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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
span	span value
D	drug loading ratio, %
E	encapsulation efficiency, %
R	release rate, %

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.

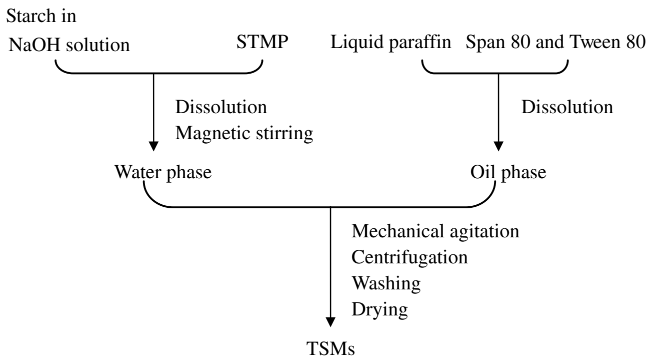

Fig. 1. Scheme of emulsification-crosslinking reaction of starch.

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,