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Preparation and characterization of crosslinked starch microspheres using a two-stage water-in-water emulsion method

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ABSTRACT

Crosslinked starch microspheres (CSMs) were prepared using a novel two-stage water-in-water emulsion method (WWEM). The results show that the yield and crosslinking density of CSMs were affected significantly ($p < 0.05$) by molecular weight and concentration of polyethylene glycol (PEG) in continuous phase, crosslinker concentration as well as incubation time. The increase in any one of these variables resulted into increase in the crosslinking density. The yield and crosslinking density of CSMs prepared by WWEM (WWEM-CSMs) were lower than those prepared by water-in-oil emulsion method (WOEM). The morphology, chemical composition and amorphous/crystalline nature of the CSMs were studied by scanning electron microscopy (SEM), Fourier transform infrared (FTIR) spectroscopy and X-ray diffraction (XRD). SEM analysis indicated that the WWEM-CSMs were spherical, compact and nonporous in nature. FTIR and XRD analysis indicated that WWEM-CSMs were PEG-free and amorphous in structure. These characteristics were very similar to the characteristics of CSMs prepared by WOEM.

1. Introduction

There is an increasing attention in the production and characterization of microspheres made from both the natural and synthetic polymers targeted to medical and pharmaceutical applications. Among various polymeric microspheres, starch microspheres have been widely investigated as hemostatic (Morise et al., 2006), embolic (Hoffmann et al., 2009) agents and drug carriers (Mao, Chen, Wei, Liu, & Bi, 2004) because of their total biodegradability, biocompatibility, non-toxicity, as well as cost-effectiveness.

In general, starch microspheres intended for medical and pharmaceutical application are synthesized using emulsion-based method. In this method, droplets of starch solution (or suspension) are solidified in a liquid continuous phase to form spherical microspheres. The crosslinking has been a predominant approach of solidification (Dziechciarek, van Soest & Philipse, 2002; Li, Wang, Li, Bhandari, et al., 2009; Yu & Liu, 1994). The solidification has also been achieved through recrystallization process in some cases (Elfstrand, Eliasson, Jönsson, Reslow, & Wahlgren, 2006; Elfstrand et al., 2007). Compared to starch microspheres obtained from recrystallization process, the crosslinked starch microspheres (CSMs) are considered to be more attractive because the swelling property and degradability of CSMs can be designed conveniently by controlling the degree of crosslinking reaction.

In the past, the water-in-oil (w/o) emulsion method (WOEM) was usually employed to prepare CSMs. However, the use of toxic organic solvents such as cyclohexane (Hamdi, Ponchel & Duchêne, 1998), toluene and chloroform (Stertman, Lundgren & Sjöholm, 2006) is a major concern regarding the safe production and usage of the microspheres. To address this concern, a novel water-in-water (w/w) emulsion method (WWEM) was invented and applied to prepare dextrin microspheres (Franssen & Hennink, 1998). This method avoids the use of toxic organic solvents. In our previous study, CSMs were prepared successfully by WWEM, and the effects of the process and formulation parameters on the particle size of CSMs were evaluated using Plackett–Burman screening design methodology (Li, Wang, Li, Chiu, et al., 2009).

Until now, publications regarding the application of CSMs prepared by WWEM (WWEM-CSMs) are still quite limited. Since the application of CSMs prepared by WOEM (WOEM-CSMs) had been studied intensively (Atyabi, Manoochehri, Moghadam, & Dinarvand, 2006; Fang et al., 2008; Malafaya, Stappers & Reis, 2006), we believe that the elucidating the distinctive features of WWEM, as well as the similarities and differences of CSMs prepared by WWEM and WOEM, can promote the application of WWEM-CSMs.

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However, to our knowledge there are no publications in open literature presenting the comparative studies on these two methods. Furthermore, in our opinion, the preparation of CSMs using WWEM could become more energy efficient with some judicious modification of the production procedure. It has been reported that mechanical stirring of about 5 h is required to avoid the aggregation of microspheres when water-in-oil method (WOEM) is used to prepare crosslinked starch microspheres (Fang et al., 2008). On the other hand, as the WWEM is water-in-water (w/w) emulsion method and the emulsions obtained from this method are relatively stable, shorter (of the order of 6 min) stirring time is sufficient.

In this study, we modified the WWEM such a way that it consisted of a two-stage procedure: a short stirring stage and a static incubation stage. The effect of this modified procedure and formulation variables on yield and crosslinking density of CSMs was studied. Besides, the morphology and structural organization of CSMs prepared by WWEM and WOEM were characterized by scanning electron microscopy (SEM), Fourier transform infrared (FTIR) spectroscopy and X-ray diffraction (XRD).

2. Materials and methods

2.1. Materials

Soluble starch was purchased from Aoboxing Biotech Co. Ltd. (Beijing, China). Commercial soluble starch used in this study is produced by the partial acid hydrolysis of potato starch. It is readily soluble in hot water, but insoluble in cold water. Trisodium trimetaphosphate (TSTP) was obtained from Alfa Aesar (Tianjin, China). Polyethylene glycol (PEG) 10000 was purchased from Shantou Xilong Chemical Company (Guangdong, China). PEG 20000 was obtained from Yili Fine Chemical Co. Ltd. (Beijing, China). Other reagents and solvents were of analytical grade and were used as received.

2.2. Preparation of CSMs by WWEM

CSMs were prepared by a two-stage water-in-water emulsion method (WWEM). There were three main steps involved in the preparation procedure:

(1) Emulsification: 18 mL of starch solution (initial dispersed phase) containing 16% soluble starch (starch/H_2O, w/w), 2% NaOH (NaOH/H_2O, w/w) and TSTP was poured slowly into a beaker containing 90 mL of PEG solution (initial continuous phase) under constant mechanical stirring (500 rpm) for 6 min using a water bath maintained at 30 °C to prepare water-in-water emulsion.
(2) Incubation: after emulsification, the beaker was transferred to an incubator (30 °C) and kept there without agitation (still).
(3) After incubation, approximately 100 mL of deionized water was used to dilute the emulsion, and the resulting microspheres (CSMs) were collected by centrifugation (5000 rpm, 5 min). Subsequently, the collected CSMS were washed with deionized water and absolute alcohol for 3 times. Finally, the microspheres were dried under vacuum at 60 °C for 12 h and kept in sealed containers prior to their use.

2.3. Preparation of CSMs by WOEM

For comparison purpose, we also prepared CSMs by WOEM as follows: 90 mL of paraffin liquid and 4.5 g Span 80 were poured into a beaker and heated to 30 °C using a water bath until the Span 80 dissolved completely. Paraffin liquid solution containing Span 80 was employed as initial continuous phase. The 18 mL of starch solution (or suspension) contained of 16% soluble starch (starch/H_2O, w/w), 2% NaOH (NaOH/H_2O, w/w) and 6% TSTP (TSTP/H_2O, w/w) was poured slowly into the beaker and subsequently stirred at 500 rpm for 3 h. The starch microspheres (CSMs) produced in this way were collected by centrifugation (5000 rpm, 5 min), subsequently washed with acetone, light petroleum and deionized water for 3 times, in sequence and then dehydrated by absolute alcohol. Finally, the microspheres were dried under vacuum at 60 °C for 12 h and kept in sealed containers before use.

2.4. Determination of yield of CSMs

The yield of microspheres was calculated using Eq. (1) given below.

$$\text{Yield (\%)} = \frac{M}{M_1 + M_2} \times 100 \tag{1}$$

where M, M_1 and M_2 are the masses of starch microspheres, soluble starch and TSTP, respectively.

2.5. Estimation of crosslinking density of CSMs

It has been reported that the relative amount of adsorbed methylene blue (RMB) of TSTP-crosslinked microspheres is proportional to their crosslinking density (Dulong et al., 2004). The higher the adsorbed RMB, the higher would be the crosslinking density. This quantification technique provides an indirect albeit convenient way to evaluate the crosslinking degree of microspheres or hydrogels crosslinked by TSTP (Liu, Fan, Wang, & He, 2007). In this study, RMB was employed to estimate the crosslinking degree of CSMs.

One hundred and fifty milligrams of CSMs were placed in a centrifuge tube containing 30 mL of methylene blue (MB) solution (1 mg/mL), and were incubated in dark for 1 h. Subsequently, the CSMs were separated from the MB solution by using a syringe driven filter (0.2 μm). The absorbance of the filtered MB solution was studied using a UV/VIS spectrophotometer (TU-1810, Beijing Purkinje General Instrument Co. Ltd., China) at 665 nm. The RMB was calculated using Eq. (2) as:

$$\text{RMB} = \frac{C_0 - C_1}{C_0 - C_2} \tag{2}$$

where C_0 is the initial concentration of methylene blue before any adsorption occurred. Similarly, C_1 and C_2 are the concentrations of the methylene blue in solutions containing CSMs and only soluble starch, respectively.

2.6. Scanning electron microscope (SEM) analysis

The morphology of CSMs prepared by WWEM and WOEM was studied using a scanning electron microscopy (KYKY-2800, KYKY, China) operating at 25 kV. The samples for SEM studies were mounted on metal stubs with double-side adhesive, and coated with gold in vacuum using an IB-3 ion coater (Eiko, Japan).

2.7. Fourier transform infrared (FTIR) spectroscopy analysis

The FTIR measurements were performed using a Nicolet iS10 FTIR instrument (Thermo Scientific, USA) equipped with a SMART iTR accessory. The powder samples were scattered on the platform of accessory and scanned in between 4000 and 525 cm^{-1} range. Prior to recording, the baseline was adjusted against the atmosphere background.

2.8. X-ray diffraction (XRD) analysis

The X-ray diffraction experiments were conducted using XD-2 XRD instrument (Beijing Purkinje General Instrument Co. Ltd., China). The X-ray powder diffraction analyses were performed at 36 kV and 20 mA with nickel-filtered Cu Ka (wavelength 1.54050 Å) radiation. The scattered intensities were measured with a scintillation counter. Powdered samples were scanned from 5° to 35° (2θ) with a scanning speed of $0.25\ \text{min}^{-1}$ and sampling interval of 0.02°. The samples were studied at ambient temperature.

2.9. Statistical analysis

All of the sample analyses were conducted in triplicate and the values were expressed as means ± SD. Statistical analysis was performed using EXCEL$^{\text{TM}}$ 2003 (Microsoft, USA) and significant differences among means were estimated at a probability level of 0.05.

3. Results and discussion

3.1. Preparation of CSMs

3.1.1. The effect of initial continuous phase on yield and crosslinking density of CSMs

In this paper, the aqueous starch solution and the aqueous PEG solution (or paraffin liquid) before emulsification were defined as initial dispersed phase and initial continuous phase, respectively. The effect of initial continuous phase on yield and crosslinking density of CSMs is shown through the data presented in Table 1. The data provided in this table show that both the yield and crosslinking density increased significantly ($p < 0.05$) with the increase in the molecular weight and concentration of PEG. When 44% PEG 20000 solution (w/w) was used as initial continuous phase, the yield and RMB (indicates the crosslinking density) reached 65.8% and 41.0, respectively. However, as can be seen from Table 1, both the yield and RMB of CSMs prepared in paraffin liquid were higher (69.4% and 46.2) than those prepared in PEG solution.

When two aqueous polymer solutions are mixed, a phase separation can occur to form domains that are rich in one polymer and poor in the other (Ding et al., 2002). This means that a mass re-distribution occurs in phase-separated aqueous mixtures. In a preliminary study, we found that the volume of dispersed phase (starch-rich phase) increased with the increase in the molecular weight and the concentration of PEG (data not shown), which indicated that less starch molecules distributed in continuous phase. As a result, the yield of CSMs increased. Regarding the crosslinking degree, it is reasonable to assume that the amount of crosslinker distributed in continuous phase (unavailable for crosslinking reaction) decreases with the increase in the molecular weight and concentration of PEG. This would result into increase in the crosslinking degree (increase in RMB). The fact that the yield and crosslinking density of CSMs prepared by WOEM were higher than those prepared by WWEM can be attributed to the limited mass re-distribution in the w/o emulsion.

Table 1
The effect of initial continuous phase on the yield and crosslinking density of CSMs.

Batch	Initial continuous phase	Yield (%)	RMB
1	PEG 10000 solution, 28%	55.0 ± 0.6	15.2 ± 0.2
2	PEG 10000 solution, 32%	57.8 ± 0.2	17.4 ± 1.7
3	PEG 10000 solution, 36%	60.3 ± 0.6	19.7 ± 0.5
4	PEG 10000 solution, 40%	62.4 ± 0.2	26.8 ± 1.4
5	PEG 10000 solution, 44%	64.2 ± 0.2	36.4 ± 1.7
6	PEG 20000 solution, 28%	55.8 ± 0.3	16.1 ± 0.9
7	PEG 20000 solution, 32%	60.2 ± 0.4	19.1 ± 1.1
8	PEG 20000 solution, 36%	62.6 ± 0.3	24.3 ± 1.8
9	PEG 20000 solution, 40%	64.9 ± 0.8	30.4 ± 0.9
10	PEG 20000 solution, 44%	65.8 ± 0.2	41.0 ± 1.6
11	Paraffin liquid containing Span 80	69.4 ± 0.3	46.2 ± 0.1

Table 2
The effect of crosslinker concentration on the yield and crosslinking density of CSMs.

Batch	Crosslinker concentration[a] (%)	Yield (%)	RMB
12	2	53.6 ± 0.5	10.2 ± 0.5
13	4	62.7 ± 1.2	13.8 ± 0.5
14	6	60.3 ± 0.6	19.7 ± 0.5
15	8	57.0 ± 0.3	25.3 ± 0.8
16	10	53.3 ± 0.2	34.7 ± 1.3

[a] Weight ratio of TSTP to H_2O.

3.1.2. The effect of crosslinker concentration on yield and crosslinking density of CSMs

As can be seen from Table 2, the yield of CSMs increased significantly ($p < 0.05$) from 53.6% to 62.7% as the crosslinker concentration (TSTP/H_2O, w/w) increased from 2% to 4%. After reaching the peak (or ceiling) yield at 4% crosslinker concentration the yield started to decline gradually and reached 53.3% when crosslinker concentration was increased to 10%. This may be due to the fact when the TSTP concentration is below its ceiling value (4% in this case), large number of starch molecules can be crosslinked into CSMs with the increase in crosslinker concentration which increases the yield accordingly. However, above 4% of TSTP concentration, a large proportion of increased TSTP did not take part in the crosslinking reaction, which resulted into decrease in the yield.

Table 2 also shows that the RMB increased steadily from 10.2 to 34.7 as crosslinker concentration rose from 2% to 10%. This indicated that the increase in crosslinker concentration accelerated the crosslinking reaction, which resulted into increase in the crosslinking density.

3.1.3. The effect of incubation time on the yield and crosslinking density of CSMs

The effect of incubation time on yield and crosslinking density of CSMs is presented in Table 3. The data presented in this table show that the variation in the incubation time affected both the yield and crosslinking density significantly ($p < 0.05$). For example, the yield of CSMs increased from 55.2% to 60.3% as the incubation time was extended from 1 h to 3 h. These observations suggest that longer incubation time favor the crosslinking of greater number of free starch molecules to CSMs. However, beyond 3 h, the increase in the yield continuously slowed down and became stable after 5 h. On the other hand, the crosslinking density of CSMs increased continuously even up to 9 h of incubation. The RMB increased steadily from 13.7 to 38.2 as the incubation time was increased from 1 h to 9 h. This can be due to the fact that more and more crosslinkages continued to form as the incubation time increased. As can be seen from Table 3, the crosslinking density was still increasing at the end of 5 h long incubation period. This result revealed that the crosslinking reaction was still in progress. The differences in trends between the yield and crosslinking density (RMB) suggest

Table 3
The effect of incubation time on the yield and crosslinking density of CSMs.

Batch	Incubation time (h)	Yield (%)	RMB
17	1	55.2 ± 0.7	13.7 ± 0.4
18	3	60.3 ± 0.6	19.7 ± 0.5
19	5	61.6 ± 0.4	24.4 ± 0.1
20	7	62.0 ± 0.5	30.6 ± 1.0
21	9	62.1 ± 0.2	38.2 ± 1.0

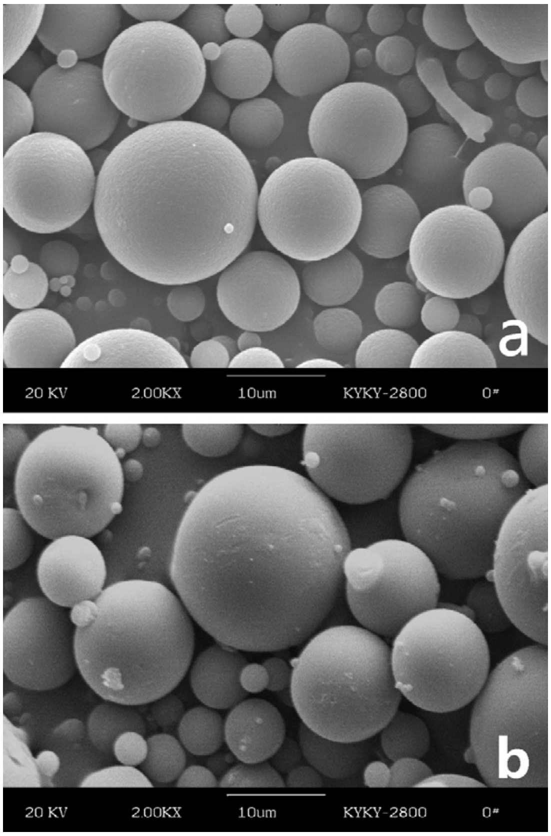

Fig. 1. SEM photographs (2000×) of (a) WWEM-CSMs (batch 3) and (b) WOEM-CSMs (batch 11).

that the crosslinking reaction continuously occurs in the interior of the microspheres even thought the crosslinking reaction between free starch molecules and TSTP almost concluded within 5 h of incubation. We noticed that the increase in the crosslinking density did not result into the increase in the yield of CSMs. This might be due to the fact that the mass (weight) of crosslinking group (phosphate group) in the microspheres might be negligible compared to the total mass of microspheres.

3.2. Morphology of CSMs

The morphology of CSMs obtained using scanning electron microscope is shown in Fig. 1. As can be seen from this figure CSMs with excellent spherical particles in discrete state can be prepared by the two-stage WWEM (Fig. 1a). This may be due to the fact that the w/w emulsion was relatively stable and the separation process including the incubation was gentle. As a consequence the aggregation of microspheres can be avoided even though the stirring was applied only for a short time.

Fig. 1 also shows that the CSMs prepared by both the w/w and w/o emulsion methods are spherical, compact and non-porous. It appears that the presence of PEG in the dispersed phase did not visibly affect the morphology of CSMs prepared through WWEM.

3.3. Chemical composition of CSMs obtained from WWEM and WOEM methods

Fig. 2 shows the infrared spectra of PEG, soluble starch, as well as CSMs prepared by WWEM and WOEM methods, respectively. As can be seen from this figure, the spectra of soluble starch and CSMs obtained from both the WWEM and WOEM methods are quite similar. It is worth mentioning here that the characteristic peaks between 1500 cm^{-1} and 750 cm^{-1} belonging to PEG did not appear in the spectrum of CSMs prepared by WWEM. This suggested that almost none of the PEG molecules remained in the structure of the CSMs. This fact can further be corroborated from the fact that there was no distinct difference between the chemical composition of CSMs obtained from WWEM and WOEM methods.

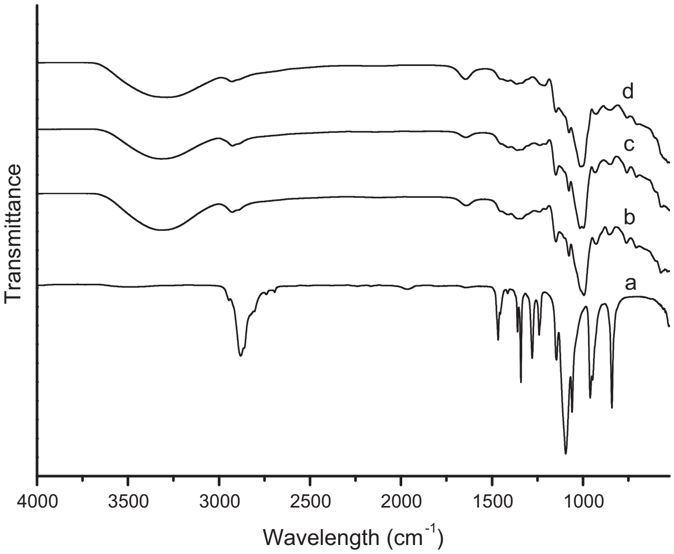

Fig. 2. FTIR spectra of: (a) PEG 10000; (b) soluble starch; (c) WWEM-CSMs (batch 3) and (d) WOEM-CSMs (batch 11).

3.4. Amorphous structure of CSMs

The XRD patterns of soluble starch, crosslinked starch microspheres obtained from WWEM and WOEM are presented in Fig. 3. A diffraction peak at 17° (2θ) can be observed in the diffractogram of soluble starch. This peak indicates that there is crystalline region in soluble starch. On the contrary, broad diffractograms similar to those of amorphous materials can be observed in the diffraction patterns of crosslinked starch microspheres obtained from both WWEM and WOEM methods. These diffractograms indicate that crosslinked starch microspheres obtained from both the WWEM and WOEM methods have amorphous structure.

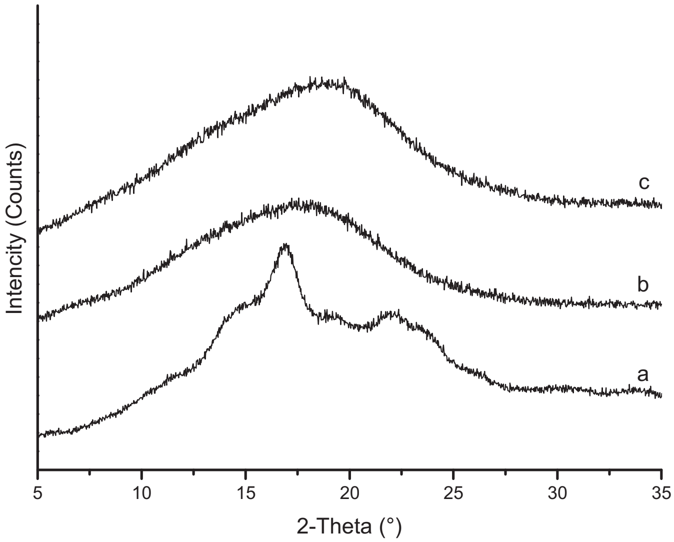

Fig. 3. XRD patterns of: (a) soluble starch; (b) WWEM-CSMs (batch 3) and (c) WOEM-CSMs (batch 11).

4. Conclusions

Crosslinked starch microspheres (CSMs) with excellent spherical shape and non-agglomerated discrete form can be synthesized by a novel two-stage water-in-water emulsion method as proposed in this study. The yield and crosslinking density of the CSMs were found to be significantly ($p < 0.05$) affected by the concentration and molecular weight of polyethylene glycol (PEG) present in the continuous phase, the composition of the crosslinker and the incubation time. The increase in any one of these variables was found to result into increase in the crosslinking density. It was found that the morphology, chemical composition and structure organization of CSMs prepared from w/w emulsion (WWEM) and w/o emulsion (WOEM) methods were identical in nature. However, the yield and the crosslinking density of CSMs obtained from WWEM were lower than those obtained from WOEM.

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