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Preparation of starch nanospheres through hydrophobic modification followed by initial water dialysis

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ABSTRACT

Starch nanospheres smaller than 200 nm were produced from hydrophobically modified starch by using initial water dialysis method. The hydrophobic modification of starch was performed by using octenyl succinic anhydride (OSA). The resultant starch nanospheres were characterized by using Fourier transformation infrared (FTIR) spectroscopy, ^{1}H nuclear magnetic resonance (^{1}H NMR) spectroscopy and fluorescence spectroscopy, scanning electron microscopy (SEM) and dynamic light scattering (DLS). Effects of degree of substitution (DS) in OSA-starch, initial water content and OSA-starch concentration on morphology and particle size of starch nanospheres were evaluated. The SEM micrographs showed that starch nanospheres with spherical shape and sharp edge can be produced at DS values $\geq$0.67. The particle size of starch nanospheres decreased significantly ($P < 0.05$) with increase in DS of OSA-starch and increase in the initial water content, whereas the particle size increased significantly ($P < 0.05$) with the increase in the concentration of OSA-starch. These OSA-starch nanospheres can be preferentially used to microencapsulate hydrophobic drugs.

1. Introduction

Nanoparticles (10–1000 nm) can be obtained in various morphologies such as nanospheres, nanocapsules and nanoliposomes (Jung et al., 2000; Pinto Reis, Neufeld, Ribeiro, & Veiga, 2006). There has been growing interest in preparing biodegradable and non-toxic nanoparticles as vehicles for drug delivery for biomedical applications (Lemarchand, Gref, & Couvreur, 2004). Partial hydrophobic modification of pullulan (Jung, Jeong, Kim, & Kim, 2004), chitosan (Vieira, Moscardini, Tiera, & Tiera, 2003; Yang et al., 2008), and dextran (Aumelas, Serrero, Durand, Dellacherie, & Leonard, 2007; Liebert, Hornig, Hesse, & Heinze, 2005) has been used to produce self-assembled nanospheres.

Starch is suitable material to produce nanoparticles for industrial applications because it is non-toxic, biodegradable, biocompatible and readily available in reasonable price (Rodrigues & Emeje, 2012). Starch nanoparticles are commonly produced using nanoprecipitation (Tan et al., 2009; Tay, Pang, & Chin, 2012), acid hydrolysis (Angellier, Choisnard, Molina-Boisseau, Ozil, & Dufresne, 2004) and emulsification (Jain, Khar, Ahmed, & Diwan, 2008; Shi, Li, Wang, Li, & Adhikari, 2011). However, these methods have disadvantages such as they take long time to produce nanoparticles, leave behind large quantity of organic solvent, require large amount of emulsifiers and particle size distribution is usually quite broad.

Dialysis is an effective way of producing nanospheres (Liu et al., 2007; Namazi, Fathi, & Dadkhah, 2011). This method is based on the exchange of solvent and water through diffusion during the

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dialysis process. The polymer precipitates into nanoparticles due to the solvent–water exchange. This method has many advantages, for example, the size distribution of nanoparticles can be controlled within a narrow range and the solvent can be completely removed at ambient condition.

The amphiphilic characteristic of a biopolymer is responsible for producing self-assembled nanospheres through the dialysis process. In aqueous solution, the amphiphilic polymers self-assemble spontaneously into a core–shell nanospherical (micellar) structure. This self-assembly is driven by intra- or intermolecular associations between hydrophobic moieties, primarily to minimize the free energy at the interface (Letchford & Burt, 2007). The formation of polymeric nanoparticles in water results from self-assembly of nanospheres containing hydrophobic domains in the core and hydrophilic domains in the shell. The micellar core creates a microenvironment which can contain many hydrophobic drugs. At the same time, the hydrophilic micellar shell provides a stabilizing interface between the hydrophobic core and the aqueous medium (Song, Zhang, Gan, Zhou, & Zhang, 2011).

Octenyl succinic anhydride (OSA)-starch is prepared by esterification of native starch or starch derivatives using OSA. It is one of the most important amphiphilic starch derivatives (Liu et al., 2008). Through esterification, the hydrophobicity of OSA is introduced and the hydrophilicity of starch backbone is retained. Although many studies on the esterification of starch have been reported, the degree of substitution (DS) of OSA-starch for food applications is typically low, and the highest DS value of OSA-starch is reported to be of the order of 0.1 in the literature (Shogren, Viswanathan, Felker, & Gross, 2000; Viswanathan, 1999). This could be explained by two reasons. Firstly, starch is polymeric molecule and forms granular structure. This means that the granule structure restricts the diffusion of reactants to the interior of the granule due to which the reaction occurs mostly on the surface. Secondly, the reaction is performed under heterogeneous conditions, which reduces the reaction efficiency between OSA and starch. For preparing OSA-starch with high DS, Shogren et al. (Shogren, 2003) performed the modification with OSA under high temperature/pressure conditions and DS of about 0.5 was achieved at 180 °C. Another way to obtain OSA-starch with a DS of 0.3 was performed using a microwave-assisted modification (Biswas, Shogren, Kim, & Willett, 2006). However, these methods are confined to improve the DS. Recently, Wang, Li, et al. (2011) used pyridine as reaction medium. These authors obtained DS values of 0.2–1.3 using OSA/starch weight ratios up to 5/1. This is because pyridine increases the initial reactivity of the starch grains and also acts as a catalyst in starch and OSA reaction as it forms a succinyl-pyridinium intermediate. This intermediate much more readily reacts with hydroxyl groups than the non-activated OSA molecules (Viswanathan, 1999).

Despite the above mentioned advantages of preparing self-assembled nanospheres using dialysis method, there are no studies on the formation and characteristics of self-assembled OSA-starch nanospheres prepared using dialysis method. This is mainly due to low degree of substation (DS) of OSA in starch as mentioned above. The low DS tends to favor supramolecular interactions through hydrogen bonds. These interactions lead to aggregation and ultimate collapse of the nanoparticles (Hornig, Heinze, Hesse, & Liebert, 2005).

In this study, we prepared OSA-starch with high DS through esterification of waxy corn starch with OSA using pyridine as the solvent. Subsequently, we prepared self-assembled nanospheres from the amphiphilic OSA-starch which had high DS. The initial water dialysis method was used to produce these nanospheres. The starch nanospheres produced in this way had narrow size distribution. We also quantified and explained the effect of DS, initial water content, concentration of OSA-starch on the particle size distribution and microstructure of the OSA-starch nanospheres.

2. Materials and methods

2.1. Materials

Waxy corn starch was purchased from Dezhou Dacheng Food Co., Ltd. (Shandong, China). Octenyl succinic anhydride (OSA) was obtained from Nanjing Golden Chemical Co., Ltd. (Nanjing, China). Dimethyl sulfoxide (DMSO) was purchased from Tianjin Fuyu Fine Chemical Co., Ltd. (Tianjin, China) and was dried over molecular sieves and then vacuum-distilled. Pyridine was purchased from Chinasun Specialty Products Co., Ltd. (Jiangsu, China). Dialysis tube with a molecular weight cut-off of 8000–15,000 g/mol was purchased from MYM Biological Technology Company (USA). DMSO-d_6 (99.9% atom D) and trifluoroacetic acid-d_1 (TFA-d_1, 99.5% atom D) were from Cambridge Isotope Laboratories, Inc. (USA). All other chemicals were of analytical grade and were used as received.

2.2. Preparation of OSA-starch

OSA-starch was used as the main material to synthesize nanoparticles. It was prepared by modifying partially hydrolyzed waxy maize starch with n-octenyl succinic anhydride.

Partially hydrolyzed waxy maize starch was prepared by an acid-alcohol treatment (Chang, Lin, & Lii, 2004). One hundred grams of waxy maize starch (dry basis) were suspended in 400 mL ethanol solution (90%, v/v). The reaction was started by adding 4 mL concentrated HCl (36%, w/w) and heating the mixture to 65 °C and maintaining this temperature for 1 h. The reaction was stopped by adding 28 mL of 1 mol/L Na_2CO_3 solution. The fully reacted mixture was cooled in an ice-bath for 5 min and centrifuged at 3500 rpm for 5 min. The precipitate was washed four times with ethanol (50%, v/v) and precipitated through centrifugation. The final precipitate was dried at 40 °C using a fan forced oven for 24 h.

The partially hydrolyzed starch (15 g, d.b.) was suspended in 150 mL pyridine solution in a 250 mL three-neck round bottom flask. This suspension was stirred using a mechanical stirrer at 85 °C for 2 h to activate the starch. Subsequently, 15 g (OSA-to-starch ratio = 1:1) to 75 g (OSA-to-starch ratio = 5:1) liquid OSA was added into the flask through a funnel. The reaction between OSA and starch was allowed to continue for 2.5 h at 85 °C. Once the reaction was completed, the flask together with its content was cooled to room temperature. The OSA-starch obtained in this way was washed three times with distilled water and twice with 70% (v/v) alcohol by means of centrifugation. This fully washed OSA-starch precipitate was oven dried at 40 °C for 24 h.

2.3. Synthesis of starch nanospheres

As mentioned in Section 1, dialysis is one of the most extensively used methods of producing self-assembled polymer nanospheres. In this work, starch nanospheres were synthesized by using water dialysis method as reported by Liu et al. (2007) with some minor modifications.

For a typical batch, a sample of 20 mg OSA-starch was placed in 10 mL of DMSO and heated to 95 °C in a water bath until the solution became clear. Then, 10 mL distilled water (referred to as initial water) was added into the OSA-starch–DMSO solution under continuous stirring. Subsequently, this mixture was transferred to the dialysis tube (molecular cut off 8000–15,000 g/mol) and dialyzed against 1.0 L of distilled water at intervals of 2 h in the first 6 h, then at intervals of 6 h during the following 42 h. This suspension containing OSA-starch nanospheres was stored at 4 °C before further analysis.

2.4. Characterization of OSA-starch

2.4.1. Fourier transform infrared (FTIR) analysis

The FTIR analysis was performed using a FTIR Bruker-Vector 33 spectrometer (Bruker, Germany). Native and OSA-starch samples were mixed separately with analytical grade KBr. The KBr-to-starch (or OSA-starch) ratio was maintained at 5/200 (w/w) and pressed into pellet. Spectral scanning was done within the wavenumbers of 4000 cm^{-1} and 400 cm^{-1}. Prior to recording, the baseline was adjusted against the KBr background.

2.4.2. Determination of degree of substitution (DS)

The DS of OSA-starch was determined by using a ^1H nuclear magnetic resonance (^1H NMR) spectrometer (Bruker, Germany) at 600 MHz. Three milligrams of native starch and OSA-starch samples were individually dissolved in 0.7 mL DMSO-d_6, and then added to 20 μL TFA-d_1 before the determination. Tizzotti, Sweedman, Tang, Schaefer, and Gilbert (2011) demonstrated that the addition of a very low amount of TFA-d_1 to the medium causes the exchangeable protons of starch hydroxyl groups and residual water to shift to higher frequency and produce clear and well-defined ^1H NMR spectra.

DS is calculated using Eq. (1):

$$DS = \frac{I_{0,86}}{3(I_{\alpha\text{-}1,6} + I_{\alpha\text{-}1,4} + I_{r\text{-}e})} \quad (1)$$

where $I_{0.86}$ is the ^1H NMR integral of the signal of the CH_3 group of OSA at 0.86 ppm, $I_{r\text{-}e}$ corresponds to the reducing ends of starch chains (α and β reducing-end signals at 5.12 and 4.05 ppm, respectively), $I_{\alpha\text{-}1,4}$ and $I_{\alpha\text{-}1,6}$ are the ^1H NMR integrals of internal α-(1 → 4) and α-(1 → 6) linkages at 5.31 and 4.78 ppm, respectively.

2.5. Determination of critical aggregation concentration (CAC)

Pyrene was used as a hydrophobic fluorescent probe. This is because it is known to be highly sensitive to the local polarity of microenvironment (Kalyanasundaram & Thomas, 1977). In the polar medium, pyrene has weak fluorescence intensity, however, it has a strong intensity in the nonpolar medium. Once the polymer aggregates form nanospheres (micelles) and pyrene preferentially partition into hydrophobic part, this results into change in the fluorescence intensity (Qiu, Feng, Wu, Zhang, & Zhuo, 2009). Tizzotti, Sweedman, Schäfer, and Gilbert (2013) demonstrated that there were no false positives caused by complexes of linear starch and pyrene. Fluorescent pyrene is an appropriate technique to determine the CAC of OSA-starch.

The measurement procedure was adapted from Qiu et al. (2009) with slight modification. One milliliter of pyrene solution (6×10^{-6} mol pyrene/L acetone) was added to containers. After the acetone was fully evaporated, 10 mL of OSA-starch nanospheres (at different concentrations) were added to the containers which contained pyrene residue. The aqueous sample solutions contained 6×10^{-7} mol/L of pyrene residue. To attain the equilibrium solubility of pyrene in the aqueous phase, the mixture was kept at room temperature for 24 h. Fluorescence spectra were recorded using an F-7000 fluorescence spectrophotometer (HITACHI, Japan). Excitation was carried at 335 nm, and emission spectra were recorded in between 350 nm and 450 nm. Excitation and emission bandwidths were 5 nm and 10 nm, respectively.

2.6. Characterization of starch nanospheres

2.6.1. Morphology of starch nanospheres

The morphology of the starch nanospheres was observed using a scanning electron microscope (Evols 10, Carl Zeiss, Germany). The suspension of starch nanoparticles was spread on the surface of a glass flake. The water content was allowed to evaporate at 40 °C for 6 h. The glass flake was stuck to the metal stub and was coated by gold before scanning.

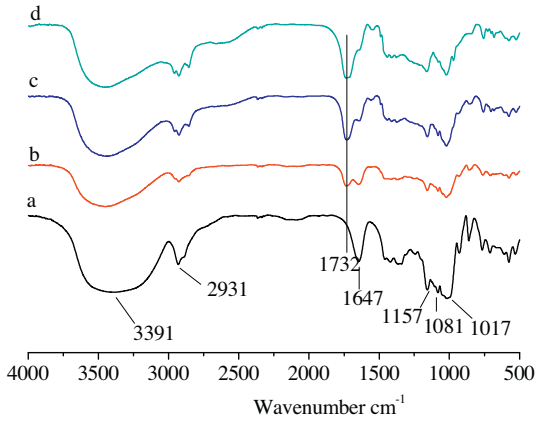

Fig. 1. FTIR spectra of: (a) native starch; (b) OSA-starch with DS = 0.14; (c) OSA-starch with DS = 0.36; (d) OSA-starch with DS = 0.67.

2.6.2. Dynamic light scattering (DLS) analysis

The average size (*Z*-average size) and polydispersity index (PDI) of starch nanospheres in water were measured using a dynamic light scattering particle size analyzer (Malvern Nano-ZS90, UK). DLS measurements were carried out at 25 °C with scattering angle fixed at 90°. Before these measurements, all samples were filtered to remove large aggregates by a polyether sulfone microfiltration membrane (0.45 μm, ANPEL Scientific Instrument, China).

2.7. Statistical analysis

All the tests were carried out in triplicate and the size of nanosphere is expressed as mean ± standard deviation. Statistical analysis was performed using EXCEL™ 2007 (Microsoft, USA) and significant difference among the means was estimated at 95% confidence level ($P < 0.05$).

3. Results and discussion

3.1. Preparation and characterization of OSA-starch

In order to prepare OSA-starch with high DS, we firstly hydrolyzed the native starch by using acid-alcohol treatment to decrease the resistance of starch's granular structure in reacting with OSA. Then, we carried out the esterification reaction in pyridine solvent. The esterification allows the reaction with starch to proceed more uniformly and efficiently.

Fig. 1 shows the FTIR spectra of native starch and OSA-starch samples. In the spectrum of native starch (Fig. 1a), the C—O bond stretching vibrations of the anhydroglucose unit (AGU) showed several discernible absorption peaks at 1157 cm^{-1}, 1081 cm^{-1} and 1017 cm^{-1}. Strong absorbance peaks due to O—H and C—H stretching vibrations were observed at 3391 cm^{-1} and 2931 cm^{-1}, respectively (Kačuráková & Wilson, 2001). Unlike native starch, the FTIR spectra of OSA-starch exhibited strong absorbance band at 1732 cm^{-1} irrespective of degree of substitution (Fig. 1b–d), due to the vibration caused by perturbation in carbonyl C=O symmetry. This new absorption band indicated that the starch was esterified in the process. The intensity of the peak at 1732 cm^{-1} increased remarkably.

The ^1H NMR has been used extensively to quantitatively determine the amount of octenyl succinyl groups attached to starch. Fig. 2 shows the typical ^1H NMR spectrum of native starch (Fig. 2A)

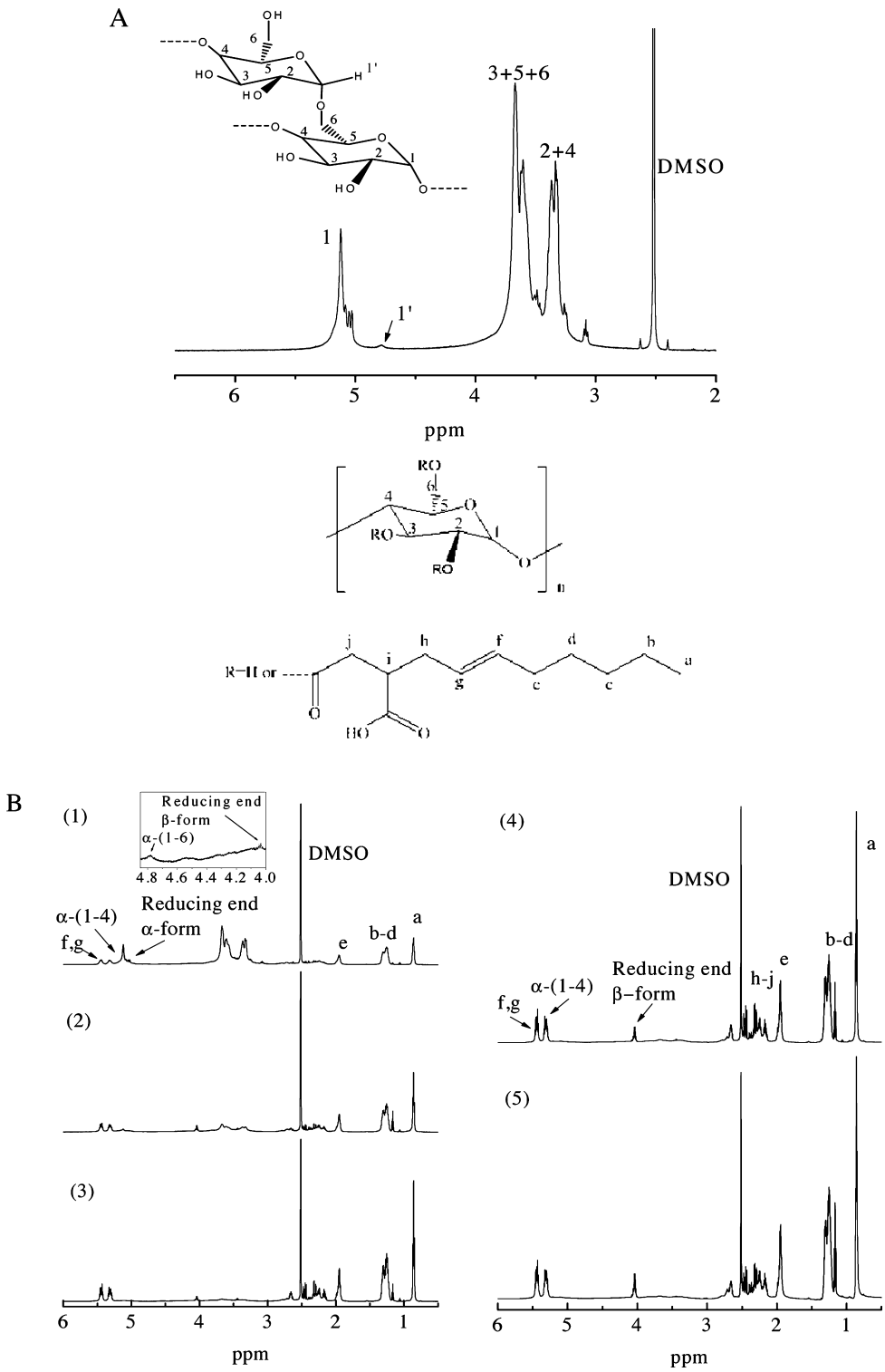

Fig. 2. ^{1}H NMR spectra of (A) native starch and (B) OSA-starch with different OSA/starch ratio: (1) 1:1, (2) 2:1, (3) 3:1, (4) 4:1, and (5) 5:1.

and OSA-starch samples (Fig. 2B). ^{1}H chemical shifts of protons observed at 5.12 ppm, 4.78 ppm were assigned to 1 and 1′, respectively, and the signals of the other protons 2–6 are present in this region (3.20–3.91 ppm) (Namazi et al., 2011; Tizzotti et al., 2011). Fig. 2B also shows that the peak (5.20–5.35) for the anomeric protons of internal α-1,4-linkages became broader as the level of OSA substitution increased, which could be attributed to the increase in substitution of octenyl succinyl group at the O-2 position. A similar phenomenon was observed by Bai, Shi, Herrera, and Prakash (2011). Moreover, the ^{1}H NMR spectra of OSA-starch samples showed three protons of the terminal methyl group of the acyl chain around 0.86 ppm (Fig. 2B). This is because the esterification process introduced acyl groups into starch, which changed the proton resonances of anhydroglucose unit. This result also demonstrated the presence of OSA.

The degree of modification by or substitution of OSA in OSA-starch was determined by the OSA/starch ratio (Table 1). As can be seen from this table, when the OSA/starch ratios were 1:1, 2:1

Table 1
Degree of substitution (DS) of OSA-starch at different OSA/starch ratio.

Sample code	OSA/starch ratio	DS
OSAS-1	1:1	0.14
OSAS-2	2:1	0.36
OSAS-3	3:1	0.67
OSAS-4	4:1	0.72
OSAS-5	5:1	0.74

and 3:1, the DS values of OSA-starch were 0.14, 0.36 and 0.67 respectively, which is a significant ($P < 0.05$) increase. This increase in DS can be interpreted in terms of greater availability of OSA molecules to react with the hydroxyl groups of starch molecules (Jeon, Lowell, & Gross, 1999). However, the DS values of OSA-starch increased only marginally (0.72–0.74) when the OSA/starch ratio was increased from 3:1 to 5:1. This observation suggested that the OSA/starch ratio >3:1 did not significantly ($P > 0.05$) increased the esterification reaction further under the prevailing conditions. It is expected that the DS is also affected by the reaction temperature and reaction time. It has been reported that a DS value as high as 1.21 was obtained when the OSA/starch ratio, reaction time and reaction temperature were maintained at 4:1, 3 h, 85 °C, respectively (Wang, Li, et al., 2011).

3.2. Determination of critical aggregation concentration (CAC)

The CAC is one of the most important parameters in synthesizing polymeric nanospheres. This is because amphiphilic polymers could only form self-assembled micelles above the CAC of these polymers in aqueous medium. As detailed in Section 2.5, the self-assembling behavior of the OSA-starch in water was investigated using fluorometry using pyrene as a fluorescent probe. The fluorescence intensity of pyrene increases with the increase in polymer concentration. The shift of I_1 (373 nm) and I_3 (383 nm) indicates the formation of hydrophobic domains by a polymer in aqueous medium. This shift brings about changes in emission spectrum as pyrene gets transferred into these hydrophobic domains (Hornig & Heinze, 2007). Thus, the CAC value could be determined by the crossover point of two straights lines as indicated in Fig. 3.

As can be seen from Fig. 3, the values of I_1/I_3 remained nearly unchanged at lower concentrations. When the concentration starts to increase, the intensity ratio starts to decrease which indicates to the formation of micelles and the transfer of pyrene into hydrophobic core of the micelles (Lu, Zhang, Wang, & Chen, 2011). The CAC values of OSA-starch at DS values of 0.67, 0.72 and 0.74 were 0.05 mg/mL, 0.0011 mg/mL and 0.0004 mg/mL, respectively. These data suggest that the CAC value is highly sensitive to the DS value and it decreases remarkably even with slight increase in the DS value. This is probably due to the relatively stronger hydrophobic interactions at higher DS values. The presence of hydrophobic core in these starch nanospheres makes very useful vehicles for microencapsulation of hydrophobic substances (Brannon-Peppas, 1995).

3.3. Morphology of starch nanospheres

Fig. 4 shows the SEM micrographs of OSA-starch nanospheres prepared at different DS. As shown, aggregates of irregularly shaped particles were observed when the DS was 0.14 (Fig. 4a). Similar morphological feature was observed when the DS was 0.36 (image not shown). However, when the DS of OSA-starch was higher (0.67, 0.72 and 0.74), neatly spherical and unagglomerated starch nanospheres with good dispersibility were synthesized (Fig. 4b–d). The particle size of these nanospheres ranged from 50 nm to 200 nm (Table 2). The particle size of these nanospheres decreased when the DS increased.

Table 2
Effect of degree of substitution (DS) on the particle size and polydispersity index (PDI) of nanospheres.

DS	*Z*-average size (nm)	PDI
0.67	127.67 ± 0.21	0.153 ± 0.071
0.72	78.78 ± 3.78	0.145 ± 0.057
0.74	65.32 ± 5.68	0.128 ± 0.020

Other reaction conditions: initial water content 10 mL and OSA-starch concentration 2 mg/mL.

The fact that higher DS favors the synthesis of spherically shaped starch nanospheres with good dispersibility can be explained as follows. OSA-starch exhibits an amphiphilic characteristic in water. OSA-starch can self-assemble into nano-sized aggregates due to the self-association of hydrophobic alkyl segment as well as hydrophilic interactions of carboxyl and hydroxyl groups with water. Starch backbone containing higher number of long chain alkyl segments tends to produce nanospheres more effectively. Our results are in accordance with those of Hornig et al. (2005) who reported that irregularly shaped particles were obtained after dialysis of a dextran furoate pyroglutamate at low DS ($DS_{Fur} = 0.12$, $DS_{Pyr} = 1.13$). When the remaining hydroxyl groups were fully modified, spherical nanoparticles were obtained. Higher DS means that there will be lower numbers of hydroxyl groups available for reaction. The intensive and uncontrolled formation of hydrogen bond decreases when the number of hydroxyl group is low, which ultimately prevents the aggregation of nanospheres.

3.4. Mechanism of synthesis of starch nanospheres

Starch nanospheres were synthesized by using dialysis method. The amphiphilic nature of OSA-starch provides an opportunity to produce nanospheres in water. In this method the solution of a polymer in water–miscible organic solvent is introduced into a dialysis tube and placed in water. When the dialysis progresses, the water content in the dialysis tube increases gradually and facilitates the self-assembly of amphiphilic OSA-starch into nanospheres. Addition of initial water into the organic polymer solution before dialysis is one of the efficient ways to control the speed of dialysis and the rate of synthesis of nanospheres. The DS of OSA-starch, the initial water content and the OSA-starch concentration influence the size of the resulting starch nanospheres.

3.4.1. Effect of DS on the size of starch nanospheres

Particle size is one of the most important characteristics of nanoparticles. It has been reported that in order to measure the size of nanoparticles reliably, the diffusion coefficients should be measured at various angles and concentrations, then these values should be extrapolated to infinite dilution and to zero scattering angle (Galinsky & Burchard, 1997; Ioan, Aberle, & Burchard, 2001; Takahashi, Kato, Saito, Matsuyama, & Kinugasa, 2008). It has also been reported that 40° is an optimal angle for DLS measurement in certain systems such as Sigma starch/DMSO. Below 40°, the background noise increases and the data is affected (Dona et al., 2007). The key objective of this study was to quantify the effect of formulation/process parameters on the size of the resultant nanoparticles. This could be adequately achieved by comparing values obtained by DLS analyzer constructed to give a fixed scattering angle of 90° (Chiou, Fellows, Gilbert, & Fitzgerald, 2005). Hence, DLS measurements were carried out at a fixed angle of 90° using Malvern Nano-ZS90 in this study. However, it has to be pointed out that the particle size determined by the software assumes that the Brownian motion is that of impenetrable hard spheres, whereas our starch

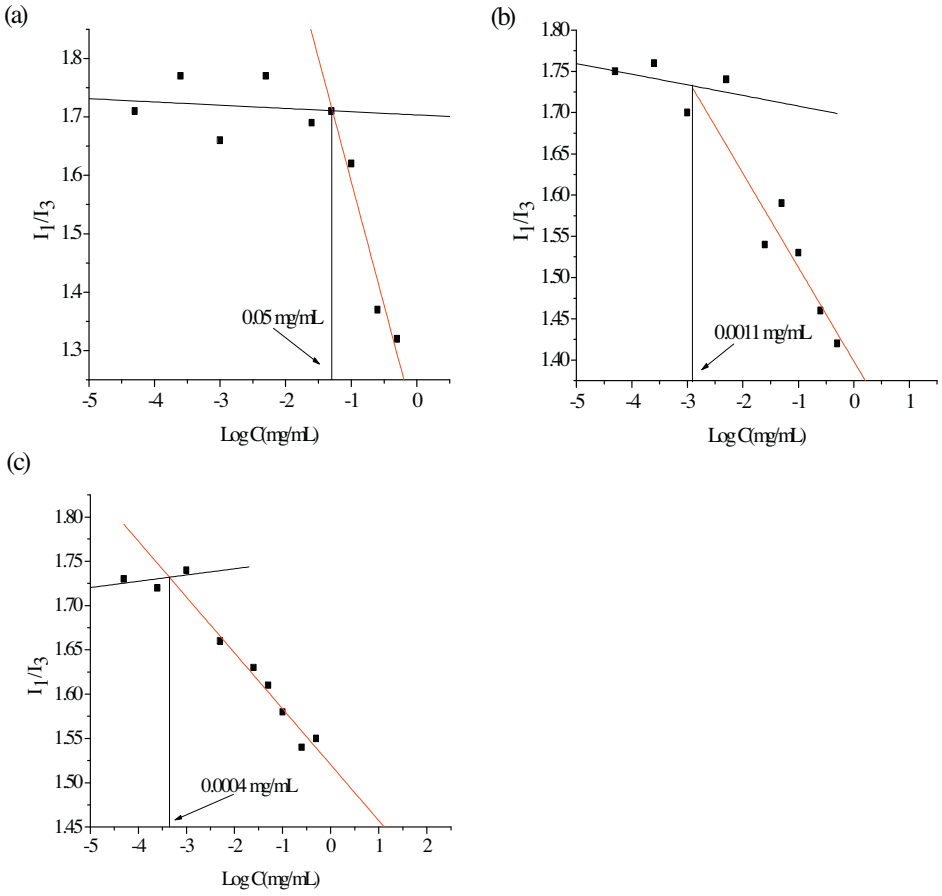

Fig. 3. Critical aggregation concentration (CAC) of OSA-starch with different degree of substitution (DS): (a) DS = 0.67, (b) DS = 0.72, and (c) DS = 0.74.

nanospheres could be classified as 'soft nanospheres' of complex branched polymers (Gaborieau, Gilbert, Gray-Weale, Hernandez, & Castignolles, 2007).

The effect of DS of OSA-starch on the size and polydispersity index (PDI) of starch nanospheres is depicted by the data presented in Table 2. As can be seen, the size of starch nanospheres decreased significantly ($P < 0.05$) with the increase in the DS. The *Z*-average size decreased from 127.67 nm to 78.78 nm and then to 65.32 nm when the DS increased from 0.67 to 0.72 and then to 0.74, respectively. It was noteworthy that PDI from 0.153 to 0.128 can be observed as the DS increased from 0.67 to 0.74.

The particle size data broadly agrees with the particle morphological features observed from SEM micrographs (Section 3.3). The decrease in particle size due to increase in the DS can be explained from the fact that the hydrophobic interaction becomes much stronger when the proportion of OSA in OSA-starch increases. This increase in the hydrophobicity leads to the formation of compact hydrophobic inner core which favors the formation of smaller nanospheres (Wang, Li, et al., 2011; Wang, Zhang, et al., 2011).

3.4.2. *Effect of initial water content on the size of starch nanospheres*

In order to study the effect of initial water content on the size of nanospheres, 0–20 mL water was added into OSA-starch–DMSO solution before dialysis. The particle size and PDI of nanospheres were measured by DLS and the results are presented in Table 3. This table reveals that the *Z*-average size decreased from 111.77 nm to 75.94 nm as the initial water increased from 0 mL to 20 mL.

The speed of dialysis can be controlled by varying the initial water content in OSA-starch/water/DMSO mixture. Liu et al. produced poly(DL-lactic acid) (PLA) nanospheres from PLA solution using initial water dialysis method and reported that the initial water content was one of the most important factors controlling the size of PLA nanospheres. When the initial water content in the PLA solution was over 20%, it slowed down the subsequent dialysis speed to such an extent that only more compact PLA chains were obtained (Liu et al., 2007). This reasoning can be used to explain our results. As can be seen from Table 3, the *Z*-average size decreased when the initial water content increased. This can be attributed the formation of more compact nanospheres at lower dialysis speed caused by higher water content in OSA-starch/water/DMSO mixtures.

Table 3
Effect of initial water content on the particle size and polydispersity index (PDI) of nanospheres.

Initial water content (mL)	*Z*-average size (nm)	PDI
0	111.77 ± 1.56	0.047 ± 0.025
2	107.40 ± 1.61	0.043 ± 0.010
5	83.11 ± 3.42	0.141 ± 0.013
10	78.78 ± 3.78	0.145 ± 0.057
20	75.94 ± 4.72	0.125 ± 0.003

Other reaction conditions: degree of substitution (DS) 0.72 and OSA-starch concentration 2 mg/mL.

3.4.3. *Effect of OSA-starch concentration on the size of starch nanospheres*

The effect of variation of OSA-starch concentration on the mean particle size and PDI of nanospheres was investigated by varying the OSA-starch concentration (1, 2, 5, 10 and 15 mg/mL) and the results are shown in Table 4. The 'OSA-starch concentration'

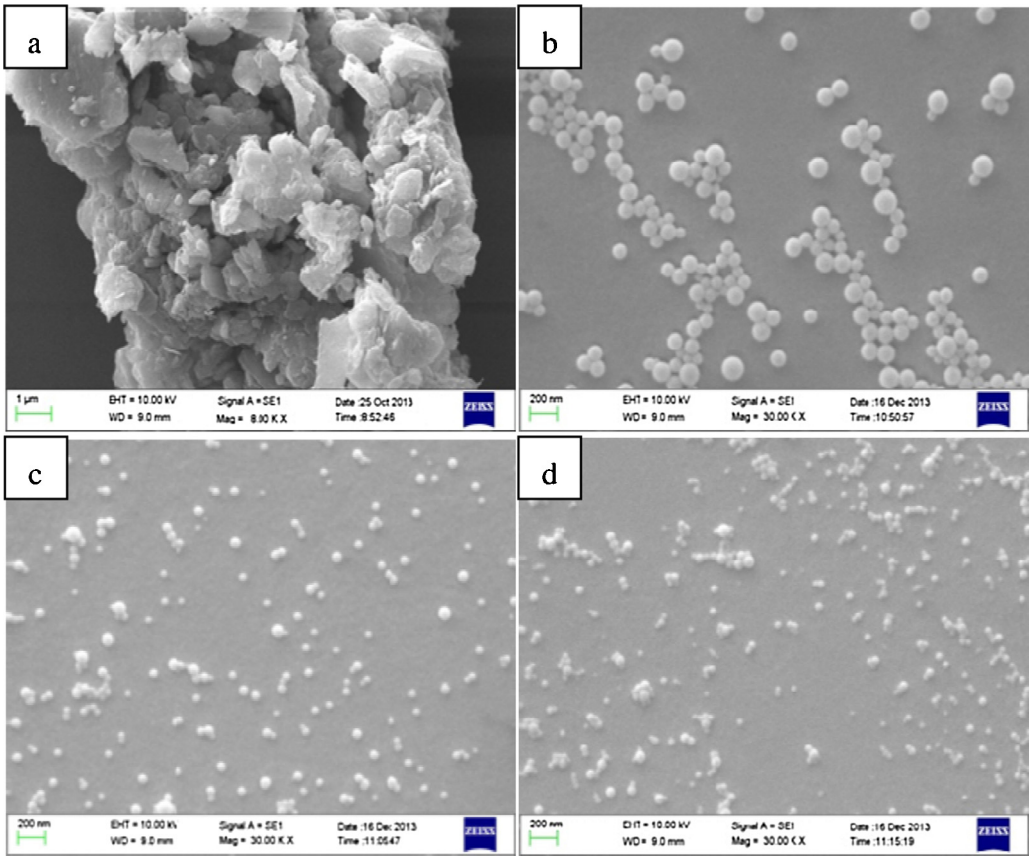

Fig. 4. SEM images of starch nanospheres based on OSA-starch derivatives with different degree of substitution (DS): (a) DS = 0.14, (b) DS = 0.67, (c) DS = 0.72, and (d) DS = 0.74.

Table 4
Effect of OSA-starch concentration on the particle size and polydispersity index (PDI) of nanospheres.

OSA-starch concentration (mg/mL)	Particle size (nm)	PDI
1	74.23 ± 1.46	0.182 ± 0.024
2	78.78 ± 3.78	0.145 ± 0.057
5	82.21 ± 6.72	0.154 ± 0.022
10	98.24 ± 6.63	0.097 ± 0.076
15	120.9 ± 7.26	0.125 ± 0.086

Other reaction conditions: degree of substitution (DS) 0.72 and initial water content 10 mL.

here refers to the concentration of OSA-starch in DMSO before adding the initial water. An increase of OSA-starch concentration from 1 mg/mL to 15 mg/mL increased the particle size of starch nanospheres from 74.23 nm to 120.9 nm (Table 4). Moreover, a somewhat smaller value of PDI is achieved for the nanospheres with a higher OSA-starch concentration (Table 4).

These results indicate that the OSA-starch concentration is an important factor affecting the size of nanospheres. This phenomenon can be explained on the basis that an increase in the OSA-starch concentration increases the viscosity of the mixture undergoing dialysis which leads to the formation of larger particles. Higher viscosity favors the formation of larger size droplets which ultimately produces larger nanospheres (Chorny, Fishbein, Danenberg, & Golomb, 2002).

4. Conclusions

OSA-starch samples with varying degree of substitution (DS) were produced by using hydrophobic modification. The critical aggregation concentration (CAC) of OSA-starch in aqueous solution decreased significantly ($P < 0.05$) from 0.05 mg/mL to 0.0004 mg/mL when the DS increased only marginally from 0.67 to 0.74. Starch nanospheres smaller than 200 nm were successfully obtained from OSA-starch using initial water dialysis method. Higher values of DS in OSA-starch favored the synthesis of starch nanospheres having spherical shape and sharp edge. The increase in DS of OSA-starch and increase in initial water content significantly ($P < 0.05$) decreased the particle size. The increase in the concentration of OSA-starch significantly ($P < 0.05$) increased the size of the OSA-starch nanospheres. These spherical starch nanospheres could potentially be used to microencapsulate hydrophobic drugs.

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