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RESEARCH ARTICLE

Ruixue SUN, Jingjing SHI, Yanchuan GUO, Lijuan CHEN

Studies on the particle size control of gelatin microspheres

Abstract A series of gelatin microspheres (GMs) were prepared through emulsification-coacervation method in water-in-oil (w/o) emulsions. The influence of preparation parameters on particle size, surface morphology, and dispersion of GMs was examined. The studied preparation parameters include concentration of gelatin solutions, concentration of the emulsifier, w/o ratio, emulsifying time, stirring speed, and so on. The surface morphology, dispersion, and particle sizes of GMs were determined by the scanning electron microscopy (SEM), SemAfore 4 Demo software, and particle size distribution graphic charts. The experimental results indicated that increasing the concentration of gelatin solution would increase the particle size of GMs. When the solution concentration increased from 0.050 to 0.200 g/mL gradually, the particle size increased correspondingly. The relationship between the two quantities was linear. On the contrary, increasing the concentration of the emulsifier would decrease the particle size of GMs. Furthermore, the particle size reduced quickly at initial time and slowed down latterly. With the increase of emulsifier concentration from 0 to 0.020 g/mL, the mean diameters of GMs decreased from 17.32 to 5.38 μm. However, the particle size dwindled slowly when emulsifier concentration was higher than 0.020 g/mL. The excellent result was obtained with the condition of 0.050 g/mL of emulsifier concentration, 0.100 g/mL of gelatin solution concentration, 1/5 of w/o ratio, 10 min of emulsifying time, and 900 r/min of the stirring speed. The GMs prepared at this condition had the smallest sizes, the narrowest size distribution, the best spherical shape, and fluidity. The w/o ratio has the same influence on particle size of GMs as that of gelatin solution concentration. With the increase of w/o ratio, the average particle sizes increased linearly, and the surface of microspheres become smoother as well. It is supposed that w/o ratio can be used to change the diameters and surface morphologies of GMs. The emulsifying time has little influence on the mean diameters of GMs, but it affects the dispersion of GMs apparently. When the emulsifying time was shorter than 5 min, the GMs had bad dispersion. After increasing the emulsifying time to 13 min, the dispersion of GMs changed greatly, whereas the dispersion of GMs became bad again when the emulsifying time was longer than 13 min. According to the experimental results, 13 min was considered to be the best emulsifying time. The stirring speed has the similar influence on GMs' morphologies as that of emulsifying time. Slow stirring rate made large size distribution and bad spherical shape of GMs; excessive stirring speed results in aggregation among GMs likewise. The smaller size distribution and better spherical shape of GMs were observed under the stirring rate between 500 and 1500 r/min by SEM. In conclusion, increasing the concentration of gelatin solution or w/o ratio would increase the particle sizes of GMs, increasing the concentration of the emulsifier would decrease the sizes of GMs at proper emulsifying time, and stirring speed would get the best spherical shape of GMs. These are the basic laws governing the design and manufacture of the GMs.

Keywords gelatin microspheres (GMs), emulsification-coacervation method, preparation parameters, particle size, surface morphology

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Ruixue SUN, Jingjing SHI, Yanchuan GUO, Lijuan CHEN (✉)
Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Beijing 100190, China
E-mail: chenlijuan@mail.ipc.ac.cn

Ruixue SUN, Yanchuan GUO
Graduate University of Chinese Academy of Sciences, Beijing 100049, China

Emulsification method was introduced first by Tanaka [1]. Comparing with other methods, it needs simple necessary facilities and can be easily operated in mild condition. With these advantages, it has become a common method in developing gelatin microspheres (GMs) in the field of biology and pharmacy [2–9]. However, in practice, with the effect of various conditions, it is hard to prepare GMs of uniform particle size. Usually, the GMs obtained with the method have large particle size, and size distribution, and the surface morphology is unsatisfactory [10, 11]. At present, GMs are mostly used as drug-loaded microspheres. Zhan *et al*. [12] prepared streptomycin sulfate gelatin microspheres with GMs as carriers and studied the effect of emulsifier, cross-linker, and stirring rate on

developing GMs with particle size in the range of 5.0 to 25.0 μm. The authors noted that the stirring rate was the key point, but there was no further discussion about that. Li *et al*. [13] also developed medical microspheres of sizes from 50 to 1000 μm based on gelatin, and they fractionated the GMs by using sieves. Elisabetta *et al*. [14] comprehensively studied the influence of concentration of gelatin, stirring rate, the selection of oil phase, and the type and concentration of emulsifier in preparing GMs, based on which, they obtained microspheres with particle size ranging from 6 to 105 μm. However, while developing microspheres, the authors focused on controlling the entrapment rate and improving the effect of drug after the microspheres were loaded with drug, and there was no specified discussion about GMs' particle size and the effect of dispersion.

Currently, there was little report on specificity research on the controlling skill for GMs' particle size, and the main purpose was to study the drug-loading quantity of medical microsphere and entrapment rate. Using emulsification coagulation method, we systematically studied the effect of the concentration of gelatin solution, concentration of emulsifier, emulsification time, water-oil (w/o) ratio, and stirring rate on the GMs' particle size and dispersion. Experiment shows that the GMs' particle size increases with increasing concentration of gelatin solution and water-oil (w/o) ratio; the GMs' particle size decreases with increasing concentration of emulsifier. The dispersion and surface smooth lever could be improved by choosing suitable emulsification time and stirring rate. Small-sized GMs with good dispersion, smooth surface, and average particle size of 3.58 μm were obtained.

1 Materials and methods

1.1 Materials

Commercial gelatin, cooking oil (Beijing Eisen-Lubao oil Co., Ltd.), Span 80 (A. R., Beijing Chemical Reagents Company), glutaraldehyde solution (50%, Biochemistry reagent, Beijing Chemical Reagents Company), acetone (A. R., Beijing Chemical Reagents Company), and distilled water.

1.2 Preparation of GMs

First, a certain volume of cooking oil was dosed in a 250-mL three-neck flask, where emulsifiers were dosed during stirring and then heated in a water bath of 50–60°C. Then, the gelatin solution was added and emulsified at a fixed time. When the emulsification was completed, the three-neck flask was moved into an ice-water bath, and the temperature of the system was set below 10°C. Then 50 μL of 50% glutaraldehyde solutions was added; after cross-linking for 60 min, the system was dehydrated for 40 min by adding 30 mL of acetone. The samples were filtered, washed with acetone, and dried at room temperature. The yield of the microspheres is above 80%.

1.3 Characterizations

First, the double conductive adhesive was pasted on the sample stage; then, a little of GM powders were put on the conductive adhesive, metal sprayed for 60 s. Then, the shape of the microspheres was observed using scanning electron microscopy (SEM). The average particle size and the standard deviation were calculated, and the distribution curve of the particle size was obtained using the SemAfore 4 Demo software.

2 Results and discussion

2.1 Effect of concentration of gelatin solution on particle size of GMs

The concentration of the gelatin solutions is one of the crucial parameters to affect the microsphere shapes. When the concentration of the gelatin solutions is below 0.02 g/mL, it is difficult for the gelatin to form spheres in the solutions. It is because when the moisture content of the gelatin solution droplets is high, unregular grains, with loose interstructures, are obtained after gelation and dehydration, as shown in Fig. 1. Therefore, seven different concentrations of the gelatin solutions (0.050, 0.075, 0.010, 0.125, 0.150, 0.175, and 0.200 g/mL) were used, and at the same time, the other parameters were as follows: the w/o ratio (1∶5), the stirring rate (900 r/min), the time of the emulsification (10 min), and no emulsifier.

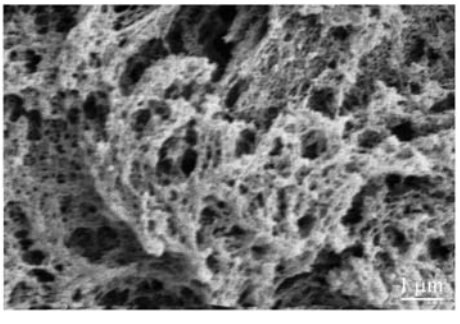

Fig. 1 The SEM image of the surface morphology of GMs (gelatin concentration: 0.020 g/mL)

Figure 2 shows the SEM images of microspheres obtained from gelatin solutions of four different concentrations. It is found that agglomeration appears when the concentration is low (Fig. 2a and Fig. 2b). This is because the low concentration may lead to high moisture content in the gelatin droplets. Furthermore, the collision between the spheres in the process of stirring may result in the shape

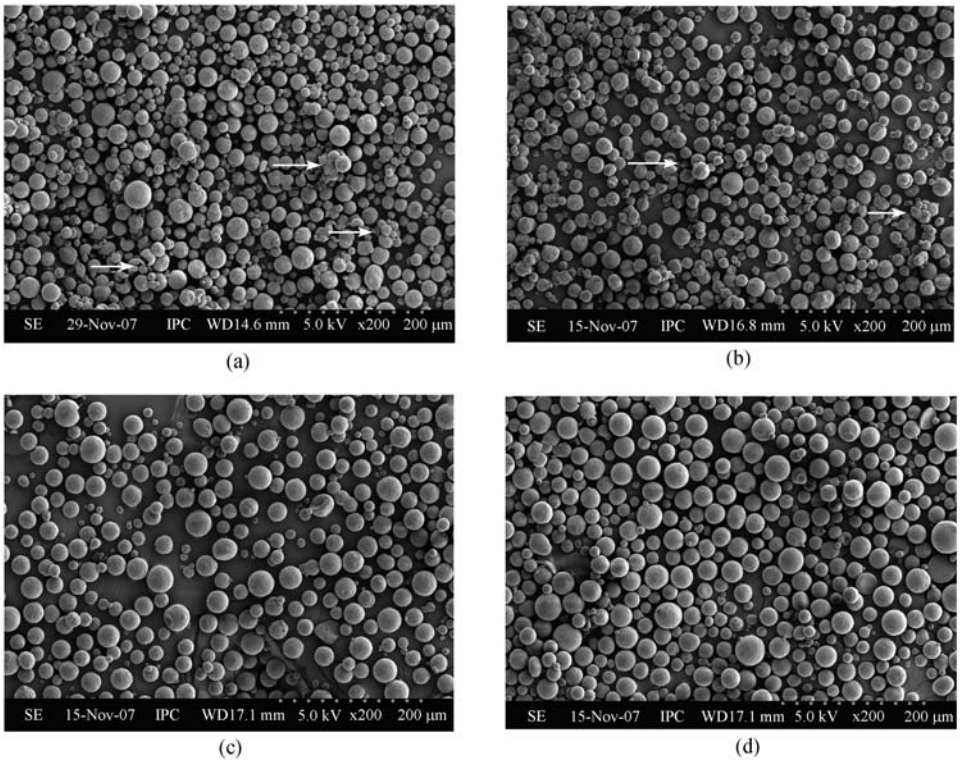

Fig. 2 The SEM images of GMs prepared at different gelatin concentrations
(a) 0.075 g/mL; (b) 0.125 g/mL; (c) 0.175 g/mL; (d) 0.200 g/mL

malformations of these loose spheres. If the concentration of the gelatin solutions increases, it will improve the shape and the dispersion of the microspheres greatly, and the phenomenon of the adhesion and the agglomeration will disappear (Fig. 2c and Fig. 2d).

The particle size distribution data are listed in Table 1. It is found that when the concentration of the gelatin solution increases from 0.050 to 0.200 g/mL, the size range of the most probable distributions are 8–12, 12–16, 12–16, 16–20, 20–24, 24–28, and 20–24 μm (bold parts in the table), respectively, which illuminates that the particle sizes increased with the increase of gelatin concentration. Figure 3 shows the relation of gelatin concentration and the average diameter of GMs; it is a linear relationship obviously.

2.2 Effect of emulsifier concentration

The purpose of using emulsifiers is to lower the w/o interfacial tension so that the dispersed phase may form smaller and stable droplets, and the introduction of the emulsifier is in favor of preparing gelatin microspheres of small particle size and good dispersion. Emulsifier is one of the crucial factors to affect the particle size and the shape of the microspheres.

The category of the emulsifier has correlation with its

Table 1 Size distribution percentage of GMs prepared with different gelatin concentrations

No.	Gelatin concentration /(g/mL)	Diameter range /μm									$\overline{d}$ [a]/μm	SD[b] /μm
		4–8	8–12	12–16	16–20	20–24	24–28	28–32	32–36	36–40		
A	0.050	31.19	**34.40**	20.18	11.47	1.83	0.46	0.46	0	0	10.80	4.77
B	0.075	25.00	16.50	**27.00**	14.00	7.00	7.50	1.00	2.00	0	13.80	7.03
C	0.010	4.55	12.27	**29.55**	26.82	20.91	5.00	0.90	0	0	16.12	5.35
D	0.125	1.00	13.50	20.50	**33.50**	19.50	9.00	3.00	0	0	17.37	5.48
E	0.150	0.50	4.00	16.50	20.50	**27.00**	23.50	8.00	0	0	20.56	5.77
F	0.175	0	2.00	10.00	17.00	24.00	**28.50**	11.00	5.00	2.50	22.95	6.41
G	0.200	0	0.92	5.50	22.02	**24.77**	22.94	15.14	5.96	2.75	23.69	6.08

[a] The average diameters of GMs; [b] the standard deviations of the GMs' diameters

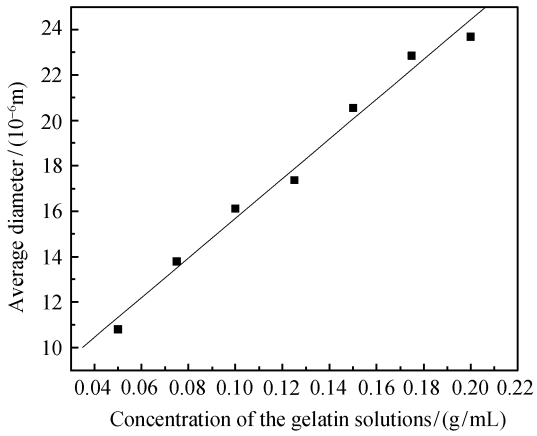

Fig. 3 The relation of gelatin concentration and the average diameter of GMs

object and performance. We chose the lipophilicity Span 80 as the emulsifier for studying the effect of emulsifier concentration on the size, shape, and dispersion of microspheres. In the experimental process, the concentration of the gelatin solutions was 0.100 g/mL, the w/o ratio was 1∶5, the stirring rate was 900 r/min, and the emulsifying time was 10 min. Figure 4 shows the curve of the average particle size of the microspheres when the emulsifier concentration increases from 0 to 0.080 g/mL. An approximately L-shaped curve is obtained. When the concentration of the emulsifier increases from 0 to 0.020 g/mL, the particle size of microspheres decreases from 17.32 to 5.38 μm. When its concentration increases to 0.040 g/mL, the decreasing speed is lowered. At this point, the average particle size of the microspheres is 4.08 μm. If the emulsifier concentration increases further, the average particle size of microspheres fluctuates between 4.0 and 5.0 μm. It is illuminated that the particle size of the microspheres is not linearly related with the emulsifier concentration, and in an adequate range of emulsifier concentration, the size of the microspheres is quite stable.

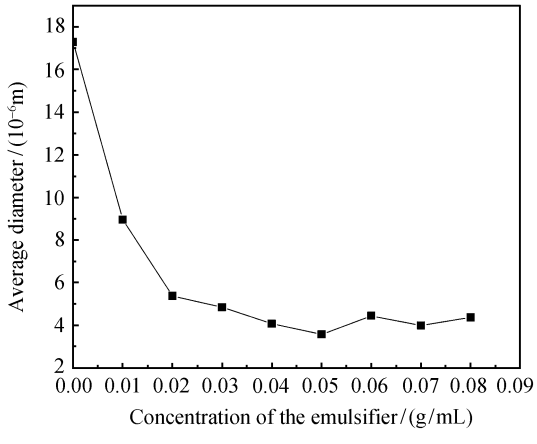

Fig. 4 The particle size at different concentration of Span 80

When the concentration of the emulsifier used was 0.050 g/mL, the average size of the GMs was 3.85 μm. Figures 5 and 6 are the SEM images and the distribution chart of the GMs' particle size for this sample, respectively. The distribution of the particle size is uniform, and the proportion of GMs with particle size between 1.0 and 5.0 μm is 94.39%.

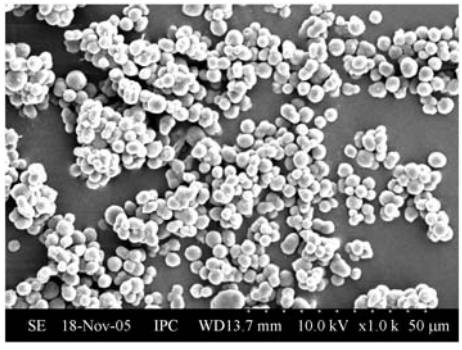

Fig. 5 The SEM image of GMs formed with 0.050 g/mL emulsifier concentration

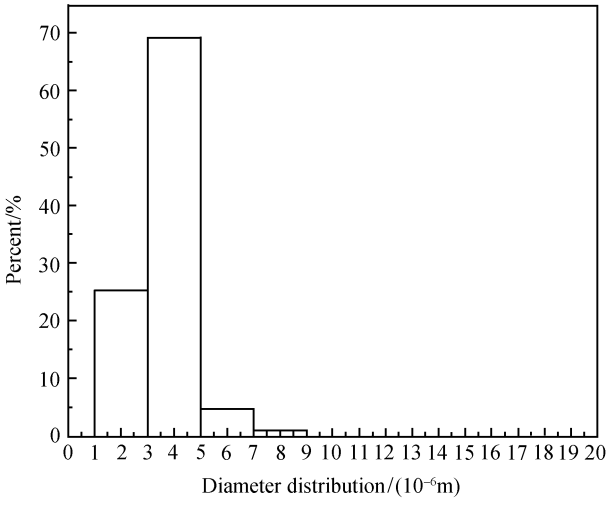

Fig. 6 The diameter distribution of GMs formed with 0.050 g/mL emulsifier concentration

Experimental results illuminated that, in the process of emulsification, the concentration of the emulsifier decreased, whereas the average size increased. This is because the addition of emulsifier breaks the normal point value of the w/o interfacial tension. As a result, surface tension falls down, and the diameter of droplets decreases; however, when the emulsifier increases further, it improves the adsorption capacity on the surface. At this moment, the interface tension decreases continually, and the diameter of the droplets is also changing; when the concentration of the emulsifier exceeds the adsorption capacity on the surface, the decrease of particle size is stopped [15].

2.3 Effect of the water/oil ratio

In the above two sections, we have discussed the effect of concentrations of gelatin and emulsifier on the particle size and morphology of microspheres. We also pay attention to another measure of value in emulsion system that is the ratio of w/o, which plays an important role in the formation of GMs' morphology.

Likewise, the other parameters would be kept constant; these were gelatin solution concentration of 0.100 g/mL, emulsifier concentration of 0.050 g/mL, stirring rate of 900 r/min, and the emulsifying time of 10 min. We just changed the ratio of w/o in the experiments. In Fig. 7, we can find that with the increase of the aqueous phase volume, the surface of the microspheres is becoming smooth, and the particle size is increased. With the w/o ratio as the x axis and the average size as the y axis, we can draw up a chart that indicates that with the increase of the w/o ratio, the average size is increased (Fig. 8). The results indicate that the lower the w/o ratio, the easier to form small droplets, and the higher the w/o ratio, the weaker ability of the oil phase' decentralization. As a result of forming bigger droplets, we can get bigger microspheres after solidification. So it is possible for us to control the dimension and the shape of the microspheres by regulating the ratio of w/o phase.

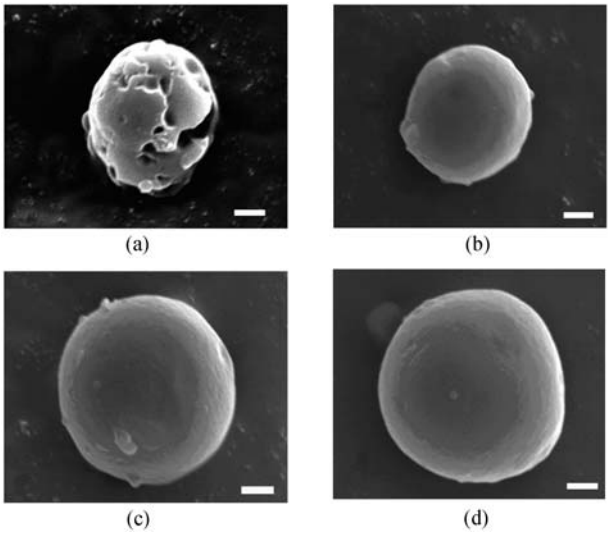

Fig. 7 The SEM image of GMs obtained at different ratios of w/o: (a) 1/10; (b) 2/10; (c) 3/10; (d) 4/10
The bar in the images represents 1 μm.

2.4 Effect of emulsifying time

From the experiments, we can find that in the process of reversed emulsion, it will take some time to stabilize the droplet formed by the disperse phase. During this time, the droplet takes place some changes like abruption, assemble, coalescence, phase inversion, and so on, which affect the size, shape, and structure of the microspheres directly. So, to get the expectation level of the gelatin microspheres, we should control the time of the emulsification. We chose ten points within 20 min to study the effect of emulsifying time, keeping other factors constant (gelatin concentration, 0.100 g/mL; emulsifier concentration, 0.050 g/mL; stirring speed, 900 r/min; and w/o ratio, 1/5).

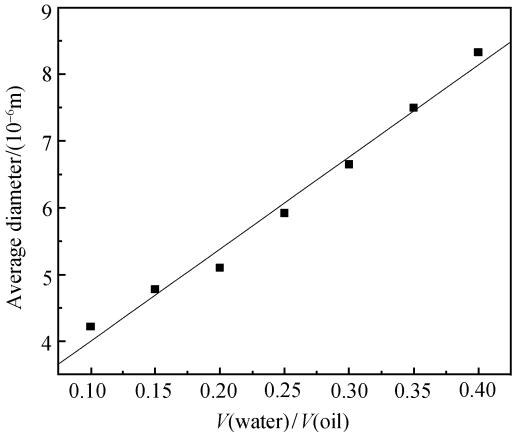

Fig. 8 The relation of w/o ratio and GMs average diameter

The relationship between the time of the emulsification and the average grain size is described in Table 2. From the table, we can find that when other experimental conditions are constant, the size of the microspheres prepared with different emulsifying time is different, and the relationship between them is not a linear one. Figure 9 gives the pictures of the GMs under different emulsifying time. It is found that from 1 to 5 min, the emulsification process exhibits a qualitative change, the microspheres become individually dispersed gradually. Beyond 5-min emulsification, there is an adjustment stage for the shape of the microspheres, and 13-min emulsification is the optimum condition for getting the better microspheres' disperse state. After 15 min, there appears adhesion again between the microspheres.

Table 2 The mean diameter of GMs prepared with different emulsifying time

Time/min	1	3	5	7	9	11	13	15	17	19
Scope/μm	3–14	2–17	2–16	3–21	3–17	4–22	3–14	3–21	3–20	3–21
Size/μm	7.72	8.32	8.20	8.56	9.42	10.09	8.34	8.26	8.22	8.19

2.5 Effect of stirring rate in the emulsification

Stirring rate is a kind of physical factor, which plays an important role in the process of reversed emulsion-coacervation.

In the process of emulsification, we chose three ranges of stirring rate, keeping the other conditions constant (the concentration of gelatin solution, 0.100 g/mL; the concentration of emulsifier, 0.020 g/mL; the emulsification time,

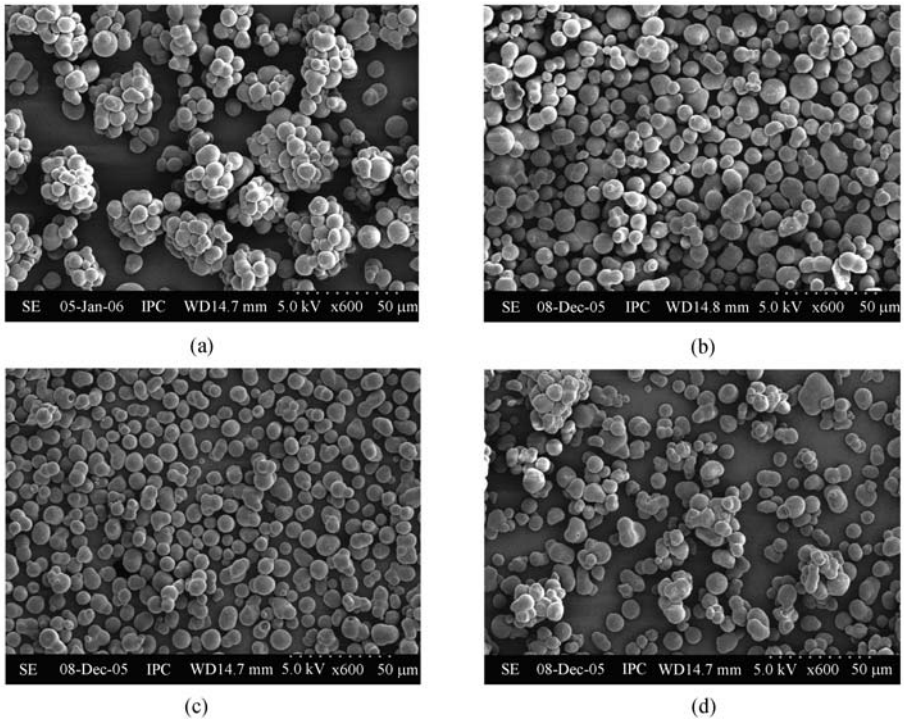

Fig. 9 The SEM image of GMs obtained at different emulsifying time: (a) 1 min; (b) 5 min; (c) 13 min; (d) 15 min

10 min; and the w/o ratio, 1∶5). Results show that, when the stirring rate is lower than 500 r/min, the microspheres have relatively large average particle size, and there are small particles adhering on the large ones. With increasing stirring rate, the dispersion is obviously improved, and the microspheres show smaller size and better shape. On the contrary, if the stirring rate reaches 1500 r/min, the dispersion becomes worse, and the microspheres would be bound seriously with each other.

The factors influencing the particle size and dispersion of GMs under different stirring rates could be as follows: with lower stirring rate, the water phase is exerted with little force, which is not enough for the water phase to be split into drops, and the formed emulsions is not stable. The droplets may connect with each other with flocculation or settlement. With increasing stirring rate, the shear stresses exerting on the water phase increase and become uniform, and even drops of small size are formed. Meanwhile, because of the stability caused by stirred centrifugal force, the drops are not easy to flocculate for settlement, which makes small particle size and uniform and good dispersion microspheres possible. However, when the stirring rate is too high, emulsion system will form turbulence, which makes drops suffer uneven forces. As a result, the drops would be out of shape for butting with each other, and the breakage rate increases; under this condition, the microspheres would be with damaged shape and unequal size [14, 16].

3 Conclusion

In short, the particle size of gelatin microspheres decreases with decreasing concentration of gelatin solution and w/o ratio or increasing amount of emulsifier. Increasing the stirring rate of emulsification and choosing the proper emulsifying time are propitious to the dispersion and spherical shape of the microspheres. The gelatin microspheres with the good surface morphology, good dispersion, and average particle size of 3.58 μm were successfully synthesized by optimizing the experimental conditions.

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