeze-dried or vacuum dried starch nanoparticles are shown in Figure 3.6. Even though some aggregations was observed in Figure 3.6.a after vacuum drying due to drying and centrifugation typically, starch nanoparticles were spherical in shape. After freeze-drying, most of the nanoparticles lost their shape and formed a fibrous structure. The results were in agreement with formation of fiber structure from chitosan nanoparticles during in freeze drying process. This may be attributed to phase seperation during occurred in freezing. Water forms ice crystal but excludes nanoparticles from the crystals. In the drying process, high mechanical stress occurs between ice crystals which eventually leads to formation of fibrous structure [7].

The effect of pH of starch solution on formation of nanoparticles and their size was studied and the pH of starch solution was adjusted to pH 7, 9, 11 and 12.6 with 1 M HCl prior to desolvation. In general, we did not observe remarkable changes in particle size depending on varying pH of starch solution but for all cases, the formation of starch nanoparticles were confirmed with SEM (data not presented here). The ultrasound-assisted nanoprecipitation method was also performed to produce starch nanoparticles, therefore, strach solution dripped into ethanol that continously stirring at 350 rpm while applying ultrasound to ethanol. The particle size measurements showed that particle size was not remarable change with or without sonication.

Table 1. The particle stability of starch nanoparticles stabilized by 1% SDS

Time (day)	Particle size (nm)
0	193.9
1	192
2	215.8
3	218.8

Table 1 shows the stability of starch nanoparticles improved using 1% surfactant, Sodium dodecyl sulfate (SDS), and particles remained stable for 3 days.

4 ACKNOWLEDGEMENT

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