

Fabrication of Size-Controlled Starch-Based Nanospheres by Nanoprecipitation

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ABSTRACT Nanometric and monodisperse starch acetate nanospheres can be prepared through a simple procedure of nanoprecipitation, by the dropwise addition of water to an acetone solution of starch acetate, without any stabilizing agent. This is the first report of the preparation of starch-based nanospheres by this method. The size of the nanospheres obtained can be easily controlled by a number of simple and efficient modifications, i.e., through regulation of the polymer concentration in acetone, the proportions of the water and organic phases, and the molecular weight and degree of substitution of the starch esters. A number of reasons are suggested to explain the observed transitions in the particle size. Fluorescence spectroscopic studies proved that these types of nanospheres could be potentially used for the encapsulation of hydrophobic drugs.

KEYWORDS: nanospheres • starch acetate • nanoprecipitation • biopolymers • size-controlled • encapsulation

INTRODUCTION

Starch, as one of the most abundant biopolymers in nature, has been considered as an alternative material for industrial applications because of its biodegradability, derivability, availability, and low cost (1). One interesting application has been the preparation of starch nanocrystals and nanoparticles by acid hydrolysis (2–5). However, the nanoplatelets produced have a number of drawbacks, including the long duration for their formation and the low final yield. Another problem also arises from the presence of a large number of unmodified hydroxyl groups, which tend to reform the supramolecular interactions characteristic of polysaccharides, yielding aggregation and, consequently, collapse of the nanoparticles (6). Polysaccharides, such as glucan (7, 8), pullulan (9), and dextran (10, 11), have also been used for the generation of self-assembling nanospheres by partial hydrophobic modification of the polysaccharide, followed by a dialysis process.

In addition, the process of nanoprecipitation, first described by Fessi et al. (12, 13), has been investigated as a procedure for the generation of nanoparticles. This process involves the successive addition of a dilute solution of polymer to a nonsolvent, which leads to polymer precipitation on the nanoscale. The method is essentially based on the interfacial deposition of polymers following displacement of a semipolar solvent miscible with water from a lipophilic solution. It has many advantages; for example, large amounts of toxic solvents and external energy sources

are avoided, and submicron particle sizes with narrow size distribution can be obtained (14). Cellulose acetate nanoparticles have been prepared by nanoprecipitation by the dropwise addition of the dissolved polymer into water under stirring. However, this method produced irregularly sized nanoparticles (15).

In this paper, we report on the production of nanometric, monodisperse, and size-controlled starch acetate (SA) nanospheres by the dropwise addition of water to an acetone solution of SA, with no added stabilizing agent. The particle size and size distribution can be easily controlled by changing the preparation conditions, such as the concentration of acetone, the proportions of the water or organic phases (V_{H_2O}), or the molecular weight (M_w) and the degree of substitution (DS) of the starch esters. The potential application of these nanospheres is explored by fluorescence spectroscopy.

EXPERIMENTAL SECTION

Materials. Waxy maize starch acetate (SA) samples with DSs of 2.97, 2.96, 2.63, and 2.62 were prepared according to a previously published method (16). All other chemicals were obtained from Beijing Chemical Works and were used as received.

Nanospheres Preparation. The nanospheres were prepared by a nanoprecipitation process. Typically, 100 mg of SA was dissolved in 20 mL of acetone. Distilled water (50 mL) was then added dropwise to the polymer solution. The resulting nanosphere suspensions were stirred at room temperature until acetone was completely vaporized from the aqueous suspension.

Nanospheres Analysis. The appearance of nanospheres was characterized by scanning electron microscopy (SEM) using a model XL 30 ESEM microscope (Philips). A droplet of suspension was placed on a silica surface. After the water was evaporated at 40 °C for 6 h, the system was covered by gold before measurement.

The particle size and polydispersity of nanospheres in aqueous solution were measured by dynamic light scattering using

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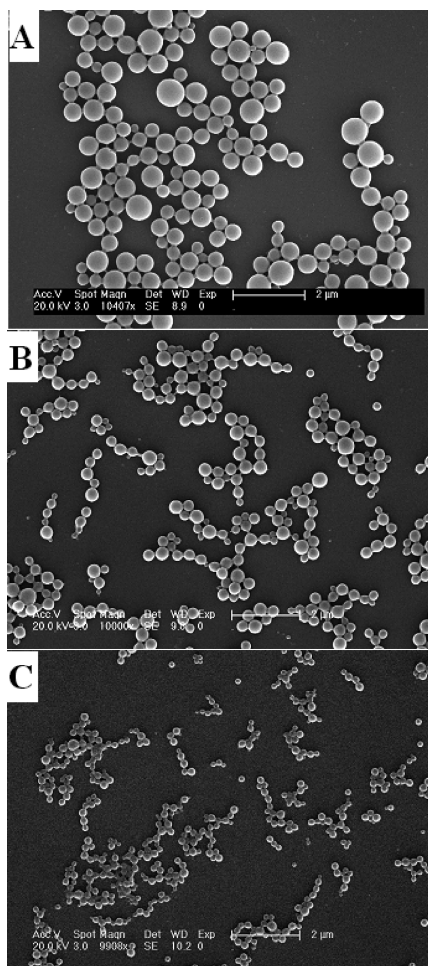


FIGURE 1. SEM images of SA nanospheres (DS 2.97) prepared by the dropwise addition of water into a polymer acetone solution with concentrations of (A) $10 \text{ mg} \cdot \text{mL}^{-1}$, (B) $5 \text{ mg} \cdot \text{mL}^{-1}$, and (C) $1 \text{ mg} \cdot \text{mL}^{-1}$.

a 90 Plus particle size analyzer (PSA; Brookhaven) at $25 \text{ }^\circ\text{C}$. The suspensions were diluted with distilled water to a concentration of about 0.01%. The mean particle size was approximated as the diameter and the combined polydispersity as the polydispersity index (PDI).

Fluorescence Spectroscopy. Stock solutions containing pyrene ($6 \times 10^{-6} \text{ mol} \cdot \text{L}^{-1}$) and ethyl ether were added to 5 mL nanosphere suspensions at various concentrations to achieve a final pyrene concentration of $6 \times 10^{-7} \text{ mol} \cdot \text{L}^{-1}$. The mixture was heated at $50 \text{ }^\circ\text{C}$ for 1 h to remove the ether. The fluorescence intensity was measured at room temperature by a fluorescence spectrometer (Hitachi F-2500) using an emission wavelength of 335 nm. The excitation and emission bandwidths were both 2.5 nm.

RESULTS AND DISCUSSION

To study the formation of nanoparticles, the particle size of a sample prepared from SA with a DS of 2.97 and a M_w of 21 500 is first investigated. Sphere-shaped particles with uniform diameter are confirmed by field emission SEM examination, and the results are shown in Figure 1. PSA measurement indicates that the mean diameter increases from 249 to 720 nm as the concentration of SA in acetone increases from 1 to $20 \text{ mg} \cdot \text{mL}^{-1}$. This effect of the polymer concentration on the nanosphere size arises from the higher resultant organic phase viscosity, which leads to larger nanodroplet formation (17).

The specific viscosity is often used as a function of the polymer concentration to determine the critical overlapping concentration (c^*) (18). When the concentration is lower than c^* , the molecules are in a dispersed state and can be separated into nanodomains by adding the nonsolvent water. Horning and Heinze reported that the abrupt transition of PDIs of nanospheres could be used to deduce the value of c^* (15). As shown in Figure 2A, a narrow size distribution with PDIs from 0.005 to 0.038 can be observed as the polymer concentration is increased from 1 to $8 \text{ mg} \cdot \text{mL}^{-1}$, while further increases in the polymer concentration lead to a rapid increase of the PDI. Coagulation occurs at concentrations above $20 \text{ mg} \cdot \text{mL}^{-1}$. An inflection is also observed, in the range from 8 to $10 \text{ mg} \cdot \text{mL}^{-1}$, in the size distribution curve. The same transformation is also observed when the polymer solution is dropped into water (Figure 2B). This experiment may possibly indicate the c^* range of SA in acetone.

As shown in Figure 2B, small particles are obtained when an acetone solution of the polymer is dropped into water, but large precipitates at the micrometer level appear as well. One reason for this aggregation is the concentration gradient that forms at the water–acetone interface. By dropping an acetone solution into water, SA precipitates rapidly in the excess of water, which results from a sharp decrease of the interfacial tension and the migration of the insoluble SA toward the two-phase interface. However, dropping water into the acetone will lead to a slow increase in the water content in the mixture and, consequently, a uniform nanoprecipitation of particles (19, 20).

The origin of the nanospheres can be explained by interfacial turbulence or the spontaneous agitation of the interface between two unequilibrated liquid phases, and it involves flow, diffusion, and surface processes (12, 21). This explanation is confirmed by the fact that the particle size varies with the volume proportion between the water and organic phases at the same polymer concentration. As shown in Figure 3, the particle size is successively reduced from 596 to 460 nm as $V_{\text{H}_2\text{O}}$ is increased from 1 to 3.5. This fact is due to the reinforcement of the interfacial interaction between acetone and water and the compression of the SA nanospheres by increasing water content.

Significantly, a good linear dependence is observed between the particle size and the theoretical value of the average solubility parameter (δ), which is calculated according to the expression (22)

$$\delta = x_1\delta_1 + x_2\delta_2 \quad (1)$$

where x_1 and x_2 are the volume fractions of acetone and water, respectively, and δ , δ_1 , and δ_2 are the solubility parameters of the mixture, acetone, and water, respectively.

As shown in Figure 4, the linear correlation coefficient of the combined line is -0.995 , which indicates that the size of the nanospheres is strongly dependent on the polarity of the mixture. When acetone–water mixtures are used as dispersion media, the average particle size generally decreases with an increase in the solubility parameter and, consequently, the polarity of the mixture. It is noteworthy

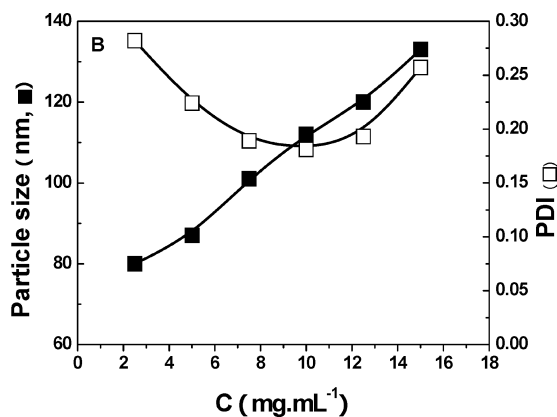
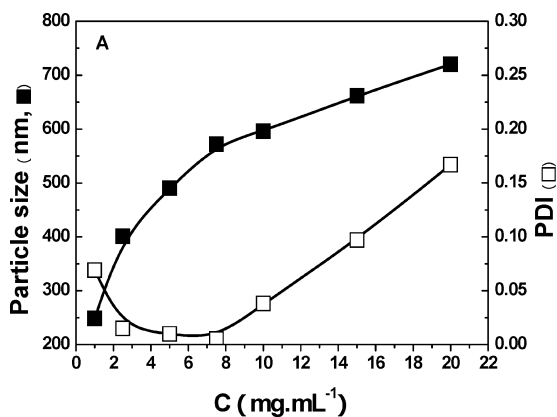


FIGURE 2. Nanosphere size and size distribution as a function of the SA concentration in an acetone solution: (A) water dropped into the polymer solution; (B) polymer solution dropped into water.

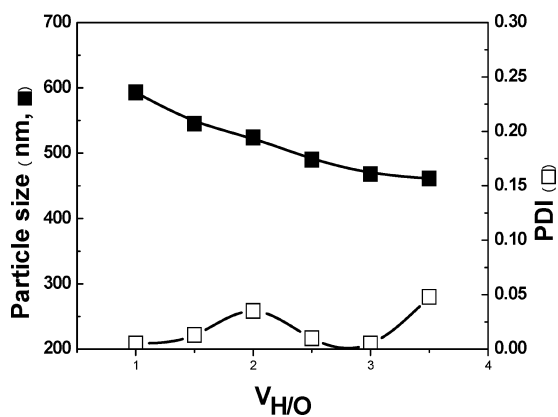


FIGURE 3. Nanosphere size and size distribution as a function of the proportion of water and organic phases ($V_{H/O}$) in mixture.

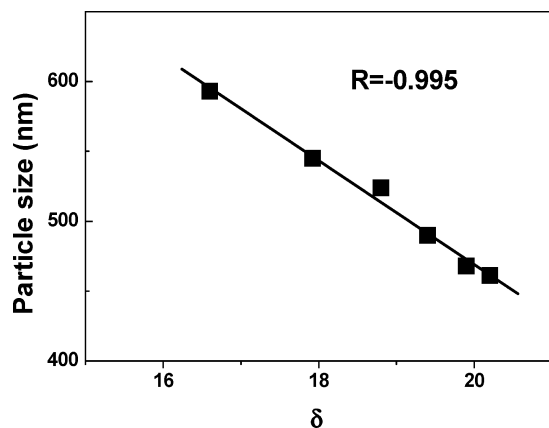


FIGURE 4. Nanosphere size as a function of the solubility parameter (δ) of the mixture with an SA concentration of $5 \text{ mg} \cdot \text{mL}^{-1}$ in the organic phase.

that all of the nanospheres maintain a lower PDI level range from 0.005 to 0.049 (Figure 3).

The influence of acetone on the particle size is also significant. When $V_{H/O}$ is 1, nanospheres with a size of ca. 590 nm and a PDI of 0.005 are obtained after the removal of acetone. After 3 days, the particle size and PDI are almost unchanged by the addition of a fresh 30 mL of water. However, the mean diameter reduces to ca. 530 nm with a PDI of 0.087 by adding 20 mL of acetone and 30 mL of water and subsequently removing the acetone. This fact demonstrates that the polarity of the mixture can act as a regulator

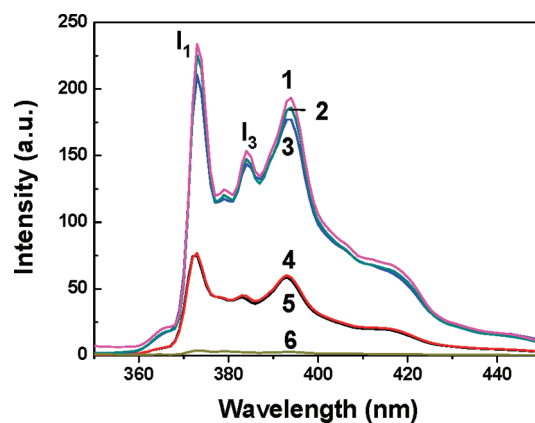


FIGURE 5. Fluorescence emission spectra of pyrene ($6 \times 10^{-7} \text{ mol} \cdot \text{L}^{-1}$) in the presence of decreasing concentrations of nanospheres (entry 1) with excitation at 335 nm. Polymer concentration in order of decreasing fluorescence intensity: curve 1 ($1 \text{ mg} \cdot \text{mL}^{-1}$), curve 2 ($10^{-1} \text{ mg} \cdot \text{mL}^{-1}$), curve 3 ($10^{-2} \text{ mg} \cdot \text{mL}^{-1}$), curve 4 ($10^{-3} \text{ mg} \cdot \text{mL}^{-1}$), curve 5 ($0 \text{ mg} \cdot \text{mL}^{-1}$), and curve 6 ($1 \text{ mg} \cdot \text{mL}^{-1}$ without pyrene).

Table 1. Molecular Mass (M_w) and Degree of Substitution (DS) of SA and Mean Diameter (D) and Polydispersity Index (PDI) of the Resulting Nanospheres Determined by a PSA

entry	M_w	DS	D (nm)	PDI
1	21 500	2.97	490	0.010
2	20 600	2.63	446	0.105
3	292 000	2.96	149	0.103
4	345 000	2.62	121	0.277

of the particle size. Therefore, the complete removal of acetone in the mixture is necessary to standardize the size of the nanospheres.

Other factors that control the size of the SA nanospheres are also considered. For SA with a DS of 2.97 (Table 1, entry 1), the size of the nanospheres is 490 nm. However, nanospheres with a similar M_w and a lower DS of 2.63 have a lower size of 446 nm (Table 1, entry 2). This may arise because the numbers of particles deposit rapidly in conditions of rather high numbers of OH groups. Moreover, a somewhat higher value of PDI is achieved for the nanospheres with a lower DS (Table 1, entries 2 and 4). This uneven distribution is due to the aggregation that results from intensive and uncontrolled hydrogen bonding (23).

Furthermore, it is significant that SA with higher M_w leads to smaller nanospheres (Table 1, entries 3 and 4). This fact can be explained by assuming that a polymer with high M_w is more likely to separate out from the medium, forming substantively small particles (24).

The vibrational structure of the fluorescence bands of pyrene is known to be sensitive to the local polarity of the microenvironment at the binding sites (25). Thus, pyrene is employed in this study as a hydrophobic probe to monitor the micropolarity of SA nanospheres by fluorescence spectroscopy (26). A nanosphere suspension of SA (DS 2.97) with a mean diameter of 490 nm is used in the fluorescence study. As shown in Figure 5, the nanosphere suspension itself shows no fluorescence (curve 6). For the nanospheres with a low concentration (10^{-3} mg · mL⁻¹) of pyrene, the fluorescence intensity is comparable to that of the aqueous pyrene solution without nanospheres. With an increasing polymer concentration, an increase in the total fluorescence intensity and a red shift of the I_1 (373.5 nm) and I_3 (384 nm) bands are observed. Moreover, the peak intensity ratios of I_1 to I_3 increase with an increase in the concentration of SA, reaching an approximate value of 1.53 at the polymer concentration range of 10^{-2} – 1 mg · mL⁻¹ (curves 1–3). These data indicate a transfer of pyrene from the polar media to the less polar microdomains (27). The presence of a relatively nonpolar domain enables these starchy nanospheres to have potential applications for the encapsulation of hydrophobic substances (28).

CONCLUSION

An efficient procedure was developed for the preparation of uniform nanospheres from SA by nanoprecipitation. The size of the nanospheres can be tuned by adjusting the SA concentration in acetone, the proportions of the water and organic phases, and the M_w or DS of the starch esters. High concentrations of SA in the organic phase will lead to the formation of larger nanospheres, and the critical overlapping concentration of SA is observed to be within the range of 8–10 mg · mL⁻¹. Moreover, a linear dependence between the theoretical value of the polarity of water and acetone mixtures and the particle size was observed, which provides further evidence for nanosphere formation in terms of an interfacial effect between two unequilibrated liquid phases and proves the strong dependence between the size of the

nanospheres and the polarity of system. Furthermore, higher numbers of OH groups and higher M_w 's result in the formation of smaller SA nanospheres. Fluorescence spectroscopy demonstrated the existence of less polar microdomains within the nanospheres, indicating that these nanospheres could be used for the encapsulation of hydrophobic drugs.

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