Research Article

Xin Wang[#], Zhe Wang[#], Shengwei Guo^{*}, Guxia Wang, and Min Xue

Sonochemical synthesis of protein microcapsules loaded with traditional Chinese herb extracts

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Abstract: Traditional Chinese herbs have attracted extensive attention due to their good efficacy, low toxicity, and minor side effects. However, some active ingredient extracts are relatively sensitive to external influence and liable to lose their effectiveness. Different from the traditional and complex encapsulation methods, one can easily prepare drug-loaded microcapsules to improve their bioavailability using the new encapsulation technology. In this work, we used the sonochemical method to prepare bovine serum albumin (BSA)/astragalus membranaceus oil (AM) microcapsules. The technology was simpler, greener, and more efficient. Thereinto, BSA and AM oil were used as the shell material and the core material, respectively. The effects of ultrasonic amplitude, ultrasonic mode, and ultrasonic time on synthetic reactions were studied. The morphology and size of the BSA/AM microcapsules were investigated using transmission electron microscopy, scanning electron microscopy,

and dynamic light scattering (DLS). The loading efficiency and drug release behavior were determined using thermogravimetric analysis (TGA) and ultraviolet (UV) spectroscopy, respectively. The best ultrasonic synthesis conditions were obtained by the above analysis, with an ultrasound amplitude of 30%, ultrasound mode of the pulse mode of 2 s, ultrasound time of 4 min. The DLS results show that the microcapsule size is 484.4 nm and the polydispersity index is small. The TGA results show that the drug loading efficiency is about 77%, and the hemolysis tests show that the BSA/AM microcapsules have no cytotoxicity at lower concentrations (lower than 50 $\mu\text{g}\cdot\text{mL}^{-1}$).

Keywords: sonochemistry, Chinese herb microcapsules, bovine serum albumin, astragalus membranaceus oil

[#] These authors contributed equally.

*** Corresponding author: Shengwei Guo**, Department of Polymer Materials and Engineering, School of Materials Science and Engineering, North Minzu University, Yinchuan 750021, China; International Scientific and Technological Cooperation Base of Industrial Waste Recycling and Advanced Materials, North Minzu University, Yinchuan 750021, China; Key Laboratory of Polymer Materials and Manufacturing Technology, North Minzu University, Yinchuan 750021, China, e-mail: shengwei@nzu.edu.cn

Xin Wang, Zhe Wang, Min Xue: Department of Polymer Materials and Engineering, School of Materials Science and Engineering, North Minzu University, Yinchuan 750021, China; International Scientific and Technological Cooperation Base of Industrial Waste Recycling and Advanced Materials, North Minzu University, Yinchuan 750021, China; Key Laboratory of Polymer Materials and Manufacturing Technology, North Minzu University, Yinchuan 750021, China

Guxia Wang: Department of Chemical Engineering and Technology, School of Chemistry and Chemical Engineering, North Minzu University, Yinchuan 750021, China

1 Introduction

Traditional Chinese medicine is precious wealth accumulated by the Chinese nation in the fight against diseases for thousands of years [1]. The ingredients of most traditional Chinese medicines are some extracted herbal plants. The active ingredients are relatively fragile.

Most Chinese medicines are directly taken orally, which causes inactivation and the change in the pH value of gastric juice due to the influence of secretions in the human body. These will change the environment in the body, thereby greatly reducing the efficacy. In addition, the interaction between some herbal ingredients also limits their scope of application. If a special carrier can be used, the fragile extracts of traditional Chinese medicine will be protected from the external environment. Therefore, the drug delivery carriers have become the focus of researchers.

As a new type of carrier, microcapsules can change a series of physical and chemical properties of the internal substance through the outer layer material; it is also an ideal choice for protecting the internal drugs [2,3]. Since its inception, microcapsules have been favored by many scholars.

They can effectively overcome the shortcomings of traditional formulas that reduce the efficacy of herbal medicines [4].

The microcapsule is a completely sealed or semi-permeable miniature substance, inside which gas, liquid, or solid are covered by one or more polymer materials [5]; its particle size is between 10 and 100 μm . Structurally, the microcapsule is composed of shell and core materials. The shell material is dominated by polymers and they are required to have a certain degree of mechanical strength, stability, and no interaction with the core material. The core materials are selected according to people's requirements. Of course, the microcapsules can also be hollow [6–8]. The color, shape, volume, density, stability, and other physical and chemical properties of the core material can be changed through microencapsulation. The core material can also be separated from the external environment, thereby protecting the core material and releasing them when needed. The research on microcapsules' synthesis technique started in the 1930s when a fishery company in the United States prepared a cod liver oil microcapsule [9]. In the 1970s, microcapsules' synthesis technique was developing rapidly and its application range gradually was expanding, which attracted widespread attention from researchers and had broad research prospects [9–11].

The traditional encapsulation technique has become perfect during several decades. Based on its formation mechanism, it can be roughly divided into three categories: physical methods (spray drying, solvent evaporation, air suspension method, etc.), chemical methods (interfacial polymerization, *in situ* polymerization, orifice-coagulation bath method, etc.), and physicochemical methods (oil phase separation, aqueous phase separation, multiphase emulsion method, etc.) [12]. Different methods would be selected depending on the properties of the core and shell materials, the size of microcapsules, the drug release performance, and the targeting of core materials [13,14].

Estevinho et al. [15] prepared biopolymer microcapsules by controlling the release of folic acid using the spray drying method. Arabic gum, modified chitosan, modified starch, pectin, and sodium alginate were used as the shell material for microcapsules. The product yield ranged from 13% to 50%, and the efficiency of encapsulation was about 100% with most of the shell used. The release results showed that the spherical microcapsules could release folic acid completely, and the release time was influenced by the encapsulant. In all cases, the total release was achieved in less than 2 h. Lim et al. [16] prepared PLC/PEG microcapsules using an oil-in-water emulsion-solvent evaporation technique and studied the drug delivery behavior. The particle size of microcapsules was between 1 and 5 μm , and its release property was excellent.

Jiang et al. [17] synthesized polydopamine nanocapsules using special interfacial polymerization, γ -ray polymerization-induced assemblies; the diameter was about 300 nm. These nanocapsules had a good loading and releasing performance, and when the loaded model drug (ciprofloxacin, CIP) was released, it could effectively inhibit the growth of *E. coli*. Huang et al. [18] prepared tea tree oil (TTO)/urea-formaldehyde (UF) resin microcapsules using *in situ* polymerization. They had a narrow size distribution and good shell cover, and the loading rate of TTO in TTO/UF microcapsules was up to 45%. After 5 days of continuous release, 90% of TTO of the maximum loaded amount was found. These results show that the microcapsules have high stability to be used as a slow-release vehicle for antibacterial application. Zhu et al. [19] prepared cell-laden hydrogel microcapsules, and sodium alginate as the shell of microcapsules. By adjusting aqueous-phase flow rates and oscillating frequencies, these microcapsules can be stably and continuously generated. Moreover, it has good biocompatibility and broad application prospects in many biomedical fields. Somani et al. [20] prepared release microcapsules of pentazocine hydrochloride using the coacervation phase separation technique. The results showed that the microcapsules show good performance related to drug content, drug retention efficiency, surface morphology, and drug release.

Recently, in addition to these traditional methods, there are other methods for the preparation of drug-loaded microcapsules. Yang et al. [21] prepared magnetic hybrid-shell microcapsules through mini-emulsion polymerization. The obtained microcapsules had an average diameter between 200 and 600 nm. The microcapsule shell had great application potential in drug carriers due to its magnetic properties. Jin et al. [22] synthesized calcium cross-linked polysaccharide hard-shell microcapsules using a layer by layer method. They had good antibacterial properties, and it can be seen from the release results that the ibuprofen (IBU) coated on the microcapsules was released quickly in the first hour and a subsequent prolonged release in 10 h for 80 wt%, and complete release needed 20 h. Della Porta et al. [23] synthesized injectable PLGA/hydroxyapatite/chitosan microcapsules by the supercritical emulsion extraction technique. They were coated with teriparatide/gentamicin inside, and the different drug loadings and microcapsule compositions could ensure controlled drug release within a wide range of time and concentration. Supercritical emulsion extraction fabricated microcapsules showed a good encapsulation efficiency, up to 90%. The particle size was between 1.4 and 2.2 μm . Zou et al. [24] studied the self-assembly process to encapsulate traditional Chinese medicines. The structure and

properties of the BJO nanocarrier were characterized by transmission electron microscopy (TEM), SAXS, and dynamic light scattering (DLS). The results showed that the particle size of nanoscale could be achieved (<200 nm), and high encapsulation efficiency was realized ($>90\%$).

Although with the above methods, some research was conducted on drug-loaded microcapsules, the common problem of these methods is that they are too cumbersome and have a long reaction time. Compared with other microcapsule synthesis methods, the sonochemical method has great advantages. The sonochemical method mainly refers to accelerating chemical reactions or triggering new chemical reactions using ultrasound [25]. The energy imparting speed is fast, and the energy is extremely high. It is green, simple, and efficient [26]. Ultrasound is a mechanical wave with a frequency range from 2×10^4 to 10^7 Hz. The chemical reactions were performed by the unique ultrasonic cavitation effect [27]. The ultrasonic cavitation effect refers to the process in which cavitation bubbles are generated by high-frequency radiation of ultrasonic waves in a liquid. These cavitation bubbles nucleate, grow, and burst. This process is extremely short. In one or several vibration cycles, the cavitation bubble expands and grows rapidly in the vibration negative pressure phase and then contracts and ruptures in the positive pressure phase [28–30]. When the cavitation bubble ruptures, a local high temperature ($5,000$ K) and high pressure (1.013×10^8 Pa) are produced, and the heating and cooling rates ($>10^9$ K·s $^{-1}$) are extremely fast. The cracking of the liquid in contact with the cavitation bubbles produces a large number of free radicals at the interface. This increases the activity of the substance $10,000\times$ [26,28,31]. Therefore, it provides possibilities for many chemical reactions that are impossible or difficult to achieve under normal temperatures and pressures.

In this article, a facile sonochemical method was used to prepare protein microcapsules with BSA as the shell material and astragalus membranaceus oil (AM) as the core material. Compared with traditional encapsulation methods, this synthesis process has short reaction times and high efficiency, and there are no by-products in the reaction system. In addition, for the first time, we obtained traditional Chinese medicine microcapsules by sonochemistry using biological macromolecules as the shell. Compared with previous studies, it is a further step toward application prospects. The effects of ultrasound amplitude, ultrasound mode, and ultrasound time on the performance of microcapsules were studied, and the best ultrasound parameters were determined. The particle size, drug loading, structure, morphology, and the cytotoxicity of the microcapsule products obtained under optimal conditions were characterized and analyzed by

DLS, TG, scanning electron microscopy (SEM), TEM, and hemolysis experiments.

2 Experimental protocol

2.1 Materials

Bovine serum albumin (BSA) was purchased from Shanghai Titan Scientific Co., Ltd., China. AM oil was purchased from Jiangxi Baolin Natural Spices Co., Ltd., China. All of the reagents were stored at $2-8^\circ\text{C}$ and required no purification before use. In the experiment, deionized water with an electrical resistivity of 18.2 M Ω ·cm was prepared by UPU-II-90HW-4C (Chengdu Youpu Biotechnology Co., Ltd., China) at room temperature.

2.2 Apparatus

The VCX-750 ultrasonic processor (Sonics & Materials Inc., USA) with a power of $0-750$ W and a frequency of 20 kHz was used in this work. It contained a detachable titanium ultrasonic probe with a diameter of about 13 mm. The probe of the ultrasonic horn was directly immersed in the reaction system for ultrasonic irradiation. To keep the temperature of the reaction system constant at $4 \pm 2^\circ\text{C}$, a Model DLSB-5/20 circulating pump (Zhengzhou Great Wall Scientific Industrial and Trade Co., Ltd.) was used during the reaction. The reaction device is shown in Figure 1.

2.3 Preparation of BSA/AM microcapsules by sonochemistry

BSA/AM microcapsules were synthesized by a one-step reaction of BSA aqueous solution and AM oil. First, 2.5 g of BSA was dissolved in 50 mL of deionized water, and then the aqueous solution was transferred to a customized ultrasonic reaction vessel. Next, 10 mL of AM was added to the reaction vessel. An oil–water interface was generated at this time, and the ultrasonic probe was placed at this interface. Then, the ultrasonic parameters (amplitude, mode, time) were adjusted, and the reaction was started. Finally, after the reaction, a white microcapsule emulsion was obtained. During the entire reaction, the cooling circulating water was turned on to ensure that the reaction temperature was maintained at $4 \pm 2^\circ\text{C}$. One

Figure 1: Schematic diagram of the experimental device for microcapsules synthesis.

portion of the resulting suspension was stored at 4°C for DLS, SEM, and TEM techniques. The other portion was freeze-dried at -20°C for TG and cytotoxicity tests.

2.4 Characterization

The particle size and distribution of microcapsules were obtained by DLS using a Zetasizer NanoZS90 (Malvern Panalytical, UK) at room temperature with three measurement cycles performed.

The core-shell structure of microcapsules was measured with an H7650 TEM (Hitachi High-Technologies Corporation, Japan). The sample for TEM was prepared by dispersing a small drop of the microcapsule emulsion that had been diluted to a certain concentration on a 400-mesh carbon-coated copper grid and drying it at room temperature.

The morphology of microcapsules was visualized by a SIGMA-500 field-emission scanning electron microscope (FESEM) (Carl Zeiss, Germany). The sample for FESEM was prepared by dispersing a small drop of the microcapsule emulsion that had been diluted to a certain concentration on a silicon wafer and drying in air.

The drug loading of microcapsules was measured with a Q50 thermal gravimetric analyzer (TA Instruments, USA). A small amount of freeze-dried sample was placed in a ceramic crucible. The test was conducted at a temperature

ranging from 25°C to 800°C and a heating rate of 20°C·min⁻¹.

2.5 AM release from microcapsules

Coumarin 6 (CR6, 0.06 mg·mL⁻¹) was dissolved in AM as a fluorescent dye. In the synthesis of microcapsules, AM containing coumarin 6 was used as the core material. The prepared microcapsule emulsion was placed in the dialysis bag, which was placed in *N,N*-dimethylformamide (DMF) shocked at a rate of 150 rpm at room temperature. About 5 mL of the dialysate was taken at intervals, and the absorbance was measured at 450 nm with a UV-2700 UV-Vis spectrophotometer (Shimadzu Co., Ltd., Japan). After the test, the dialysate was put back into the original dialysate to ensure that the total volume remains unchanged.

2.6 Cytotoxicity performance

In the cytotoxicity test, the hemolysis of the microcapsules was mainly measured. About 1 mL of mouse blood was taken in an anticoagulant blood vessel and centrifuged in a centrifuge at 2,000 rpm for 10 min to aspirate the upper plasma. Then, 2 mL of physiological saline was added to the centrifuge tube, mixed well gently with a pipette, and centrifuged to remove the supernatant. The obtained red blood cells were used to prepare a 2% suspension with physiological saline and stored at 4°C until use. The freeze-dried microcapsules were prepared into suspensions of 100, 50, 25, and 12.5 µg·mL⁻¹ with the physiological saline, each with 1.2 mL in volume. About 300 µL of red blood cell suspension was added to deionized water, microcapsule groups of different concentrations, and physiological saline, sequentially, incubated at 37°C for 2 h, and centrifuged at 4,000 rpm for 2 min. Finally, after centrifugation, the liquid state in the centrifuge tube was photographed.

3 Results and discussion

3.1 Effects of ultrasonic amplitude on BSA/AM microcapsules

To study the effects of ultrasonic amplitude on the performance of BSA/AM microcapsules, the ultrasonic time

Figure 2: Effect of ultrasonic amplitude on the particle size of BSA/AM microcapsules.

of 5 min and the ultrasonic mode of the pulse mode remained unchanged, and ultrasonic amplitudes (30%, 40%, 50%, 60%, 70%, respectively) were set for preparing microcapsules. The particle size distribution, core-shell structure, drug loading, and other properties of the microcapsules were studied using DLS, TEM, and TG techniques.

Figure 2 shows the particle size variation curves of microcapsules prepared at different amplitudes. It can be seen that the particle size of the synthesized microcapsules is increasing gradually with the increase in the ultrasonic amplitude. When the amplitude was set to 30%, the microcapsules had the smallest particle size with a diameter of 537.9 nm. When the amplitude was

set to 70%, the microcapsules had the maximum particle size with a diameter of 718.1 nm. From the perspective of polydispersity index (PDI), 30% amplitude had the smallest PDI (<0.3), indicating the monodisperse distribution of the particle size, and the others had PDI much >0.3 , indicating the polydisperse distribution of the particle size.

Figure 3 shows TEM photographs of microcapsules prepared at different amplitudes, and the particle size is consistent with the DLS results. It can be seen from the photographs that the overall particle size is smaller than the DLS results due to the difference in sample conditions between the two measurement methods used in the test. In DLS testing, liquid samples were used and the particle size was excessively large due to the hydration of the microcapsules and the solvent. In TEM testing, the samples were dried in air, making the particle size of the microcapsules smaller. But, the variation trend is the same for these measurement methods, and they are all increasing gradually with the increase in amplitude. The diameters of microcapsules prepared with an amplitude of 30% were also relatively uniform. Moreover, the shell layer and the core layer of the microcapsule had pronounced contrast differences, in particular, the core-shell structure.

Figure 4 shows the TG curves of BSA and AM. It can be seen that the shell and core materials have different thermal decomposition temperatures. When the temperature was increased to about 96°C, the core material AM was decomposed first. When heated to 210°C, the AM was

Figure 3: TEM photographs of BSA/AM microcapsules at different amplitudes: (a) 30%, (b) 40%, (c) 50%, (d) 60%, and (e) 70%.

Figure 4: TG curves of BSA and AM.

completely decomposed and the weight loss rate achieved was 100%. When the temperature was increased to 220°C, the BSA curve began to show significant thermal weight loss, indicating the start of decomposition. Therefore, based on the different decomposition temperature intervals of the two, the mass ratio of the two in the microcapsules could be measured to obtain the drug loading of the microcapsules.

Figure 5 shows the TG curves of microcapsules prepared at different amplitudes. The core material of the microcapsules started to lose weight at 96°C. Among the five curves, as the amplitude was increasing, the weight loss amplitude was gradually decreasing. For microcapsules with an amplitude of 30%, the weight loss was up to about 78% and this figure decreased to 70% at an amplitude of 67%. The other quality was BSA. This shows that under the same preparation conditions, the microcapsules prepared with a 30% amplitude have

Figure 5: TG curves of BSA/AM microcapsules at different ultrasound amplitudes.

more drug loading. With the increase in amplitude, the drug loading gradually decreased and the mass proportion of the protein shell gradually increased. In other words, the increase in amplitude will promote the cross-linking reaction of the sulfhydryl group and cause more BSA to be deposited into capsules, so that the mass of the drug core material will become smaller, which also explains the increase in the DLS results with the amplitude. Therefore, from the results of the drug loading, the microcapsules prepared with a 30% amplitude are in the ideal “thin-skinned stuffing” state.

Based on the DLS, TEM, and TG results, microcapsules prepared with a 30% amplitude have the smallest particle size, the most uniform distribution, and the highest drug loading. Therefore, adjusting the amplitude to 30% is the best comprehensive index for preparing microcapsules.

3.2 Effects of the ultrasonic mode on BSA/AM microcapsules

To study the effects of the ultrasound mode on the performance of BSA/AM microcapsules, the fixed ultrasound amplitude of 30% and ultrasound time of 5 min remained unchanged, and the ultrasound mode was set to the continuous mode and pulse mode (pulse 2 s, intermittent 2 s), respectively, to prepare microcapsule samples separately. The particle size distribution, core-shell structure, drug loading, and other properties of the microcapsules were studied using DLS, TEM, and TG techniques.

Figure 6 shows the particle size distribution curves of BSA/AM microcapsules synthesized under different

Figure 6: Particle size distribution curves of BSA/AM microcapsules under different ultrasound modes.

Figure 7: TEM photographs of BSA/AM microcapsules under different ultrasound modes: (a) continuous mode and (b) pulse mode.

ultrasound modes. It can be seen that the particle sizes are 551.3 and 559.8 nm, respectively. The particle sizes are relatively close but the PDI is different. With the pulse mode, the microcapsules had a smaller PDI of 0.167, and the samples prepared through the continuous mode had a larger PDI of 0.783. The results showed that the microcapsules prepared in the pulse mode tend to be monodisperse, while the microcapsules prepared in the continuous ultrasound mode tend to be polydisperse.

This is more significant in their TEM photographs. Compared with the microcapsules prepared in the continuous mode, the microcapsules prepared in the pulse mode were distributed uniformly, as shown in Figure 7.

This is associated with the dispersion state of the core material in the aqueous phase by ultrasound. The ultrasonic energy distribution inside the entire reaction system is not uniform. The energy density directly below the ultrasonic probe is larger, and the energy density at the obliquely lower and lateral sides of the ultrasonic probe is smaller. During ultrasonic irradiation, the liquid in these different areas forms a regional circulation flow, which leads to strong emulsification in some areas and forms small dispersion of oil droplets. In the other areas, there is weak emulsification, resulting in large dispersion of oil droplets. For these reasons, the nonuniform particle size of microcapsules in the continuous ultrasonic mode was formed. The pulse mode can break this regional flow in the system due to the intermittent time so that the system has a suitable period to complete the Ostwald ripening process. As a result, the emulsification caused by ultrasound is more uniform and the dispersion of oil droplets is more uniform. Therefore, the microcapsules prepared in the pulse mode show a trend of monodisperse distribution, and the microcapsules prepared in the continuous mode show a trend of polydisperse distribution.

Figure 8 shows the TG curves of BSA/AM microcapsules synthesized in different modes. It can be seen from this figure that the two curves show two significant weight loss intervals and the weight loss ratios of the

Figure 8: TG curves of BSA/AM microcapsules under different ultrasound modes.

two intervals are very similar. This shows that the ultrasound mode basically has no effects on the drug loading of microcapsules.

Based on DLS, TEM, and TG results, the ultrasonic mode has a greater impact mainly on the uniformity of the particle size distribution of the microcapsules, and almost no effects on drug loading. Compared with the continuous mode, the pulse mode is more conducive to the synthesis of more uniform protein microcapsules. Therefore, the microcapsules prepared in the pulse mode have the best overall performance.

3.3 Effects of ultrasonic time on BSA/AM microcapsules

To study the effects of ultrasound time on the performance of BSA/AM microcapsules, the fixed ultrasound amplitude of 30% and ultrasound mode of the pulse mode remained unchanged, and the ultrasound times

Figure 9: Effect of ultrasonic time on the particle size of BSA/AM microcapsules.

were set to 3, 4, 5, 6, and 7 min, respectively, to prepare microcapsules. The particle size distribution, core-shell structure, drug loading, and other properties of the microcapsules were studied using DLS, TEM, and TG techniques.

Figure 9 shows the particle size variation curve of the synthesized BSA/AM microcapsules at different ultrasound times. It can be seen that with the prolonged ultrasound time, the particle size of the microcapsules first decreased to a minimum and then gradually increased. The smallest particle size of 484.4 nm could be achieved when the ultrasound time was set to 4 min. This is because the ultrasound time was too short when the

ultrasound was set to 3 min, and the AM oil had not been fully emulsified to small droplets during the process. As the ultrasonic irradiation time was extended to 4 min, the particle size of the AM oil was the smallest in the aqueous phase, resulting in the smallest particle size of the synthesized microcapsules. As the ultrasound time elapsed, the microcapsules were destroyed. Partial microcapsules were broken under the action of ultrasound. It became a trend that the internal oil droplets were combined with the external oil droplets to form bigger droplets, and the protein was deposited to the new bigger oil droplets at the same time.

Figure 10 shows the TEM photographs of BSA/AM microcapsules synthesized at different ultrasound times. It can be seen that the particle size variation trend was consistent with the results of the DLS analysis. In these five photographs, the microcapsules synthesized at 4 min not only have the smallest particle size but also have the most uniform particle size.

Figure 11 shows the TG curves of microcapsules prepared at different ultrasound times. It can be seen that all the curves show two significant weight loss intervals. Among the five curves, the weight loss of the AM oil sample with an ultrasound time of 4 min had the largest percentage of 77%. With the increase or decrease in time, the weight loss range was gradually decreasing. When the ultrasound time was only 3 min, the drug loading was only 40%. As the time elapsed, the weight loss ratio was also gradually decreasing. This shows that the

Figure 10: TEM photographs of BSA/AM microcapsules at different ultrasound times: (a) 3 min, (b) 4 min, (c) 5 min, (d) 6 min, and (e) 7 min.

Figure 11: TG curves of BSA/AM microcapsules at different ultrasonic times.

ultrasound response time was too long, which would destroy the optimal structural state of the microcapsules. The result was consistent with DLS. Therefore, from the results of drug loading, the microcapsules prepared with an ultrasound time of 4 min had the best performance.

Based on the DLS, TEM, and TG results, the microcapsules prepared with an ultrasound time of 4 min had the smallest particle size (484.4 nm), the most uniform distribution, and the highest drug loading (77%). Therefore,

the comprehensive performance of microcapsules was the best when the ultrasonic time was adjusted to 4 min.

3.4 Performance analysis for BSA/AM microcapsules

Figure 12 shows TEM photographs of BSA/AM microcapsules. It can be seen from the photographs that the particle size of the microcapsules was about 400 nm, and it had an obvious core-shell structure with a shell thickness of about 30 nm. Figure 13 shows the SEM photographs of BSA/AM microcapsules. It can be seen that their diameter was similar to that of TEM, and the surface of the microcapsules was smooth without any obvious depression.

Figure 14 shows the particle size distribution curve of BSA/AM microcapsules. The main particle size was 484.4 nm and the PDI was 0.3. The particle size was larger than the diameter of the microcapsules shown in SEM and TEM photographs. This is because when the SEM and TEM photographs were taken, the sample was in a dry state, and protein dehydration could lead to reduced particle size. In addition, the protein microcapsules were constantly bombarded by

Figure 12: TEM photographs of BSA/AM microcapsules at different magnifications: (a) 10,000 $\times$ and (b) 20,000 $\times$.

Figure 13: SEM photographs of BSA/AM microcapsules at different magnifications: (a) 10 $\times$ and (b) 50 $\times$.

Figure 14: Particle size distribution curve of BSA/AM microcapsules.

electron beams during photographing of SEM and TEM. This would also cause the size of the photographed microcapsules to become smaller. Particularly, when photographing at high magnification, the microcapsules shrank sharply at a visible speed. In the DLS test, the microcapsules were in the state of suspension and there was hydration between the protein shell and the solvent. Thus, the size was larger compared with the TEM and SEM results.

Figure 15 shows the release curve of CR6 in microcapsules. The release of CR6 was mainly caused by shock. With the extension of the shock time, the release of CR6 gradually decreased and reached a maximum at 24 h. As CR6 was dissolved in AM, its release amount could represent the amount of the core material in the microcapsules. It can be seen in Figure 15 that the maximum release could be reached within 24 h, and the release within 4 h reached half of the maximum. This shows that the drug loaded in the microcapsules can be almost completely released within 25 h.

Figure 15: Drug release curve of BSA/AM microcapsules.

Figure 16: Hemolysis test of BSA/AM microcapsules.

The freeze-dried microcapsule powder was dissolved in PBS buffer to prepare microcapsule solutions of different concentrations, and then the solutions were mixed with blood to observe hemolysis. Figure 16 shows the hemolysis results of microcapsule suspensions of different concentrations. It can be seen that the obvious hemolysis of the normal saline was due to the osmotic pressure. A large amount of normal saline entered the red blood cells and caused swelling. No hemolysis was observed in the PBS buffer used as the pressure of the buffer (pH = 7.4) was consistent with the osmotic pressure in the blood. By changing the concentration of the microcapsule suspension, it can be seen that hemolysis occurred when the concentration increased to $100 \mu\text{g}\cdot\text{mL}^{-1}$, and the hemolysis rate was close to 10%. When the concentration of the microcapsules was below $50 \mu\text{g}\cdot\text{mL}^{-1}$, there was no cytotoxicity. It shows that the concentration of $50 \mu\text{g}\cdot\text{mL}^{-1}$ was the best value. If the concentration continued to increase, the cells would burst and the microcapsules would have certain cytotoxicity.

4 Conclusion

The BSA/AM microcapsules were successfully prepared by a sonochemical method. This process is clean, simple, and efficient. For the ultrasonic amplitude, the particle size gradually increased and the drug loading efficiency gradually decreased with its increase. For the ultrasound mode, it only had a greater impact on the particle size distribution (not size itself) of the microcapsules. The microcapsules prepared in the pulse mode were relatively uniform. For the ultrasound time, the microcapsule size first decreased and then gradually increased when it

increased. The microcapsules had the minimum size and the highest drug loading efficiency at 4 min. In brief, the best ultrasonic parameters were determined as the ultrasonic amplitude of 30%, ultrasonic mode of pulse 2 s, and ultrasonic time of 4 min. The size of the BSA/AM microcapsules prepared under the above conditions was 484.4 nm, and the loading efficiency of the AM oil was approximately 77%. The drug release test suggested that most of the AM oil could be released within 10 h. Based on the hemolysis test, the BSA/AM microcapsules had no cytotoxicity when its concentration was below 50 $\mu\text{g}\cdot\text{mL}^{-1}$.

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