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Preparation of crosslinked starch microspheres and their drug loading and releasing properties

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ABSTRACT

Crosslinked anionic starch microspheres were prepared with sodium trimetaphosphate (STMP) as crosslinking agent through 5 h w/o emulsification-crosslinking reaction at 50 °C. Laser diffraction technique and scanning electron microscopy revealed that microspheres had narrow size distribution, good sphericity and fine dispersibility. In addition, drug loading and releasing properties were investigated with Methylene Blue as a model drug on the basis of single-factor study. It was found that the loading ratio of MB was significantly influenced by loading time, dissolution medium, loading temperature as well as MB concentration ($P < 0.05$). Either the increase of loading time or drug concentration could lead to the increase of drug loading amount of microspheres, however, drug loading amount reached its maximum in NaCl (0.9%) dissolution medium at room temperature. Furthermore, the release profile contained two main expulsion processes: an initial burst release followed by a sustained swelling-controlled release.

1. Introduction

Starch is a biodegradable carbohydrate consisting of glucose units and abundant in a wide range of farm products such as rice, wheat, maize and potatoes (Chan et al., 2007; Zhou et al., 2006). It is applied to food and industrial fields as thickener, gelling agent, bulking agent and water retention agent (Che et al., 2007; Tester, Karkalas, & Qi, 2004). Starch is modified to overcome some of its limitations and broaden its applications, for instance, low shear resistance, low thermal resistance, low thermal decomposition and retrogradation tendency (Jobling, 2004; Raina, Singh, Bawa, & Saxena, 2007). Among various modifications, crosslinked starch microspheres show high stability towards swelling, high temperature, high shear and acidic conditions (Kim & Lee, 2002) and have been the most investigated drug carriers due to their total biodegradability, biocompatibility, non-toxicity, stability on storage, cost-effectiveness as well as simple fabrication method (Mundargi, Shelke, Rokhade, Patil, & Aminabhavi, 2008). Therefore, they are promising vehicles in drug delivery systems especially in the intranasal drug delivery system (Mao, Chen, Wei, Liu, & Bi, 2004). During application, the drug loaded microspheres contact with nasal mucus, with high swelling degree, form a gel-like system, deliver drugs at controlled and pre-determined rate, prolong the residence time of drugs and maintain their therapeutically effective concentrations in the nose, minimize the drug-related side effects, hence improve the clinical efficacy of the drug (Mao et al., 2004; Mundargi et al., 2008; Perswetoff-Morath, 1998).

Several preparation approaches of starch microspheres have been investigated, such as spray drying, precipitation, solvent evaporation and emulsion-crosslinking techniques (Bezemer et al., 2000; Kawashitaa et al., 2005; Luck et al., 1998; Sturesson & Carlfors, 2000), among which water-in-oil (w/o) emulsion-crosslinking technique has been extensively used and rapidly developed.

The water-in-oil emulsion system is composed of water (dispersed phase), oil (continuous phase) and emulsifier. Since emulsifier reduces interfacial tension between water and oil phases, the emulsion system becomes a thermally stable system. While mechanical energy is introduced during the emulsion preparation, water phase is dispersed as droplets surrounded by oil phase. The crosslinking reaction between starch molecules and crosslinking agent takes place in the droplets, so crosslinked starch microspheres take shape. This crosslinking generates intra-and inter chemical bonds which form network structures in starch microspheres, therefore, stabilize and reinforce the microspheres (Singh, Kaur, & McCarthy, 2007).

In recent years, many research works have covered the preparation of neutral starch microspheres and their physicochemical

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Nomenclature

$d_{4,3}$	mean diameter, μm	E	encapsulation efficiency, %
span	span value	R	release rate, %
D	drug loading ratio, %		

properties (Adebowale & Lawal, 2003). However, a few works has been done on anionic starch microspheres. The anionic microspheres have high affinity to positively charged drugs, thus enhance drug loading ability. Moreover, few studies covered the drug loading property of anionic starch microspheres.

In this study, anionic starch microspheres were prepared by 5 h w/o emulsion-crosslinking method. Water soluble starch was dissolved in sodium hydroxide solution which served as water phase, and liquid paraffin was taken as oil phase. Mechanical stirring was used to generate droplets. Among the crosslinkers, sodium trimetaphosphate (STMP), a salt of low toxicity with no adverse effects on humankind, had been reported to be an effective crosslinker for starches (Hirsch & Kokini, 2002; Muhammad, Hussin, Ghazali, & Kennedy, 2000). For the above reason, it was chosen as a crosslinker in the starch microspheres preparation. In addition, preliminary work was undertaken with varying mechanical stirring rate and time, concentration and type of both starch and crosslinker, HLB of emulsifier as well as emulsification-crosslinking temperature in order to obtain optimal microspheres with narrow size distribution, good sphericity and fine dispersibility. All these variable parameters were done on the basis of single-factor study. Morphology of starch microspheres were examined through scanning electron microscope (SEM) and particle size and distribution were measured with laser diffraction particle size analyzer (Mastersizer 2000).

Moreover, Methylene Blue (MB) was chosen as model drug, and drug loading characteristics of suitable starch microspheres were studied in-depth with variations of dissolution medium, drug concentrations, drug loading temperatures and time. Besides those, drug releasing property of drug loaded microspheres was also investigated.

2. Materials and methods

2.1. Materials

Water soluble starch and Methylene Blue were purchased from Beijing Aoxing Biological Technique Company. Span 80, Tween 80, NaCl and HCl were provided by Beijing Yili Chemical Company. STMP was obtained from Tianjing Dengfeng Chemical Company (Tianjing, China). The above reagents were of analytic grade and used without further purification. Liquid paraffin was also chemically pure, purchased from Shantou Xilong Chemical Factory. Deionized water was used throughout the work.

2.2. Preparation of starch microspheres

Starch microspheres were prepared according to water-in-oil emulsification-crosslinking technique with STMP as crosslinking agent. The reaction scheme for crosslinking starch microspheres is depicted in Fig. 1. There were four main steps involved in the microsphere preparation. (1) The water phase (W solution) was prepared by dissolving 5 g of water soluble starch and 1 g of STMP in 50 g of NaOH solution (NaOH:H_2O = 1:100, w:w), then homogenized by magnetic stirring for 3 min. (2) The oil phase (O solution) was prepared by dissolving 7.5 g of the mixture consisting of Span 80 and Tween 80 (Span 80:Tween 80 = 90.65:9.35, w:w) in 150 mL of liquid paraffin, and then poured into a thermostated water container equipped with a reflux column and a mechanical stirring device. The entire assembly was maintained at 50 °C accurately. (3) 15 mL of W solution was added into O solution dropwise under mechanical agitation of 450 rpm (Digital display force increasing agitator JJ-6, China). The water-in-oil (w/o) emulsion was formed while mechanical agitation was maintained for 5 h. (4) The emulsion was centrifuged and purified sequentially with acetone, petroleum ether, 9% NaCl solution (NaCl:H_2O = 9:100, w:w) and ethanol twice. The TSMs were obtained after vacuum-drying of centrifuged emulsion at 40 °C for 6 h. The dried microspheres were kept in a desiccator for further analysis and utilization.

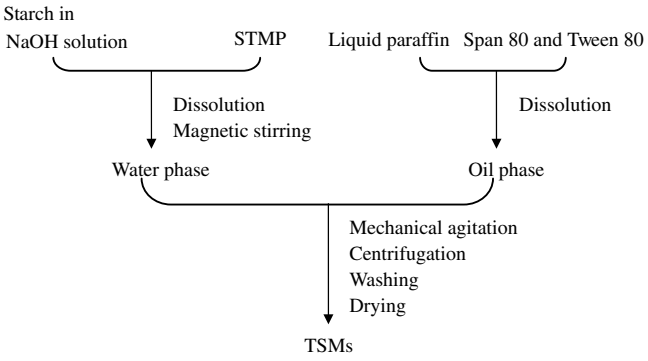


Fig. 1. Scheme of emulsification-crosslinking reaction of starch.

2.3. Morphology examination

Morphology of crosslinked starch microspheres were examined at a magnification of 200× and 5000× using a scanning electron microscopy (KYKY-2800 scanning electron microscopy, China). Samples were mounted on round brass stub then sputter-coated with gold-palladium in argon atmosphere using an IB-3 ion coater (Eiko, Japan) before morphology measurement (Liu, Sun, Wang, Zhang, & Wang, 2005).

2.4. Particle size and distribution analysis

The volume mean diameter ($d_{4,3}$) and particle size distribution (span value) of the TSMs were measured by laser light diffraction technique (Masterparticle sizer 2000, Malvern Instruments, UK). The measurement procedure and principle were as follows: moderate amount of TSMs (1 g or so) were immersed into 50 mL of anhydrous ethanol for ultrasonic dispersion so as to prevent agglomeration before used as samples. 500 mL of anhydrous ethanol was added into a beaker as dispersing medium for measuring the background concentration and then 30 mL of TSMs sample was poured into another beaker and measured until the obscuration seemed to be in the selected range, in the process of measurement, the sample was circulated in company with liquid flow. The particle size distributions were calculated from the intensity of light diffracted at each angle using Mie theory. Refractive indices of ethanol and TSMs used were 1.320 and 1.200 at 23 °C, respectively. The absorbance of TSMs was taken as 0 (Singh, McCarthy,

& Singh, 2006; Zhou et al., 2006). Particle size characteristics were indicated with Mastersizer 2000 Software v. 5.22.

The volume mean diameter ($d_{4,3}$) was evaluated according to Eq. (1), where n_i is the number of particles of diameter d_i.

$$d_{4,3} = \sum n_i d_i^4 \Big/ \sum n_i d_i^3 \quad (1)$$

The particle size distribution was evaluated with the span value according to Eq. (2), where d_N ($N = 10, 50, 90$) means the volume percentage of microspheres with diameters below of d_N is equal to N%. The smaller span value indicates the narrower particle size distribution.

$$span = (d_{90} - d_{10})/d_{50} \quad (2)$$

2.5. Drug loading analysis

Standard curves of Methylene Blue in different dissolution medium including HCl (0.1 mol/L), phosphate buffered saline (PBS, pH 7.4), as well as NaCl solution (0.9%) were obtained using the following approaches: 0.01 mg/mL MB of the above three dissolution medium were scanned at the wavelength between 360 and 760 nm with ultraviolet–visible spectrophotometer (TU-1810, Beijing Puxi General Apparatus, Ltd., China). Starch microspheres (TSM-B2) suspensions of 0.2 mg/mL were prepared with these dissolution medium and kept for 2 h at room temperature, then the suspensions were centrifuged and supernatant was scanned from 360 to 760 nm. Comparing the scanning results, the wavelengths at which starch microspheres absorbed the least while MB absorbed the most were selected as the testing wavelength for latter experiments. Then, 0.001, 0.002, 0.003, 0.004, 0.005 mg/mL of MB in the above dissolution medium were measured at their corresponding testing wavelengths to obtain standard curves of MB absorbance to concentration for each solution.

2.5.1. Effect of dissolution medium on drug loading

About 0.2 g starch microspheres (TSM-B2) were weighed and suspended in 100 mL of the three diffusion medium containing 0.5 mg/mL MB each. The resulting suspensions were gently shaken for 2.5 h at room temperature. Then suspensions were centrifuged and 1 mL of each supernatant was extracted and diluted to certain volume to determine the drug loading amount and encapsulation efficiency with ultraviolet–visible spectrophotometer according to corresponding standard curves of MB absorbance to concentration. The drug loading ratio (D) and encapsulation efficiency (E) were calculated with Eq. (3) and Eq. (4), respectively

$$D = (C_0 - C_1V_1)V_0/(1000W_{TSM}) \quad (3)$$

$$E = (C_0 - C_1V_1)/C_0 \quad (4)$$

where C_0 means initial concentration of MB in diffusion medium, C_1 means diluted concentration of MB in diffusion medium, V_1 means diluted volume of diffusion medium, V_0 means initial volume of diffusion medium, and W_{TSM} means the weight of starch microspheres dissolved in diffusion medium.

2.5.2. Effect of loading time on drug loading

About 0.2 g starch microspheres (TSM-B2) were weighed and suspended in 100 mL, 0.9% of the NaCl solution with 0.5 mg/mL MB. The resulting suspensions were gently shaken at room temperature. Then 1 mL of the suspension was extracted every 30 min. The drug loading ratio and encapsulation efficiency of different loading time were calculated according to the method described in previous Section 2.5.1.

2.5.3. Effect of loading temperature on drug loading

About 0.2 g starch microspheres (TSM-B2) were weighed and suspended in 100 mL, 0.9% of the NaCl solution with 0.5 mg/mL MB. The resulting suspension was gently shaken for 2.5 h at different temperatures of 6 °C, room temperature (25 °C), 37 °C and 50 °C. The drug loading ratio and encapsulation efficiency under different temperatures were calculated according to the method in the previous Section 2.5.1.

2.5.4. Effect of drug concentration on drug loading

About 0.2 g starch microspheres (TSM-B2) were weighed and dissolved in 100 mL, 0.9% of the NaCl solutions with 0.25, 0.5, 0.75, 1, 1.25 mg/mL MB each. The resulting suspensions were gently shaken for 2.5 h at room temperature. The drug loading ratio and encapsulation efficiency under different MB concentrations were calculated according to the method in the previous Section 2.5.1.

2.6. Drug release analysis

About 0.2 g starch microspheres (TSM-B2) were weighed and dispersed in 100 mL of 0.9% NaCl solution with 0.5 mg/mL MB. The resulting suspension was gently shaken for 2.5 h. Then 1 mL of the suspension was extracted and diluted to determine the total drug loading amount in microspheres according to Eq. (3). Then these loaded microspheres were dried and immersed in 100 mL of 0.9% NaCl solution which acted as release medium in a 25 °C incubator. 1 mL of NaCl solution with microsphere was taken out and the sample drawn was replaced by fresh 0.9% NaCl solution to maintain a constant volume. The mass of MB released from microspheres to NaCl solution was determined according to standard curve of MB absorbance to concentration in 0.9% NaCl solution and Eq. (3). The release rate of MB (R) was calculated by Eq. (5).

$$R = M_1/M_0 \quad (5)$$

where M_1 is the cumulative mass of MB released from starch microspheres at a given time, and M_0 is the total loading amount of MB in microspheres.

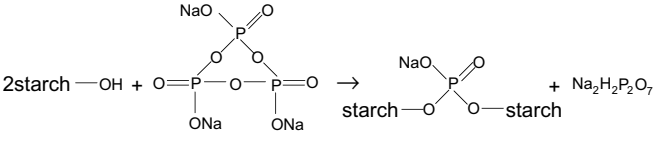
2.7. Statistical analysis

All of the sample analyses were conducted in triplicate and the values were expressed as means ± standard error of the mean, Statistical analysis were done using SAS v.6.12 developed by the SAS Institute Inc. (Cary, NC, USA). Duncan's multiple range tests were used to estimate significant differences among means at a probability level of 0.05.

3. Results and discussion

3.1. Preparation of starch microspheres

During the emulsification-crosslinking reaction, STMP reacts with hydroxyl groups of the starch molecules in the droplets, resulting in the intra-and inter ester linkages and phosphate groups provide negative charges in starch molecules. The emulsification-crosslinking reaction can be described as follows (Liu, 2001):



The morphology of TSMs under scanning electron microscope was shown in Fig. 2, which revealed that most of TSMs had good sphericity with compact surfaces and fine dispersibility, though some

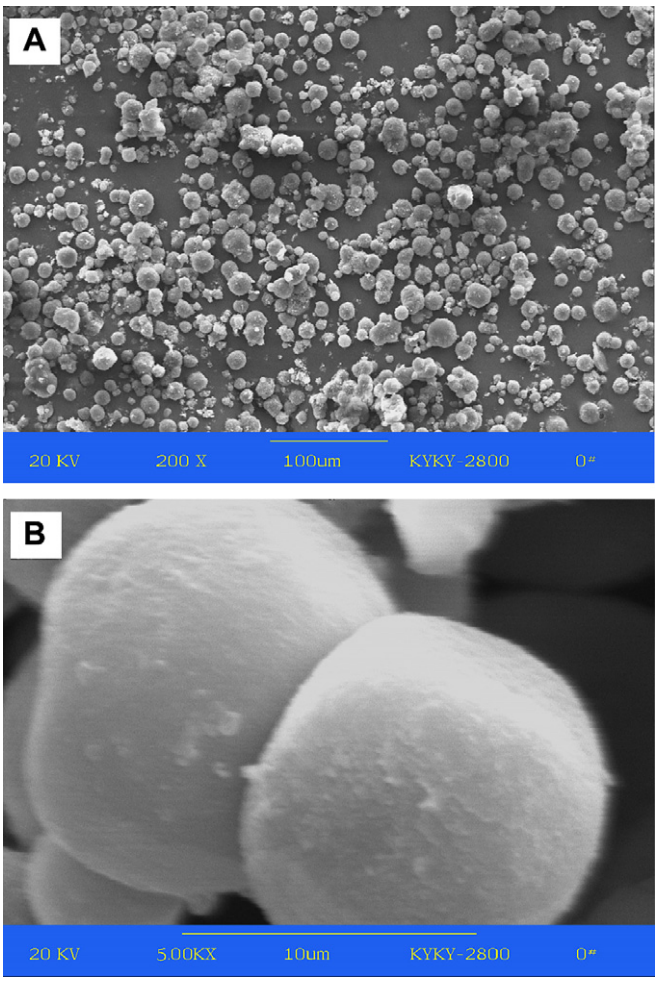


Fig. 2. Scanning electron micrograph of TSMs: (A) 200×; (B) 5000×.

particles congregated together due to strong van der Waals force and electrostatic attraction. In terms of particle size, the $d_{4,3}$ and span value of TSMs were 19.046 and 1.252 μm, respectively, and size distribution of TSMs could be seen in Fig. 3.

Preliminary work indicated that HLB of emulsifier had great effect on the stability of emulsion system. As the HLB of Span 80 is merely 4.3 which shows strong adhesion to oil phase but weak adhesion to water phase, while the HLB of Tween 80 is as high as 15 which shows quite the opposite tendency. When the HLB of mixed emulsifier approached 5.3 in this experiment, its adhesion to both phases reached a balance and reduced the interfacial tension between water phase and oil phase appropriately, thus the microspheres under this condition appeared small particle size, narrow size distribution and good sphericity. Besides that, proper stirring rate, moderate emulsification-crosslinking temperature also facilitated microspheres preparation.

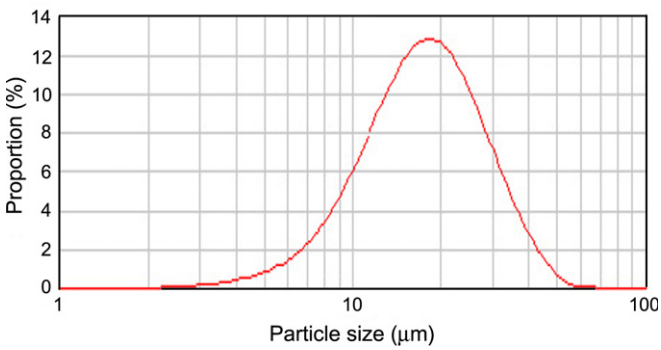


Fig. 3. Particle size distribution of TSMs.

3.2. Drug loading analysis

According to the scanning results and comparisons, the testing wavelengths of HCl (0.1 mol/L), phosphate buffered saline (PBS, pH 7.4) and NaCl solution (0.9%) were 662, 664 and 664.5 nm, respectively. Besides, standard curves of MB absorbance to concentration (from 0.001 to 0.005 mg/mL) in these three solutions were $y = 273\times - 0.003$ ($R^2 = 0.09982$), $y = 178.7\times + 0.0267$ ($R^2 = 0.9908$), $y = 188.2\times + 0.0548$ ($R^2 = 0.9984$), respectively.

3.2.1. Effect of loading time on drug loading

The influence of varying of loading time on drug loading ratio and encapsulation efficiency was performed in Table 1, which revealed that rise in time facilitated drug loading ratio and enhanced encapsulation efficiency of MB significantly ($p < 0.05$). To be exact, the drug loading ratio increased steadily from 15.51% to 17.58% as time was prolonged from 0.5 to 2.5 h, with encapsulation efficiency of MB rising from 62.04% to 70.32%. However, in the following 24 h, the drug loading ratio and encapsulation efficiency of MB did not show distinctive change; hence time had limited influence on drug loading ability of starch microspheres.

3.2.2. Effect of dissolution medium on drug loading

The effect of medium on the loading of MC could be observed in Table 2. It was obvious that different dissolution medium resulted in significant changes in drug loading ratio and encapsulation efficiency of MB ($p < 0.05$). In the acidic condition, the microspheres loaded nearly 11% less of MB than that of the neutral solution and alkaline condition. Meanwhile, the encapsulation efficiency of MB indicated the same trend. The possible reason might be that starch microspheres were inclined to degrade in acidic condition and as a result drug loading ability decreased. In the alkaline solution, the presence of quite a few salt ions lowered the osmotic pressure between MB and microspheres (Bajpai & Bhanu, 2007), thus a smaller amount of MB were absorbed into the microspheres compared with that of neutral solution.

Table 1
Effect of loading time on MB loading

MB loading time (h)	MB loading ratio (%)	Encapsulation efficiency (%)
0.5	15.51 ± 0.27^{D}	62.04 ± 1.08^{D}
1	16.49 ± 0.38^{C}	65.96 ± 1.52^{C}
1.5	$16.73 \pm 0.21^{B, C}$	$66.92 \pm 0.84^{B, C}$
2	$17.29 \pm 0.29^{A, B}$	$69.16 \pm 1.16^{A, B}$
2.5	17.58 ± 0.49^{A}	70.32 ± 1.96^{A}

Values represent the means ± SD; $n = 3$. Values in a column followed by different capital letters as superscripts were significantly different from each other according to Duncan's multiple range tests ($p < 0.05$).

Table 2
Effect of dissolution medium on MB loading

MB loading media	MB loading ratio (%)	Encapsulation efficiency (%)
HCl	6.61 ± 0.16^{C}	26.44 ± 0.64^{C}
PBS	14.44 ± 0.72^{B}	57.76 ± 2.88^{B}
PS	17.58 ± 0.49^{A}	70.32 ± 1.96^{A}

Values represent the means ± SD; $n = 3$. Values in a column followed by different capital letters as superscripts were significantly different from each other according to Duncan's multiple range tests ($p < 0.05$).

3.2.3. Effect of loading temperature on drug loading

The influence of temperature on drug loading was depicted in Table 3, which suggested that variations in temperature influenced drug loading of MB significantly ($p < 0.05$). At temperature of 25 °C, microspheres loaded with more MB than that of higher temperatures. The reason was that the sorption of MB was mainly attributed to the existence of opposite charges and high affinity between them. This process released a great deal of heat which would be hindered by high temperature, thus the drug loading ratio at 50 °C reduced to 1/10 of that of 25 °C. But too low temperature weakened still the affinity between microspheres and MB, therefore drug loading ratio and encapsulation efficiency at 6 °C was only 2/3 of that of 25 °C.

3.2.4. Effect of drug concentration on drug loading

As can be seen in Table 4, drug loading ratio ascended significantly from 9.70% to 31.64% as the concentration of MB rose from 0.25 to 0.75 mg/mL ($p < 0.05$), then the increase slowed down and peaked at 32.73% when the concentration of MB reached 1.25 mg/mL ultimately, but the encapsulation efficiency peaked at 84.40% when the concentration of MB achieved 0.75 mg/mL, after which the increase of MB caused the decline in encapsulation efficiency. The results could be explained by the fact that the loading of MB reached its saturation in the neighborhood of 0.75 mg/mL of MB, hereafter the increase of MB had little effect on microspheres drug loading amount, but decreased the efficiency of MB encapsulation significantly.

3.3. Drug release analysis

The release profile of starch microspheres was presented in Fig. 4. It could be seen that drug release amount increased significantly in 24 h of release time ($p < 0.05$). Initially, a burst release was observed in the first 1 h after the starch microspheres were immersed into release medium, high release rate of 33.87% was associated with the immediate dispersing of the MB close to starch microspheres surfaces. In the following 2 h, the crosslinked starch microspheres formed a swelling-controlled and sustained release system, in which the release rate was tailed off, and 62.30% of the MB contained in the starch microspheres was released, because on the one hand, MB in the microspheres which occupied lots free volume spaces inside the swollen microspheres created tortuous paths to assist the transportation of water molecules, which facilitated starch microspheres to absorb water and swell sufficiently. On the other hand, increasing swelling impulsed a great number of MB molecules to diffuse out of the starch microspheres and pass into the release medium through numerous pores and channels in the microspheres. However, as the microspheres swelled in NaCl solution, a gel diffusion layer took shape gradually which hampered the outward expulsion of MB (Chiao & Price, 1994) and brought a sustained slowdown to the MB release. Similar results had been made by Bajpai and Bhanu (2007), who also found that release profile of drug was greatly influenced by percent loading of drug, with increase in drug loading ratio, the release of drug increased. From the 3 to 24 h, drug release experienced a slight but slow rise. 84.52% of the MB was released into NaCl solution which kept almost unchanged in the next 24 h. Since the pores and channels for water transportation in the microspheres decreased dramatically as the gel diffusion layer enhanced, consequently less and less MB was impelled out of the microspheres. Finally, the concentration of MB reached a balance between starch microspheres and NaCl solution as time was prolonged. However, tiny amount of MB was released due to gradual but sluggish degradation of starch particles.

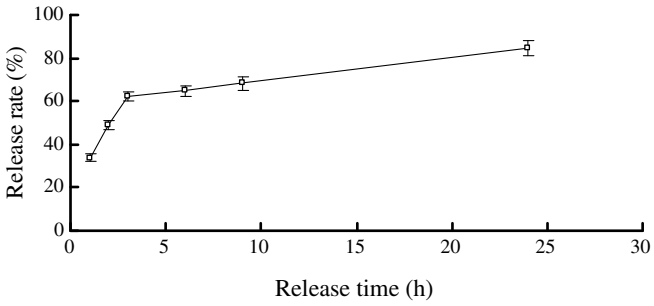


Fig. 4. MB release of TSMs in NaCl (0.9%) solution.

Table 3
Effect of loading temperature on MB loading

MB loading temperature (°C)	MB loading ratio (%)	Encapsulation efficiency (%)
6	11.60 ± 0.31^{B}	46.40 ± 1.24^{B}
25	17.58 ± 0.49^{A}	70.32 ± 1.96^{A}
37	3.13 ± 0.28^{C}	12.52 ± 1.12^{C}
50	1.81 ± 0.14^{D}	7.24 ± 0.56^{D}

Values represent the means ± SD; $n = 3$. Values in a column followed by different capital letters as superscripts were significantly different from each other according to Duncan's multiple range tests ($p < 0.05$).

Table 4
Effect of MB concentration on MB loading

MB concentration (mg/mL)	MB loading ratio (%)	Encapsulation efficiency(%)
0.25	7.70 ± 0.40^{C}	61.60 ± 3.20^{C}
0.5	17.58 ± 0.49^{B}	70.32 ± 1.96^{B}
0.75	31.64 ± 0.85^{A}	84.40 ± 2.27^{A}
1.00	32.49 ± 0.67^{A}	64.97 ± 1.34^{C}
1.25	32.73 ± 0.60^{A}	52.37 ± 0.96^{D}

Values represent the means ± SD; $n = 3$. Values in a column followed by different capital letters as superscripts were significantly different from each other according to Duncan's multiple range tests ($p < 0.05$).

4. Conclusions

Crosslinked anionic starch microspheres were prepared with sodium trimetaphosphate (STMP) as crosslinking agent through w/o emulsification-crosslinking reaction. Microspheres revealed comparatively uniform size distribution, better sphericity and dispersibility with water soluble starch, HLB of emulsifier up to 5.3 and 5 h mechanical agitation at 50 °C. Moreover, neither high temperature nor high HLB benefited the generation of microspheres as they caused the agglomeration and irregular morphology of microspheres. Besides that moderate mechanical stirring time and rate facilitated microspheres preparation.

In terms of drug loading capacity of anionic starch microspheres, it was found that the loading ratio of MB was significantly influenced by loading time, dissolution medium, loading temperature as well as MB concentration ($p < 0.05$). Either the increase of loading time or MB concentration could lead to the increase of MB loading ratio, however, overmuch use of MB decreased encapsulation efficiency of microspheres. And drug loading ratio reached its maximum in NaCl (0.9%) dissolution medium at room temperature, comparing with that of the other medium or temperatures.

Furthermore, the release profile contained two main expulsion processes: an initial burst release and expulsion of active compounds from the microspheres surface followed by a sustained swelling-controlled release with release rates dependent on swelling and degradation of starch microspheres.

Acknowledgments

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