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Characterizations and microsphere formulation of polysaccharide from the marine clam (*Mactra veneriformis*)

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ABSTRACT

Powder like samples named MVPS, containing 95.8% of polysaccharide, were extracted from a well-known marine bivalve *Mactra veneriformis*. Some in vitro tests were carried out to characterize its physicochemical properties. X-ray diffraction and thermodynamics tests indicated that MVPS were noncrystalline but thermostable polymers. Solubility and rheology tests showed that MVPS could easily dissolve in aqueous media and its solution obviously belonged to non-Newtonian fluids even at relatively high concentration. Furthermore, via blending MVPS solution with other polymers and then spray drying the blends, several composite microparticles were prepared. The chitosan/MVPS particles are available not only with spherical morphology but also with higher production yield. As a model drug, metformin can be encapsulated into the composite microsphere and then achieve the sustained release. The chitosan/MVPS composite microspheres loaded with drug might be an appropriate for nasal formulation since its particle sizes are in the range of 1–10 μm.

1. Introduction

Natural polysaccharides widely exist in the plants, microorganism (fungi and bacteria), algae, and animals (Yang & Zhang, 2009). They are long carbohydrate molecules of repeated monomer units joined together by glycosidic bonds and their molecule weight could arrive to one hundred kilo Dalton and even more. They range in structure from linear to highly branched pattern. They can provide the uppermost functions in energy storage and structural protection to living organisms. Proverbial examples were mentioned including starches, glycogen, cellulose, chitosan, and arabinoxylans. Almost all natural polysaccharides are biodegradable and safe concerning their industrialized utilizations. Moreover, they possess various special properties, such as solubility, rheological behavior, gelation, adsorption and emulsification. These properties make them become one of the most important and extensively investigated natural biomaterials. Thus they were widely used in the fields of food processing, cosmetics and pharmacy. In pharmacy, many kinds of natural polysaccharide were used as excipients to combine with some drug to form various well-defined formulations, such as tablets, films (Honary, Hoseinzadeh, & Shalchian, 2010; Murata, Isobe, Kofuji, Maida, & Miyamoto, 2010), hydrogels (Bortolin et al., 2012), emulsions (Li et al., 2012) and beads or microspheres (Kofuji, Isobe, & Murata, 2009; Singh, Sharma, Dhiman, & Gupta, 2011).

Delivering drugs through microsphere has numerous advantages compared to conventional delivery systems because they can offer three key advantages: (1) localized delivery of drug, (2) sustained delivery of drugs, and (3) stabilization of the sensitive drug. In recent years there is significant interest in using polysaccharide to prepare microsphere for drug delivery. Chitosan microsphere is used to provide controlled release of many drugs or to improve the bioavailability of degradable substances such as protein and nucleic acid. Chitosan microsphere is being investigated both for parenteral and oral drug delivery (Gungor, Okyar, Erturk-Toker, Baktir, & Ozsoy, 2010; Umadevi, Thiruganesh, Suresh, & Reddy, 2010). Chitosan microsphere shows better tolerability and can enhance the uptake of hydrophilic substances across the epithelial layers. The drug release form chitosan microsphere exhibits sustained release manner with a low initial burst effect (Dreve et al., 2011). Alginate also is an appropriate natural polysaccharide to prepare microsphere as colonic drug carrier because it is manufacturable, nontoxic and biodegradable to colonic flora, and can protect the mucous membranes of the upper gastrointestinal tract. The dried alginate microsphere has re-swelling property, which is susceptible to pH and can protect the acid sensitive drug from gastric juice (Md et al., 2011; Pal & Nayak, 2012). Other examples for natural polysaccharides in design of microsphere as drug carrier have been reviewed in several articles by other research fellows (Hojjati, Razavi, Rezaei, & Gilani, 2011; Jain, Gupta, & Jain, 2007;

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Vasir, Tambwekar, & Garg, 2003; Wang et al., 2006), which are not specified here.

Mactra veneriformis, a typical marine bivalve mollusk, is a delicious sea food and a traditional Chinese medicine while being low-cost, ubiquitous and abundant in Chinese coastal areas, especially in the coastal shoals of Jiangsu province. This marine clam was found containing high content of polysaccharide (Kasai, Horie, & Sakamoto, 2004; Luan, Wang, Wu, Jin, & Ji, 2011). The crude polysaccharide with about 3.2% of yield can be acquired through the process of boiling water extraction and ethanol precipitation. The crude polysaccharide contains about 25% protein impurities and can be deproteinized by trichloroacetic acid. Both the crude and deproteinized polysaccharides had been proved to possess strong hyperglycemic and immunity-enhancing activities by our research group (Wang, Wu, Chang, & Zhang, 2011; Wang, Zhang, Di, Liu, & Wu, 2011). Afterwards, three homogeneous polysaccharides (MVPS-1, MVPS-2 and MVPS-3) were successfully isolated from the deproteinized polysaccharide, and their average molecular weights were 446, 426 and 452 kDa, respectively, as well as their molecules structures are very similar in short and long configurations. In addition, MVPS-2 as main component was analyzed for its absolute structure by NMR. The results indicated that MVPS-2 contained two kinds of α-glucosidic bond-linked glucose residues and had a repeating unit as $[\rightarrow 4Glc1\rightarrow 4Glc1\rightarrow 4Glc1\rightarrow 2Glc1\rightarrow 4Glc1\rightarrow 4Glc1\rightarrow 4Glc1]_n$.

In this study, some basic physicochemical properties of the polysaccharide extract of *M. veneriformis* were investigated for full understanding. Furthermore, towards the purpose of utilization, the natural polymer was used as a type of functional material to produce drug load microsphere via blending and spray drying technologies. During this researching route, the relations between basic nature of the polysaccharide and properties of the produced microsphere were also discussed.

2. Materials and method

2.1. Materials

The specimens of bivalve *M. veneriformis*, cultured 3 years, were harvested from the Lvsi aquaculture farm of Jiangsu province. The collected specimens were starved in an aquarium for 24 h to evacuate their gut contents, and then flesh was excavated from the shell and stored at −10 °C condition for further using.

Polyvinyl alcohol (PVA-124) was purchased from Shanghai Chemical Reagent Co. (China). Polyethylene glycol (PEG 6000) was purchased from Merck Co. (USA). Chitosan sample with 400×10^3 Da of average molecule weight and 90% of deacetylated degree was purchased from Biochemical Medicine Plant of Qingdao (China). Analytical grade trichloroacetic acid (TCA) was purchased from Sigma Chemical Co. (USA). Powder like sample of metformin hydrochloride with 99% of purity was supplied by Shijiazhuang Polee Pharmaceutical Co., LTD. (China) and used as received. All other chemical reagents were of analytical grade and commercially available.

2.2. Extraction and deproteinization

The pre-washed flesh materials of *M. veneriformis* were cut into pieces by a mincer and then decocted 2 h by sixfold volumes of boiling water. The decoctions were centrifuged at 6000 rpm for 20 min, and the supernatant was concentrated to double volumes, and then precipitated by the addition of quadruple volumes of alcohol at room temperature. After overnight disposal, the precipitates were collected by filtration with 400 mesh fabric. The products were dehydrated by 95% ethanol, and then freeze dried to give the crude polysaccharide of *M. veneriformis*. The protein impurities in crude polysaccharide were removed using trichloroacetic acid (TCA) deproteinizing method (Gast, Zirwer, Muller-Frohne, & Damaschun, 1999; Rajalingam, Loftis, Xu, & Kumar, 2009). Briefly, the concentrated solution of the crude polysaccharide was adjusted to pH 3 with 10% TCA solution, and kept overnight. The sample was centrifuged at 6000 rpm for 10 min, and the supernatant was collected to obtain the deproteinized solution. This procedure was repeated 2–3 times. The final deproteinized solution was dialyzed against distilled water for 24 h, and then freeze dried to give the pure polysaccharide of *M. veneriformis*, which were record as MVPS.

2.3. General analysis of MVPS

First of all, the substance contents of polysaccharide and protein impurities in MVPS were precisely measured by the anthrone sulfuric acid method with glucose as standard (Pons et al., 1981; Volpi, Maccari, & Linhardt, 2008) and Kjeldahl determination (Gao et al., 2012), respectively.

Secondly, X-ray diffraction pattern of the MVPS sample was recorded with an X-ray diffractometer (Philips X-ray generator, Holland), which has an X-ray generator of 3 kW, and CuK α radiation. The samples were scanned at 0.5°/min under the diffraction angle 2θ in the range of 1–40°.

Then, differential thermal analysis and thermogravimetric measurements (TG-DSC) were carried out simultaneously using a Netzsch Simultaneous Thermal Analyzer STA 449C Jupiter equipped with a TG–DSC sample carrier type S supporting a PtRh10-Pt thermocouple (Netzsch Co. Germany). TG–DSC thermograms were taken using a standard Al_2O_3 pan. Nitrogen was used as a sweeping gas, and the heating rate was 5 °C/min. The polysaccharide sample (20 mg) was loaded in a pan without further treatment. The initial and end temperatures are −50 °C and 100 °C, respectively.

2.4. Solubility test of the MVPS

For the solubility test, a simple method was used as the following descriptions. Fully excessive dried powder of MVPS (about 3000 mg) was weighed precisely (W_0) and dissolved stepwise in 5 mL of solvents contained in a screw-capped test tube, then the mixture was sufficiently shaken at room temperature (25 °C) for 2 h in order to achieve complete polymer hydration and dissolution. After that, the mixture solution was centrifuged at 6000 rpm for 10 min, the supernatant solution were poured out and the non-dissolved remains were washed out from the tube with 5 mL of alcohols, and dried at 105 °C for 12 h to balance weight (W_1). Thus, the solubility of polysaccharide in given solvents at room temperature was calculated with the equation as $(W_0 - W_1) \times 100/4$. The given solvents included methanol, acetone, ethyl acetate, cyclohexane, petroleum ether and a series of alcohol/water co-solvents (alcohol concentration (v/v) ranged from 10 to 80%).

Meanwhile, two experiment protocols involving the effects of temperature and pH value on solubility of MVPS in aqueous media were also carried out. In the first protocol, 5 mL of distilled water in tubs was used as solvents and their temperatures were respectively set at 4, 10, 15, 20, 25, 30, 40, 60, 80 °C in a constant temperature incubator (WPL-45, Huanghua Faithful Instruments Co., China) for the solubility test. In the other experiments, 5 mL of dilute saline solution with various pH values was used as the dissolving solvents, their pH value was respectively adjusted at 1, 3, 5, 7, 9, 11 and 13 by addition of 0.1 mol/L of NaOH or HCl into distilled water. The remaining operations in the two protocols were same as above and the solubility of MVPS was calculated accordingly.

2.5. Rheological test of the MVPS

The rheological behavior was characterized by testing the apparent viscosity of polysaccharide solutions. The viscosity was measured by using a digital rotational viscometer (Brookfield DV-II+PRO, Brookfield Engineering Labs., Inc., Middleboro, USA) that equipped with a series of stainless steel rotors. About 50 mL of tested solution was added into one glass cylinder vessel with 7.0 cm of diameter and 10 cm of height. The cylinder was placed in a water bath (B-491 Water Bath, Buchi Co., Switzerland) to control the temperature during the viscosity measurements. The chosen rotor was dipped into the tested solution and rotated under set speeds at least for 30 s until a stable apparent viscosity was read on the output panel of the apparatus. Three test protocols were arranged. Firstly, the apparent viscosities of various MVPS solutions with five concentrations (ranged from 10 to 200 mg/mL) were measured at 60 rpm of rotate speed and 25 °C. Secondly, testing the apparent viscosities of two MVPS solutions (50 and 200 mg/mL) were performed at different temperatures (in the range of 0–100 °C) with 60 rpm of rotate speed. Thirdly, the effect of shear rate on the apparent viscosities of MVPS solution were also investigated for two samples (50 and 200 mg/mL of MVPS) at 25 °C by decreasing the rotate speed from 60 to 6 rpm.

2.6. Preparation of MVPS microsphere and composite microspheres

Polysaccharide microspheres were prepared by a spray-drying technique. The MVPS was dissolved in distilled water to prepare 100 mg/mL feeding solution. The solution was then spray dried with a 0.7 mm two-fluid pressurized atomizer at a feeding rate of 3 mL/min in a Buchi-B190 spray dryer (Buchi, Switzerland). The atomizing air flow rate was 650–700 NL/h. The inlet temperature was controlled at 110 °C and the outlet temperature was determined by the inlet temperature and relative factors such as air and liquid flow rates, varying between 75 and 85 °C.

In order to overcome the defects of microsphere in mechanical and appearance, other normal polymers such as PVA-124, PEG-6000, and chitosan were used as additives in microsphere preparation. Therefore, the according blend solutions were obtained by mixing the 100 mg/mL of MVPS solution with other polymer solution (50 mg/mL of PVA or PEG in water, 50 mg/mL of chitosan in 1% of HCl solution) at 1:1 of volume ratio (2:1 of solid materials) with stirring in slow rate (300 rpm) for 8 h. Subsequently, the blend solutions were spray dried as above to obtain the composite microparticles.

The effects of blending MVPS with other polymer on characteristics of composite microspheres such as yield, surface morphology, and particle size were investigated. The yield was calculated by percent ratio of the weight of harvested microspheres dividing the total weight of used solid materials in processing. Particle size distributions were determined using a Coulter LS-130 particle size analyzer (Beckman Co., USA). All microspheres were observed by scanning electron microscope (S-3400 N, Hitachi Co., Japan) with a 100 Å gold–palladium coating.

2.7. Preparation of drug loaded microspheres

After determination of the optimum composite formulation for preparing microsphere, another aim was to reach drugs loading into the composite microsphere, thus metformin hydrochloride was used as model drug considering that it activates strong hyperglycemic activities and is frequently used in clinic. For that, defined amount of drug was dissolved in 100 mL of blend polymer solution in 1:4 ratio of drug versus materials. Then the mixture containing drug were spray dried through a 0.7 mm two-fluid pressurized atomizer at a feeding rate of 3 mL/min in a Buchi-B190 spray dryer (Buchi, Switzerland). The other parameters in preparing the drug loaded microspheres were set as above. The drug-loaded microspheres were collected and its production yield was calculated by percent ratio of the weight of harvested microspheres dividing the total weight of used solid materials in processing. Particle size distributions were determined using a Coulter LS-130 particle size analyzer (Beckman Co., USA). The morphology of drug-loaded microspheres was observed by scanning electron microscope (S-3400N, Hitachi Co., Japan) with a 100 Å gold–palladium coating. The status of drug existing in microsphere was investigated by comparing the X-ray Diffraction pattern (PW-1380 X-ray generator, Philips Co., Holland) of the drug-loaded microspheres with simple mixture of drug crystals and polymers.

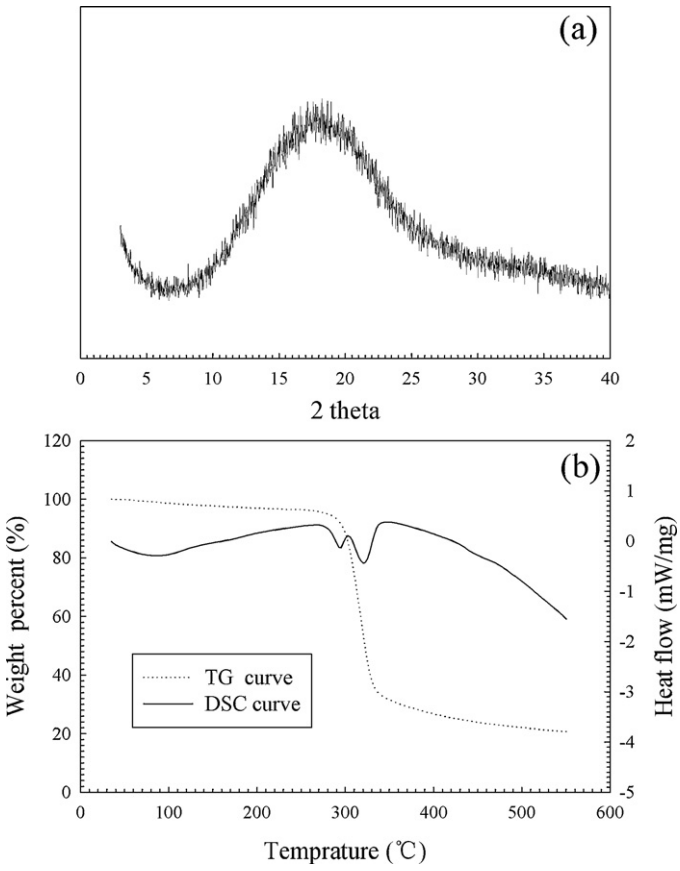

Fig. 1. X-ray diffraction (XRD) patterns (a) and TG-DSC combined analysis (b) of the MVPS.

2.8. Characterization of drug loading and release

For the determination of metformin contents in microspheres, exact amount of microsphere (10 mg) was added to 10 mL of 1% HCl solution, shaken in water bath for 2 days and filtered through membrane filter (0.45 μm). The concentration of metformin in the filtrate was detected by HPLC method (Alltima Silcon Chromatographic Column, 0.4 mL of injecting volume, 40% of acetonitrile aqueous solution as fluent phase, 1 mL/min of flow rate, and detecting at 233 nm) in a chromatograph apparatus (Agilent 1100, Agilent Co., USA). The amount of drug loaded in microspheres, given as a percentage, indicates the loading amount (mg) of metformin per 100 mg of microspheres. And the encapsulation efficiency of the process indicates the percentage of metformin encapsulated with respect to the total amount drug used in the preparation.

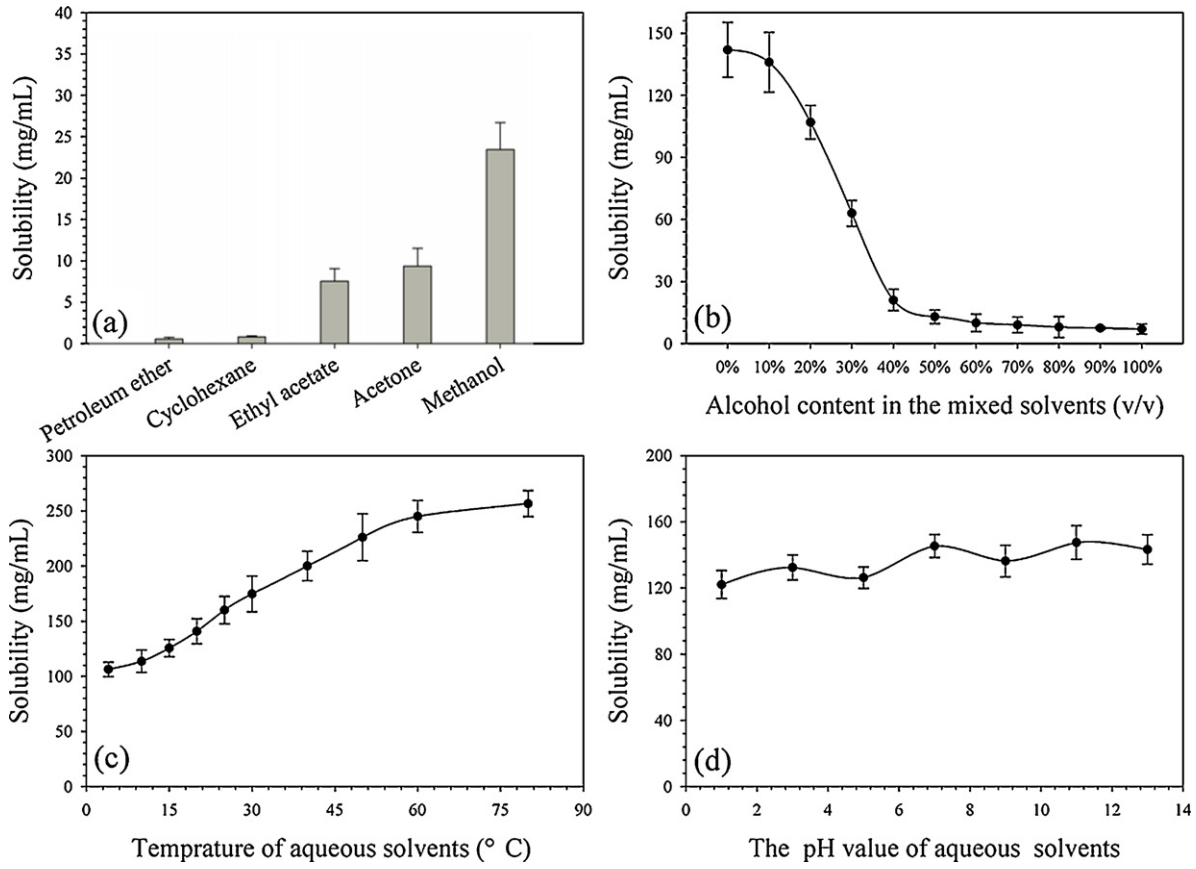

Fig. 2. Solubility tests for the MVPS dissolved in organic solvents at room temperature (a), in alcohol and water co-solvents at room temperature (b), in distilled water at different temperatures (c) and in aqueous media with various pH values at room temperature (d).

The in vitro metformin release profile of the drug-loaded microspheres and the pure drug crystal powders was detected as follows. Determined amount of microspheres and pure drug crystal powders was incubated into test tubes containing 10.0 mL of phosphate buffer (0.1 M, pH = 7.4). These tubes were stored in the same shaking air bath and set at 35 °C and 100 rpm. At appropriate intervals, 0.2 mL of release medium was withdrawn and same amount of fresh buffer was supplemented to the test tubes. The concentration of metformin in the released medium from the two systems was measured with HPLC method.

3. Results and discussion

3.1. Basic properties of the MVPS

The total macromolecule extracts obtained from the flesh of *M. veneriformis* by boiling water extraction combined with alcohol precipitation contain two primary components, polysaccharides and protein impurities. The weight percent of polysaccharide and protein in the total extracts is about 70% and 25%, respectively. After the disposal with TCA deproteinization, the crude extracts were refined and changed into MVPS. The protein content in the MVPS was lower than 1.0% and the polysaccharide content in it was higher than 95.8%, indicating that MVPS is a relatively pure polysaccharide ingredient.

X-ray diffraction (XRD) patterns are determined by the structure of substance. The XRD pattern of MVPS shows a "bun-shaped" curve (Fig. 1a), indicating that the polysaccharide is a noncrystalline polymer. This result might be attributed to the complex composition in molecules type and/or conformations. Moreover, preparing methods could also influence the structure and accordingly made the noncrystalline state (Zia, Bhatti, Barikani, Zuber, & Sheikh, 2008). However, which reasons played a more important role in resulting to the noncrystalline state of MVPS is still uncertain now.

The TG and DSC curves of MVPS were illustrated in Fig. 1b. The DSC curve exhibited three endothermic peaks at 88 °C, 285 °C and 333 °C. The two peaks at low temperatures could be ascribed to the evaporation free and bonding waters while the third peaks could be attributed to the decomposition of polymer with low molecular weight. This result accords with the TG analysis. In the TG curve of MVPS, weight loss took place in three stages. The first one starts at 60 °C with a slow weight loss of 7%. The second stage starts at 285 °C and reaches a maximum at 333 °C with a sharp weight loss of 65%. The third stage starts at 333 °C and bears a slow weight loss again with the increase of temperature. The first stage is assigned to the loss of water. The second and the third correspond to the decomposition (thermal and oxidative) of polysaccharide, vaporization and elimination of volatile products. According to the literature (Nieto et al., 1991), pyrolysis of polysaccharides starts by a random split of the glycosidic bonds, followed by a further decomposition forming acetic and butyric acids and a series of lower fatty acids, where C2, C3 and C6 predominate. Anyhow, the structures of polysaccharides in MVPS are thermostable if the temperature dose is not above 280 °C.

3.2. Solubility of the MVPS

The dry powder of polysaccharide can easily dissolves in water, while it is slightly soluble in methanol and hardly dissolve in other selected organic solvents. In various organic solutes, the solubility of MVPS was obviously influenced by the polarity of solvents.

Increasing the polarity of solvents would help to increase the solubility of MVPS (Fig. 2a). This regularity was also observed during dissolving the polysaccharide in various alcohol/water mixed solvents. The solid polysaccharide hardly dissolved in the mixed solvents with high alcohol content (>40%) but it can be wetted and become swollen by adsorbing a lot of solvents. Once by increasing the water content in the mixed solvents, this swollen polysaccharide became completely dissolvable. As we can see in Fig. 2b, the solubility of polysaccharide was sharply increased when the alcohol content got reduced lower than 40% in the mixed solvents, and the value reaches a platform at 135.0 mg/mL when the alcohol content decreases to 10%. In pure distilled water, the solubility of polysaccharide was about 142.0 mg/mL at room temperature, and this value might be the maximum in all the tested solvents. In aqueous media, the solubility of polysaccharide was obviously affected by temperature in the range of 10–90 °C, which increased with the increase of temperatures like a lot of other hydrophilic polymers such as dextran and polyvinylpyrrolidone, and the highest value can even reach 275.0 mg/mL at 80 °C. The pH value of solvents was also considered as an impotent factor for impacting on the solubility of polysaccharide in aqueous media. However, it was not found that the solubility of polysaccharide was affected by the pH in the set extent of 1–13, and the solubility value was kept in a relatively stable range from 125.0 to 140.0 mg/mL.

The solubility of a given polymer in various solvents is largely determined by its chemical structure. Polymers will dissolve in solvents whose solubility parameters are not too different from their own. This principle has become known as 'like dissolves like', and, as a general rule, structural similarity favors solubility (Miller-Chou & Koenig, 2003). Except not including crystalline phases in solid state, MVPS contains large amount of hydroxyls in its molecules, which can form strong hydrogen bonding with water, thus the solubility in water is greatest. According to the theory of dissolution of an amorphous polymer in a good solvent (Wang, Ellis, & Ross-Murphy, 2008), increasing the temperature must be smaller than the entropic term, meaning that the dissolving process will occur spontaneously and resulting that more MVPS dissolved.

3.3. Rheological behavior of the MVPS solution

The viscosity of various concentrated solutions of MVPS has been firstly investigated with a rotational viscometer at room temperature and 6 s^{-1} of shear rate (Fig. 3a). As a result, very low apparent viscosities were tested with the various MVPS solutions when their concentrations were lower than 100 mg/mL. This means that these MVPS solutions displayed obvious Newtonian behaviors which usually occurred in the non-polymer solutions (Gravanis, Milas, Rinaudo, & Clarke-Sturman, 1990; Renaud, Belgacem, & Rinaudo, 2005). In spite of this, the more concentrated solutions showed a little yield stress towards increasing the viscosities. This appearance makes the polysaccharide inclining to like a common polymer. Such results might be attributed to the conformational aspects of MVPS. It was established that MVPS is rod like, having some more flexibility because of the α-glycosidic bond linkages between glucose units (Wang, Wu, et al., 2011; Wang, Zhang, et al., 2011). Thus the macromolecules in solution appear line round state. This state cannot arouse strong interaction forces between molecules. If the concentration increased, the molecules' interactions would be strengthened because the shorting of distance among the adjacent molecules. Fig. 3b and c also gives the rheological model describing the dilute and semi-dilute solutions of MVPS (see below). Fig. 3b shows curves of the viscosity change of MVPS with the shear rate at room temperature. It was shown clearly that the MVPS solution behave as Newtonian fluids at 50 mg/mL

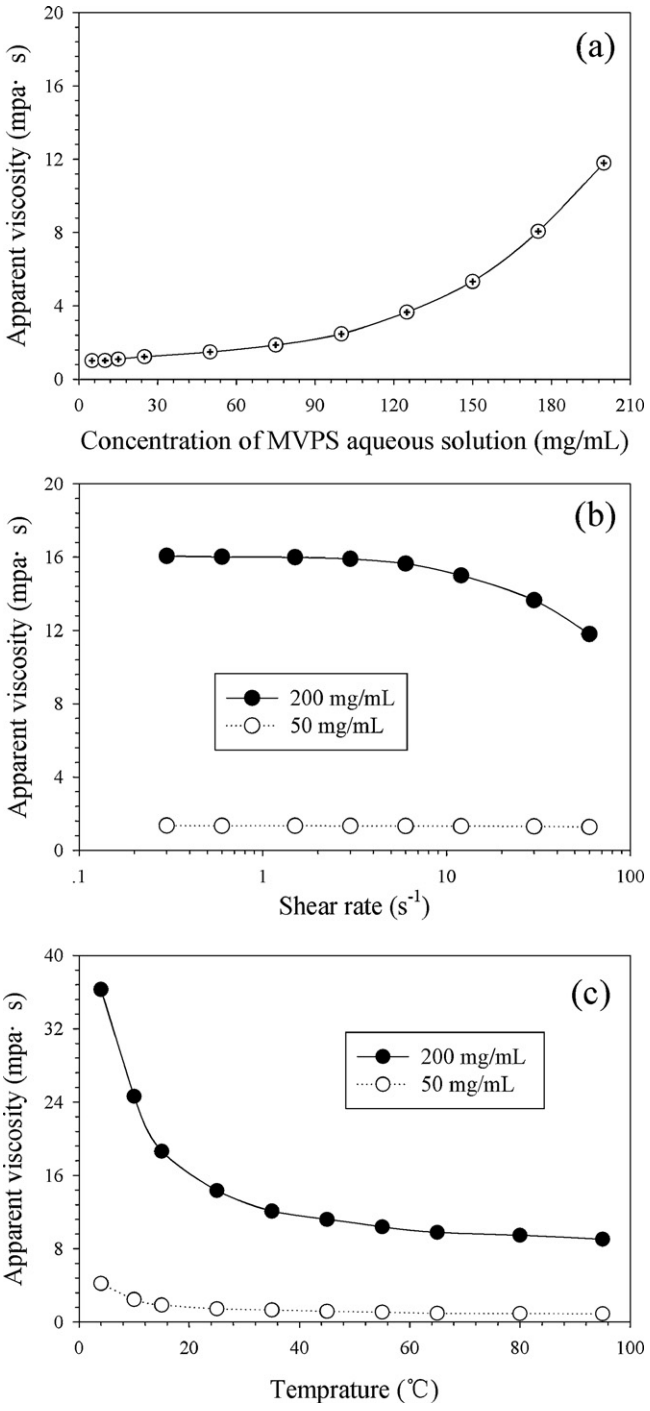

Fig. 3. Changes of apparent viscosity with the concentration of MVPS solutions at room temperature and 6 s^{-1} of shear rate (a), with the shear rate at room temperature (b) and with the temperature at 6 s^{-1} of shear rate (d).

of concentration while became rheo-thinning fluids at 200 mg/mL of concentration in high rate of shearing. Fig. 3c investigated the viscosity of MVPS dependence on temperature changes at 6 s^{-1} of shear rate. It was also established, for dilute and semidilute solutions, a unique slope curve for the gently reduced apparent viscosity as a function of temperature in range of 4–40 °C, as the temperature of MVPS solution went on increasing, the reduction in apparent viscosity will not became obvious.

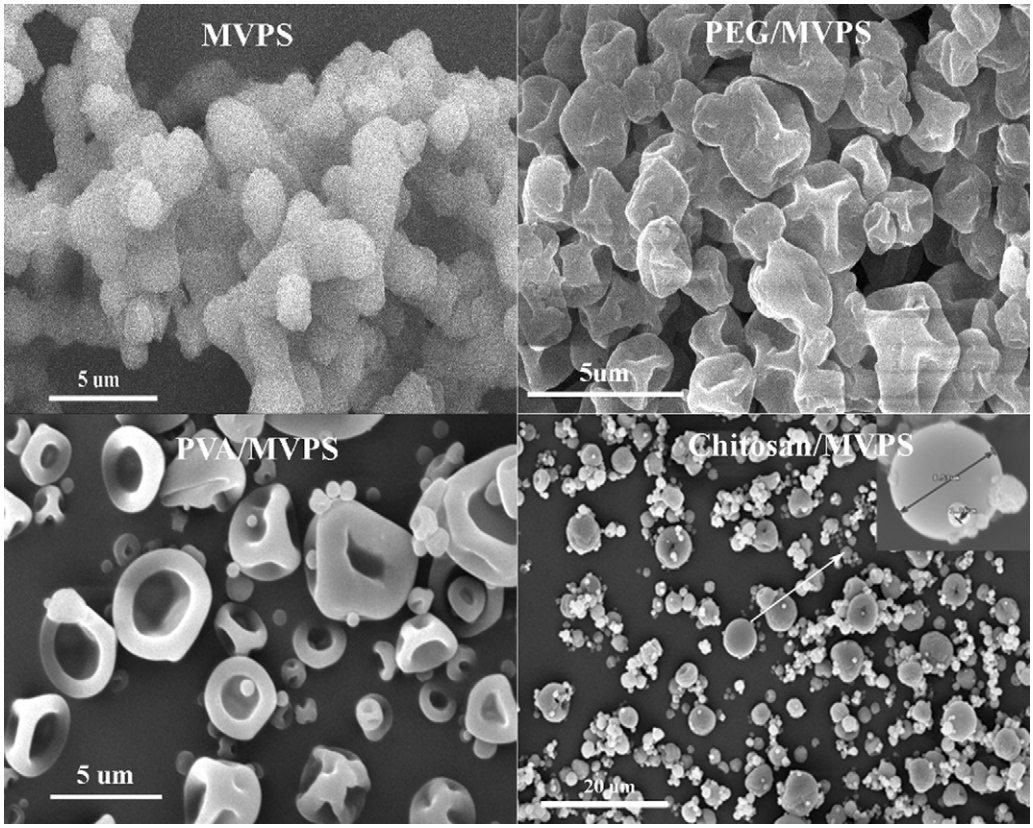

Fig. 4. SEM observations of the MVPS microparticle and three composite microparticles.

3.4. Characterization of MVPS microsphere and composite microspheres

Composition and overall characteristics of the microspheres are reported in Table 1. Satisfactory production yields of about 45–70% were found for all the formulations.

Shape and morphology of the microparticles were investigated by scanning electron microscopy (Fig. 4). MVPS microspheres prepared by spray drying were similar to spherical particles (seen MVPS image in Fig. 4), whereas large polymer aggregates were found in microspheres preparation. The co-encapsulation of PEG-6000 with MVPS produced microparticles with some surface sinks which made it seems like shriveled microcapsules (PEG/MVPS image). While the co-encapsulation of PVA with MVPS cannot improve the shape of the formulation, because when the feeding solution was spray dried, microcapsules with red blood cell shapes were obtained (PVA/MVPS image). Surprisingly, when the feeding solution containing chitosan was spray dried, smooth and spherical microspheres were obtained (chitosan/MVPS image). This could be attributed to the initial viscosity of the polymer feeding solution. If the initial solution has been made with a high dissolvable polymer or if there is too much solvent, the solution will not be viscous enough so the native droplet which leaves the nozzle will 'collapse' during the drying process. This would produce a microparticle rather than a microsphere.

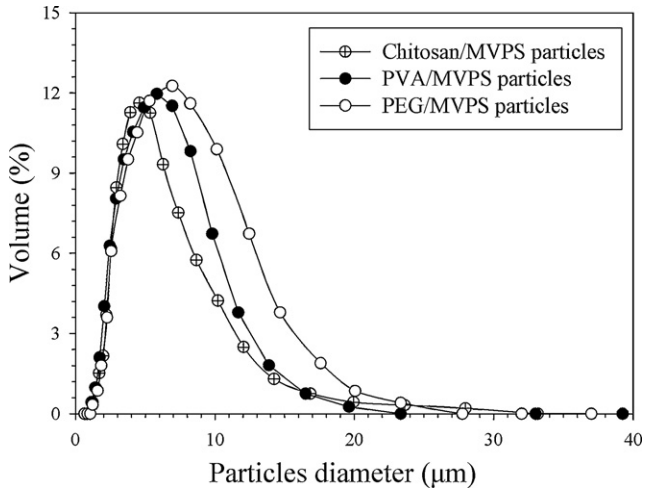

Fig. 5. Particle size distributions of the three types of microparticles.

Microspheres prepared with pure MVPS were aggregated and could not be dispersed to measure size. The other formulations displayed a monomodal size distribution (Fig. 5). The corresponding mean diameters are reported in Table 1. Microspheres prepared from the chitosan and MVPS blends showed a mean diameter of 4.2 μm, while microparticles prepared from the PVA and MVPS blends showed a mean diameter of 5.5 μm. Because of a slight aggregation, the co-encapsulation of MVPS with PEG resulted in lager microparticles with a mean diameter of 6.8 μm.

Table 1
Production yield, mean size and shape description of MVPS blending spray dried microparticles.

Samples	Production yield (%)	Mean size (μm)	Shape description
MVPS	45.5 ± 2.14	–	Aggregative particles
PVA/MVPS	67.4 ± 2.57	5.5 ± 2.6	Red blood cell shape
PEG/MVPS	70.2 ± 3.05	6.8 ± 1.3	Shriveled microcapsules
Chitosan/MVPS	69.7 ± 2.64	4.2 ± 0.2	Smoothed microspheres

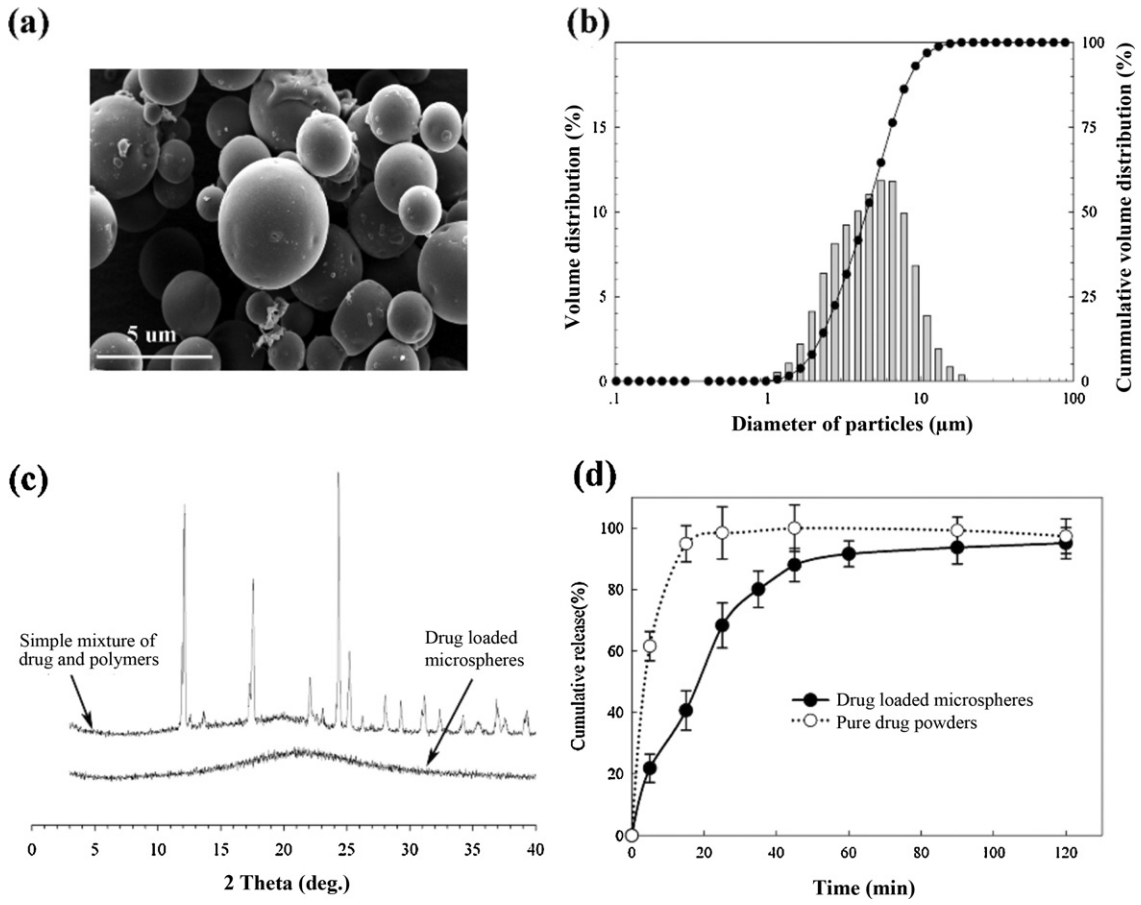

Fig. 6. Characterizations of metformin-loaded chitosan/MVPS microsphere: (a) SEM observation, (b) particles size distribution, (c) XRD analysis and (d) drug release profile.

3.5. Drug encapsulation and release of from microspheres

The chitosan/MVPS composite microsphere is the appropriate carrier materials via morphological observation, thus studies on the drug (metformin) loaded in it should also be investigated. Preparation of drug loaded chitosan/MVPS microspheres was carried out with the same methods. In the preparing process, satisfactory and over 70% of production yields were acquired. Microencapsulation of metformin in chitosan/MVPS blends by spray-drying resulted in spherical, smooth and solid particles (Fig. 6a). The sizes of the drug loaded microsphere formulations were about 1–10 μm (Fig. 6b) and were hardly affected by the change of process parameters. This range of particle size has been reported to be appropriate for nasal administration (Liu, Jia, & Xing, 2007; Varde & Pack, 2004). The actual loading of metformin in the dose was 22.31 ± 0.38%; this value was very close to that of theoretical loadings. The encapsulation efficiency of the chitosan/MVPS microsphere for metformin was 89.24 ± 1.53% by calculation. The status of drugs existed in the microspheres were examined with XRD analysis by comparing the drug loaded microspheres with the simple mixture of drug and polymers. The XRD patterns (Fig. 6c) showed that metformin was totally incorporated into the chitosan/MVPS microsphere because all crystallization peaks of metformin were entirely disappeared. The in vitro release profiles of drug-loaded microspheres are plotted in Fig. 6d.The release rate of microsphere formulations was lower than that of the pure drug powders. When metformin was incorporated into chitosan/MVPS microspheres, its release was significantly decreased ($p < 0.05$), resulting in only 65% release within 25 min compared to almost 100% release of drug in powder form. In spite of this, the metformin release from the microsphere still belongs to fast release and about 90% drugs were released during 90 min. It was because chitosan and MVPS are both hydrophilic polymers which can hydrogen bond with water, this hydrophilic surface is expected to reduce the contact angle and lead to the increase of wettability (Kobayashi, Shimizu, Kaizuma, & Konishi, 2011). Moreover, MVPS are highly dissolvable polymers, could enhance drug dissolution by breaking the microspheres once contacted with aqueous media. However, on the other hand, the prompt release of metformin from the microspheres was easily cleared from the nasal cavity and could be helpful in reaching the desired drug concentration in plasma after administration.

4. Conclusion

This study aimed at collecting different data relative to physicochemical properties of the polysaccharides from *M. veneriformis* and to primarily demonstrate that this natural polymer can be used to process microsphere formulations for loading drugs. XRD and thermodynamics tests indicate that the animal polysaccharides are noncrystalline but thermostable polymers. The MVPS are water-soluble polymers that can easily and fast dissolve in aqueous media. The polysaccharide solution belongs to obvious non-Newtonian fluids even at relatively high concentrations. It seems that the polymer is not a suitable material to prepare microspheres just by spray-dried processing, which can be proved by SEM picture of the spray dried powders of single MVPS. However, the problems in preparing microspheres can be resolved by blending the polysaccharide with other polymers, such as chitosan. The chitosan/MVPS composite microspheres were prepared and they showed smooth and spherical morphology. The composite microspheres can load

some drugs to achieve the sustained drug release. The sizes of the drug loaded microsphere formulations were about 1–10 μm and might be an appropriate for nasal formulation. Thus, the next more detailed investigations will focus on bioactivities evaluation of the drug-loaded chitosan/MVPS microspheres in diabetic rats via nasal administration.

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