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Preparation and characterization of sorafenib-loading microcapsules by complex coacervation of gum Arabic with chitosan or modified chitosan

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ABSTRACT

Sorafenib is an oral multi-target kinase inhibitor that has been used to treat unresectable hepatocellular carcinoma and advanced renal cell carcinoma. The aim of the study was to prepare gum Arabic-chitosan (GA-CS) and gum Arabic-modified chitosan (GA-MCS) microcapsules containing sorafenib as the core phase by complex coacervation. The fluorescence microscopy, dynamic light scattering (DLS), drug loading, and encapsulation efficiency of the microcapsules were clarified. The GA-MCS microcapsule was successfully performed at approximate pH of 4 with a 1% modified chitosan -to- 5% gum Arabic ratio of 5:1 (v/v), while the GA-CS microcapsule was successfully prepared at pH 3.5 with a volume ratio of 1% chitosan -to- 5% gum Arabic 1:1 (v/v). Sorafenib was encapsulated in the microcapsules as shown through the fluorescence microscopy images. The formation of GA-CS and GA-MCS microcapsules with hydrodynamic sizes of 6.31 μm and 6.56 μm, respectively, was successfully achieved. The drug loading and encapsulation efficiency of the GA-MCS microcapsule was greater than that of the GA-CS microcapsule. The findings indicated that the GA-MCS microcapsule could be an appropriate formation to load sorafenib with a high encapsulation yield.

Keywords: Microcapsule; Gum Arabic; Chitosan; Drug loading, sorafenib.

1. INTRODUCTION

Microcapsules with size from 1 to 1000 μm started to be studied in 1929 [1], spherical gelatin has been successfully synthesized through a coacervation [2]. This method is used to load compounds in pharmaceutical, cosmetic, or food products, for example, drugs, proteins, hormones, flavors, dyes [3]. Microcapsule has the role of protecting and stabilizing the substances inside it from oxidizing agents, or external pressure [4]. Due to the thin film formed during the interaction, it is possible to encapsulate the substances inside and isolate them from the external environment [5]. Thus, the microcapsule enables the controlled release of substances into the human gastrointestinal tract. Although microencapsulation has been studied for a long time, the coacervation mechanism has only been studied recently. The coacervation is determined by electrostatic attractive forces of polymeric pair, which is affected by their surface charges. Thus, the pH, the ratio between polymers and stirring time considered are key factors for the coacervation process [6].

Recently, the microcapsules prepared from gum Arabic and chitosan have been reported by many researchers [7, 8]. Gum Arabic (GA), is one of the most used polysaccharides in industry, has an active surface and low viscosity. GA is an additive material with a negatively charged surface because it contains carboxyl groups (–COOH). Therefore, it could form complexes with proteins or other positively charged polysaccharides through coacervation [9].

Chitosan (CS) is a natural polysaccharide compound extracted through the deacetylation of chitin [10]. CS has a large number of amino ($–NH_2$) and hydroxyl (–OH) groups, so the surfaces of CS could be ionized to be a positive charge. Microcapsules from CS can control the release of drugs, thereby increasing the bioavailability of drugs, especially water-insoluble drugs [11].

Modified chitosan (MCS) is similar to natural chitosan; it has a positively charged surface with lots of primary, secondary, and even tertiary amine groups. In addition, MCS has a higher buffering capacity than natural chitosan which might be an advantage in coacervation [12].

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Sorafenib (Sor) has been used as a targeting therapeutic agent for a large range of tumor types, but it belongs to biopharmaceutics classification system class II, which has a very poor aqueous solubility, and thus lead to a poor bioavailability [13].

To enhance drug delivery into the gastrointestinal tract, Sor is loaded by microencapsulation. In this study, gum Arabic-chitosan microcapsule and gum Arabic-modified chitosan microcapsule containing Sor as core were prepared by complex coacervation. The zeta potential of GA, CS, and MSC dispersions is essential to survey for determining the ratio of the polymers and the pH. The morphology of microcapsules were also analyzed based on optical images. Particle size was measured by DLS, and drug loading capacity and encapsulation efficiency were also examined.

2. MATERIALS AND METHODS

2.1. Materials

The materials used included gum Arabic (GA, MW ~ 25,000 g/mol) and chitosan (CS, MW ~ 5,000 g/mol) being provided from Yuhuan Marine Biochemistry Co., Ltd. (Yunhuan, China). Modified chitosan (MCS, MW ~ 9,786 g/mol) was a gift from Dr. Guojun Huang (Zhejiang University). Sorafenib (Sor, purity > 98%) obtained from Eastchina Pharm. Co., Ltd., Zhejiang, China was used as a core material. Glutaraldehyde was purchased from Merck Chemicals Co. (Darmstadt, Germany). Hydrochloric acid (HCl) and sodium hydroxide (NaOH) were bought from Sigma-Aldrich (St. Louis, MO, USA). All other materials were of analytical grade, and deionized water was used in all experiments.

2.2. Zeta potential of polymeric solutions

The dispersions of GA 5%, CS 1%, and MCS 1% in deionized water were stirred for 6 hours and stored overnight at 4 ºC. The pH of the above polymeric solutions was adjusted between 3 and 6 by HCl 0.1 mol L^{-1} and NaOH 0.1 mol L^{-1}. Their zeta potentials at room temperature were determined using a Zetasizer 3000 (Malvern Instrument, Worcestershire, UK).

2.3. Microcapsules preparation

According to the methodology described by PRATA and GROSSO [14], the stoichiometric ratio of the substances should bring the system to electrostatic neutrality. Therefore, the ratio of polymers was calculated based on their zeta potential. The ratio of total volume of reaction to Sor solution was 10:1. The stoichiometric ratio and pH of solutions were listed in Table 1.

At first, the emulsifier phase GA was heated to 50 °C and stirred at 1000 rpm for 5 min by a thermostatic magnetic stirrer ZNCL-BS140 (Henan, China). Then V (mL) of Sor in DMSO was dropped while stirring. After 5 min of stirring, the complexing phase (CS or MCS), also at 50 °C, was added and kept stirring for 1 h. Then the pH of the dispersion was adjusted up to the pH of coacervation of the polymeric pair with HCl 0.1 mol L^{-1} and NaOH 0.1 mol L^{-1}. The mixtures were continuously stirred for t h (t = 0.5 h; 1.0 h; 2.0 h and 3.0 h) at 50 ºC to investigate the effect of stirring time on aggregation of microcapsules. The mixture was slowly poured into the distilled water preheated at the temperature of 40 ºC. The ratio of the mixture and the water was 1:1. To stabilize the microcapsules, 10 mL of glutaraldehyde 4% was dropped. After stirring for 1 h, the mixture was cooled to ambient temperature.

Table 1: Conditions for the preparation of systems.

MICROCAPSULE	GA-CS-Sor	GA-MCS-Sor
Phase Emulsifying	GA (5%) 20 mL	GA (5%) 10 mL
Sorafenib in DMSO	Sor (1, 2, 3, 4, 5 và 6 mg mL^{-1}) 4 mL	Sor (1, 2, 3, 4, 5 và 6 mg mL^{-1}) 6 mL
Phase Complexing	CS (1%) 20 mL	MCS (1%) 50 mL
pH	3.5	4.0

2.4. Microcapsules characterization

2.4.1. Morphology and size

Morphology of the microcapsules at Sor concentration of 4 mg mL^{-1} was observed using a fluorescence microscope Nikon DS-Ri2 (Nikon Corporation, Tokyo, Japan) with a magnification of 40×. The DLS of microcapsules in dispersion was determined using a laser Light Scattering (Beckman Coulter, USA) with sample suspension unit.

2.4.2. Drug loading (DL%) and encapsulation efficiency (EE%)

The result mixture was then centrifuged slowly at 400 rpm for 5 min and the supernatant was decanted. The solid was washed with methanol three times to remove unreacted-Sor, and was then washed with water to remove unreacted phases. The microcapsules were freeze dried, weighed and suspended in deionized water.

The content of Sor loaded in microcapsules was determined by spectrophotometry. Methanol (10 ml) was added to dried and crushed microcapsules (~2.0 g) in a tube with a lid. The system was vigorously agitated for 15 min, and agitation of the tube was maintained in the next 4 hours. The mixture was filtered and then washed by using methanol. The filtrate was poured into the 25 mL volumetric flask and the methanol was added to fill up the volumetric flask. Absorbance was measured at a wavelength of 266 nm using a Thermo Scientific Evolution 300 UV–Vis spectrometer (Thermo Scientific, Waltham, MA). The amount of Sor loaded in the microcapsules was calculated by appropriate calibration curve of free Sor in methanol y = 132.0*× + 0.036 and R^2 = 0.9998. The experiments were measured in triplicate. The drug loading (DL%) and encapsulation efficiency (EE%) was calculated as:

$$DL\% = \frac{S}{m_{ms}} \cdot 100\% \tag{1}$$

$$EE\% = \frac{S}{S_o} \cdot 100\% \tag{2}$$

With S is mass of Sor in microcapsules, S_o is mass of initial Sor added in the process, and m_{ms} is mass of microcapsules.

3. RESULTS AND DISCUSSION

3.1. Zeta potential of gum Arabic, chitosan, and modified chitosan

The electrostatic forces between oppositely charged polymers determine the performance of the reaction. The pH ranges of a reaction could be assessed through the surface charge of polymers. The regions of pH chosen for the reaction between two polymers were highlighted (Figure 1).

The surface charge analysis results showed that GA had a negative charge, while CS and MCS had a positive charge in the pH range of 3 – 6, thus, GA could react with CS or MCS in this pH range. This result was also consistent with their structures. Moreover, the curve of zeta potential of chitosan was very sheer, ranging from 61 mV at pH 3.1 to 3.0 at pH 6.1. While the buffering capacity of MCS was higher than that of CS [12], its curve of surface charge is plain in the pH 3 – 6 region, from 12.5 mV to 8.9 mV, respectively. This indicated that the interaction of GA and MCS occurred successfully in a larger range of pH in comparison to the pH range of GA and CS.

Since the complex coacervation was determined by electrostatic interactions, the ratio of polymers for obtaining charge neutrality could be estimated [15]. From the zeta potential curve between the polymers (Figure 1), the ratio of GA (~ –8.9 mV) to chitosan (~44.8 mV) 5:1 and the pH of 3.5 were expected. For the pair GA (–11 – –13 mV) and MCS (~12 mV), the ratio of GA to MCS of 1:1 at pH range of 4.0 – 4.5 was appropriate.

On the other hand, the coacervation of two polymers was verified at the concentration of 3% w/w of total mass [14]. Therefore, the volume ratio between 5% GA and 1% CS used at pH 3.5 was 1:1 (v:v) while a volume ratio of 1:5 of 5% GA and 1% MCS at pH 4.0 was chosen for the interaction to be occurred.

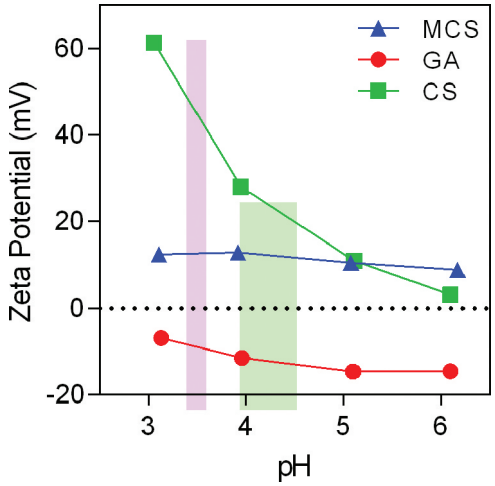

Figure 1: Zeta potential of three polymers (GA, CS and MCS) in pH 3 – 6.

3.2. Morphology and hydrodynamic size of microcapsules

3.2.1. Effect of stirring time on the morphology and hydrodynamic size of microcapsules

After adjusted pH of coacervation, the dispersion was stirred for 0.5 h; 1.0 h; 2.0 h and 3 h for examining the effect of stirring time on hydrodynamic size and aggregation of microcapsules, all other conditions were remained.

The result showed that, the coacervation of GA and CS was successful after stirring for 1.0 h or 2.0 h (Figure 2a and 2b). When the stirring time was 0.5 h, the microcapsules was not formed, might due to the short time of interaction, both phases were mostly not aggreated. After stirring for 1.0 h to 3.0 h, the microcapsules were presented as single-core spheres. Their hydrodynamic size were larger when the stirring time was longer (Figure 2b). In details, the d_{50} values of hydrodynamic size of microcapsules were 1.45 µm, 2.70 µm and 12.6 µm at stirring time of 1.0 h, 2.0 h and 3.0 h, respectively. The sizes of microcapsules at 1.0 h of stirring time were as small as that of nanoparticle, while after 3.0 h of stirring, the aggregation of microcapsules occurred, resulting in the difference in sizes. At 2.0 h, the sizes of microcapsules were identical, thus 2.0 h was chosen for the coacervation between GA and CS to load Sor.

The same way with GA-CS microencapsulation, at 0.5 h of stirring time, the GA-MCS microcapsules loaded Sor was not occurred. While the stirring time of 1.0 h, the microcapsules were formed as multi-core spheres, with a uniform size of d_{50} of 3.67 µm and d_{90} of 6.56 µm. When the stirring time was longer, the aggregate of microcapsules was occurred, leading to a varied size (Figure 2c) and an irregular morphology of microcapsules (Figure 2d). Therefore, the stirring time of 1 h for the coacervation of GA and MCS to load Sor would be chosen. The formation of two types of microcapsules might have been caused by differences in molecular weight and surface charge of CS and MCS. The molecular weight of CS used was 5,000 g/mol, while that of MCS was a larger 9,786 g/mol with many amino groups on the surface, which might lead to an easier coacervation.

3.2.2. Effect of pH on morphology and hydrodynamic size of microcapsules

To prove the effect of the pH on the coacervation of polymeric pair, the pH of the interaction of GA and CS was increased to 4.0, and the pH of the interaction of GA and MCS was adjusted to 4.5 (Figure 2). The results exhibited that increasing slightly the pH from 3.5 to 4.0, where the values of zeta potential of GA and CS were ~11.5 and ~28.0, respectively. For the complex coacervation occouration, the ratio of GA and CS should be 2.4:1, but it remained at 5:1. It can be deduced from theory that, the coacervation between GA and CS did not occurred due to the biased electric charge of the system. Meanwhile, the GA-MCS microcapsule was also formed at the pH of 4.5, where the values of the zeta potential of two polymers were ~11.0 and ~12.5. Thus, the ratio of the two polymers was the same as the ratio in the experiment. Indeed, their sizes and shapes were almost as the same as the microcapsule at pH of 4.0. This result coincided with the zeta potential curves of polymers, which were decided by their buffering capacity. In other words, the wider pH range in the interaction of a polymeric pair can be obtained if the buffering capacity of the polymeric pair is high.

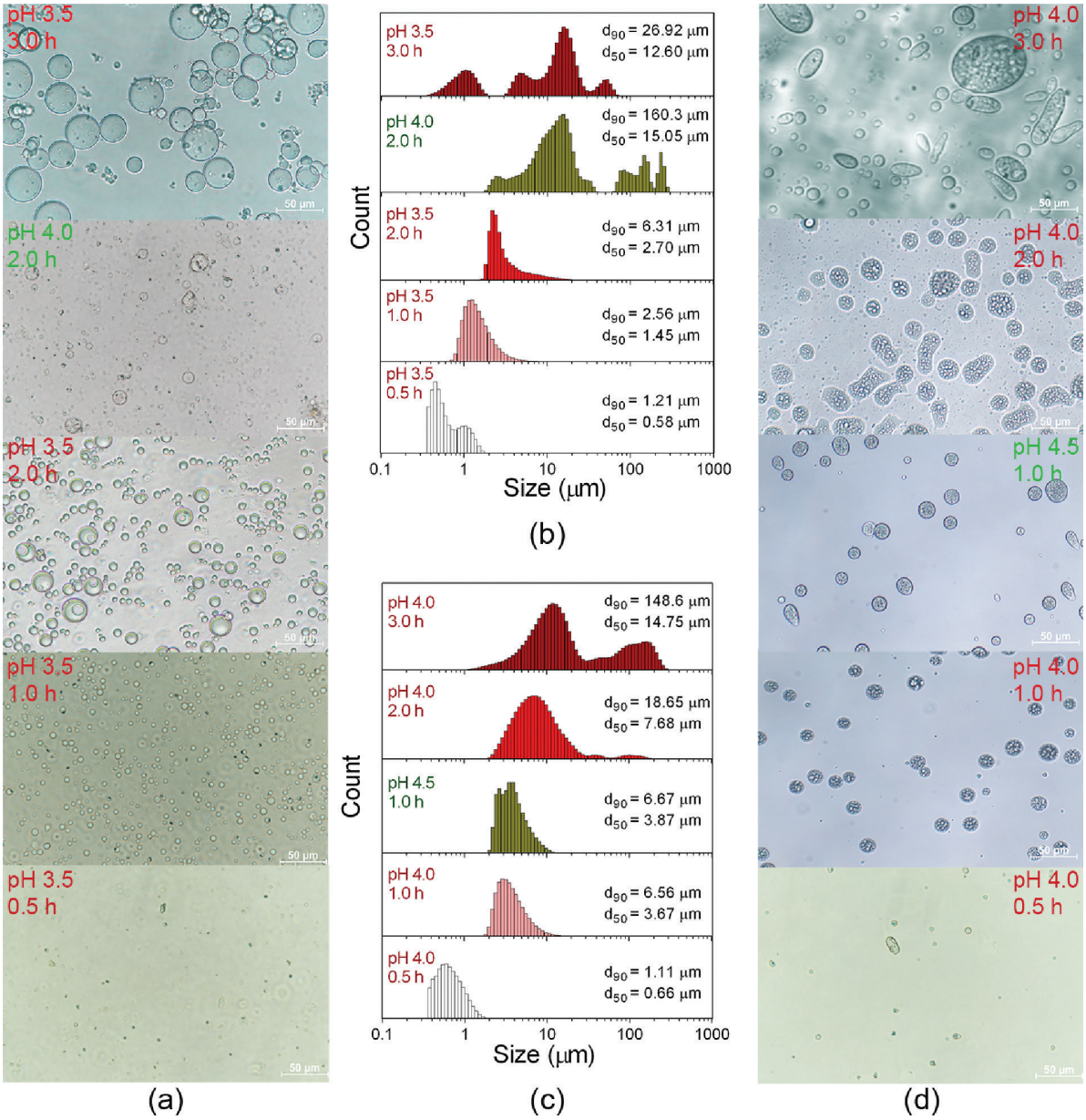

Figure 2: Effect of stirring time and pH on morphology and hydrodynamic size of microcapsules (a) morphology and (b) The hydrodynamic size of GA-CS-Sor microcapsules; (c) The hydrodynamic size and (d) morphology of GA-MCS-Sor microcapsules at different stirring time and pH.

3.3. Drug loading and encapsulation efficiency analysis

From the above findings, GA-CS-Sor and GA-MCS-Sor microcapsules achieved a suitable morphology and hydrodynamic size at stirring time of 2.0 h, pH of 3.5, and 1.0 h, pH of 4.0, respectively. The fluorescence imaging with UV light of GA-CS-Sor and GA-MCS-Sor microcapsules at the initial Sor concentration of 4 mg mL^{-1} was shown in Figure 3. The results demonstrated that Sor was successfully encapsulated in microcapsules.

The effects of drug concentrations on the Sor loading (DL%) and encapsulation efficiency (EE%) of GA-CS and GA-MCS microcapsules were shown in Figure 4.

The curves of DL% presented that the amount of Sor loaded by the microcapsules increased when the amount of Sor added increased. However, when the concentration was higher than 5 mg mL^{-1}, the Sor loading capacity of the microcapsule did not increase further (Figure 4a). This result was consistent with the EE% of the microcapsule (Figure 4b). Initially, the EE% of the microcapsule increased with an increase in concentration and decreased when the concentration of Sor was higher than 4 mg mL^{-1}. This can be explained by the precipitation

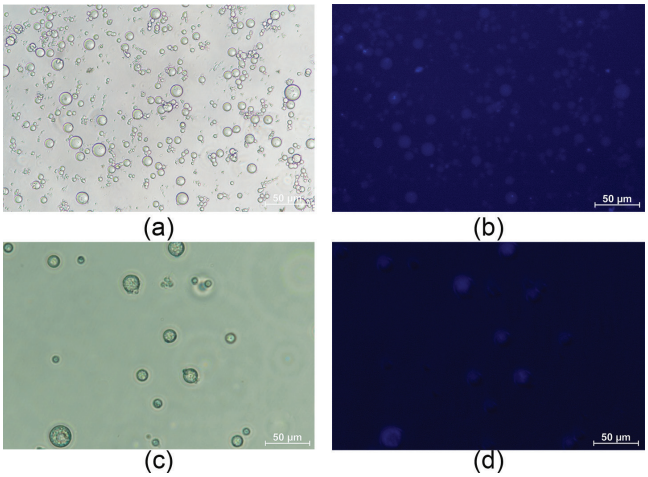

Figure 3: Imaging of GA-CS-Sor (a), (b) and GA-MCS-Sor (c), (d) under white light and florescence with UV light.

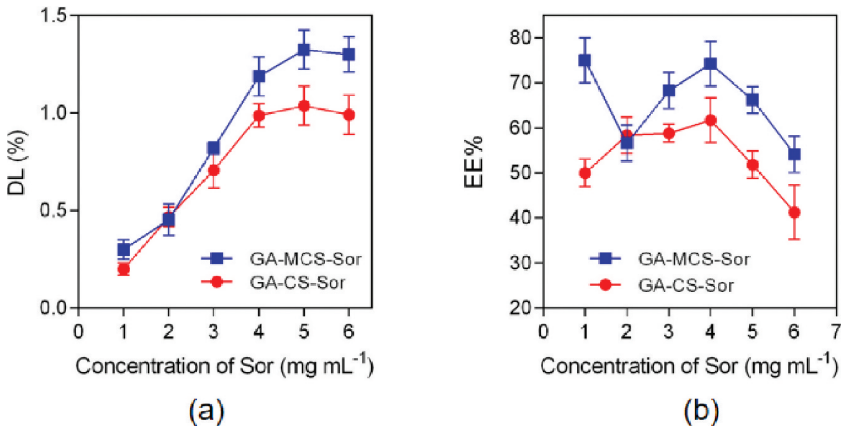

Figure 4: Drug loading (a) and encapsulation efficiency (b) of GA-CS-Sor and GA-MCS-Sor.

of Sor in water; when dropped from DMSO solvent to mixture, or may be due to the reduction in free space in the microcapsule after loading a large amount of Sor. That result revealed that GA-MCS-Sor was loaded with a higher amount of Sor, and the microcapsules should form at a concentration of Sor of 4 mg mL^{-1}.

4. CONCLUSIONS

Gum Arabic-chitosan and gum Arabic-modified chitosan microcapsules were successfully prepared to load Sor. The results showed that the microcapsules were mostly spherical in the shape, and the particle size was about 6 µm. In addition, gum Arabic-modified chitosan microcapsule was formed at a wider pH range, and had a higher drug loading capacity. Therefore, the gum Arabic-modified chitosan microcapsule loaded Sor could be applied in pharmaceutical industry to use as an anti-cancer drug.

5. ACKNOWLEDGMENTS

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