Colloids and Surfaces B: Biointerfaces 87 (2011) 399–408

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Fabrication strategy for amphiphilic microcapsules with narrow size distribution by premix membrane emulsification

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ARTICLE INFO

Article history:
Received 24 January 2011
Received in revised form 29 April 2011
Accepted 27 May 2011
Available online 17 June 2011

Keywords:
PELA microcapsules
Amphiphilic polymer
Premix membrane emulsification
Narrow size distribution
Size controllable

ABSTRACT

Amphiphilic co-polymer, which can maintain the stability of proteins and increase the protein loading efficiency, is considered as an exploring-worthy biodegrade polymer for drug delivery. However, amphiphilic microcapsules prepared by conventional methods, such like mechanical stirring and spray-drying methods, exhibit broad size distributions due to its hydrophilic sequences, leading to poor reproducibility. In this study, we employed poly(monomethoxypoly ethylene glycol-co-D,L-lactide) (mPEG-PLA, PELA), one of common amphiphilic polymers, as model to focus on investigating the process parameters and mechanisms to prepare PELA microcapsules with narrow size distribution and regular sphericity by combining premix membrane emulsification and double emulsion technique. The coarse double emulsion with broad size distribution was repeatedly pressed through Shirasu Porous Glass (SPG) membrane with relatively high pressure to form the fine emulsion with narrow size distribution. Then, the microcapsules with narrow size distribution can be obtained by solvent extraction method. It was found that it was more difficult to obtain PELA microcapsules with narrow size distribution and smooth surface due to its amphiphilic property, compared with the cases of PLA and PLGA. The smooth surface morphology was found to be related to several factors including internal water phase with less volume, slower stirring rate during solidification and using ethyl acetate as oil phase. It was also found that mass ratio of hydrophilic mPEG, stabilizer PVA concentration in external water phase and transmembrane pressure played important role on the distribution of microcapsules size. The suitable preparation conditions were determined as follows: for the membrane with pore size of 2.8 μm, the mass ratio of PLA/mPEG was 19:1, volume ratio of W_1/O was 1:10 and O/W_2 was 1:5, PVA concentration (w/v) was 1.0%, magnetic stirring rate during solidification was 60 rpm and 300 kPa was chosen as transmembrane pressure. There was a linear relationship between the diameter of microcapsules and the pore size of the membranes. Finally, by manipulating the process parameters, PELA microcapsules with narrow size distributions (coefficient of variation was less than 15%), smooth morphology and various sizes, were obtained. Most importantly, the key factors affecting fabrication have been revealed and mechanisms were illustrated in detail, which would shed light on the research of amphiphilic polymer formulation.

1. Introduction

In recent years, the entrapments of peptide and protein drugs in microspheres or microcapsules have been extensively investigated [1], because biodegradable microcapsules not only prolonged the half-life time of drug, but also favored to the increase bioavailability of drug in vivo by controlling the release rate [2,3]. Among those biodegradable polymers, the amphiphilic polymers have evoked considerable interests as protein drug carriers [4–6]. Compared to hydrophobic matrix, such as PLA and PLGA, amphiphilic polymers yielded a more stable interfacial layer at the oil and water interface, and thus were more suitable to stabilize primary emulsion and prevent protein in the inner droplets merging into external water phase [5,7]. Therefore, amphiphilic microcapsules would obtain higher encapsulation efficiency than hydrophobic microcapsules. Furthermore, its amphiphilic property can prevent protein contacting with the oil/water interface and the hydrophobic network, which can improve the bioactivity retention of protein drugs [8].

Until now, the most popular method to prepare microcapsules as drug delivery system is double emulsion water-in-oil-in-water solvent evaporation method. However, in conventional processes, the double emulsions were usually prepared by mechanical stirring, homogenization or sonication techniques [9,10], and thereby the size distributions of microcapsules obtained were very broad.,

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doi:10.1016/j.colsurfb.2011.05.051

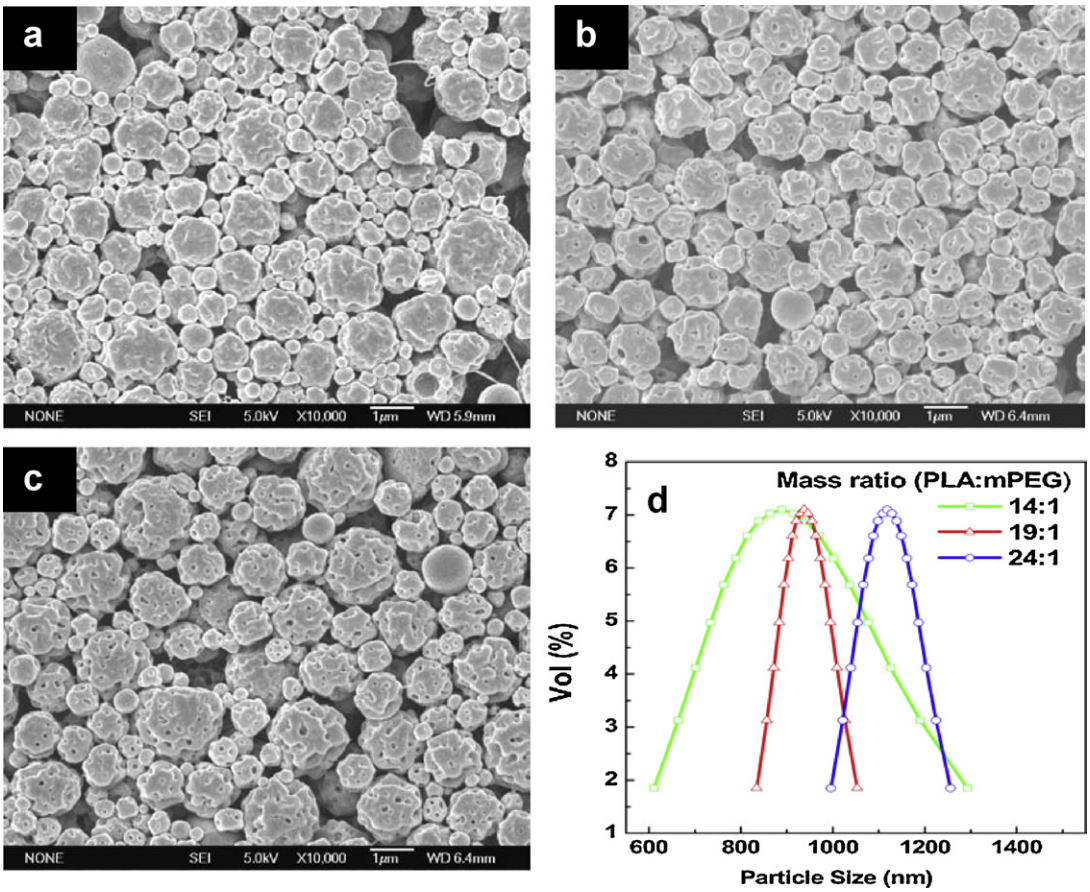

Fig. 1. SEM micrographs (a–c) and size distributions (d) of microcapsules prepared with different mass ratios of PLA/mPEG: (a) 14:1; (b) 19:1; (c) 24:1.

which will result in poor repeatability of preparation, release behavior and drug efficacy in vivo among batches [11,12]. In order to obtain microcapsules with narrow size distribution, selective centrifugation has been considered [13], but the process was relatively tedious and inefficient, and expensive protein drugs had to be wasted. Therefore, it is necessary to develop a novel method in order to provide amphiphilic microcapsules with narrow size distribution.

In this study, we try to use poly(monomethoxypoly ethylene glycol-co-D,L-lactide) (PELA), one of common amphiphilic polymers, to prepare microcapsules with narrow size distribution containing bovine serum albumin (BSA) as model protein by premix membrane emulsification technique combined with double emulsion method. Although uniform PLA and PLGA microcapsules have been prepared by premix membrane emulsification technique in our group [14,15], it was found it was a big challenge to control the shape and size distribution of PELA microcapsules because of its amphiphilic characteristics, which was very different from the previous hydrophobic PLA systems [14]. Firstly, introducing hydrophilic sequence of PEG to PLA chains led to remarkable change in viscosity of PELA under the same molecular weight [9]. As a result, the viscosity of PELA coarse double emulsion had great difference with that of PLA, which further changed the transmembrane pressure and other key factors for preparing uniform microcapsules [16]. In addition, PEG has been reported to be a pore-forming agent [17], which resulted easily in macropores on surface of PELA microcapsules. Furthermore, the solidification rate also showed great difference with PLA system due to the existence of hydrophilic sequence of PEG [18], which would have significant influence on morphology of the PELA microcapsules. Therefore, it is necessary to investigate and optimize the main process parameters that influence the uniformity and surface morphology of microcapsules when using amphiphilic PELA as polymer materials. Herein, we mainly manipulated the physical factors such as polymer viscosity, volume ratio of W_1/O and O/W_2, PVA concentration and type of oil phase. We also evaluated the operational conditions including stirring rate during solidification and transmembrane pressure. The mechanisms responsible for distribution of particle size and surface morphology were elucidated in detail when amphiphilic polymer used as material.

2. Materials and methods

2.1. Materials

PELA with different mass ratio of PLA/mPEG (30, 40, 50 kDa) were purchased from the Dai Gang Company (Shandong, China), in which the mPEG block has a molecular weight of 2000 Da. Poly(vinyl alcohol) (PVA-217, degree of polymerization 1700, degree of hydrolysis 88.5%) was provided by Kuraray (Japan). Bovine serum albumin (BSA) was from Merck (Germany). SPG membrane (pore size of the membrane was 1.4, 2.8, 5.2, 18 μm) was provided by SPG Technology Co. Ltd. (Japan). All other reagents were of analytical grade.

2.2. Preparation of microcapsules

Microcapsules loaded with BSA were prepared by two-step procedure [19]. The first step was coarse double emulsions preparation. At first, BSA aqueous solution was mixed with dichloromethane

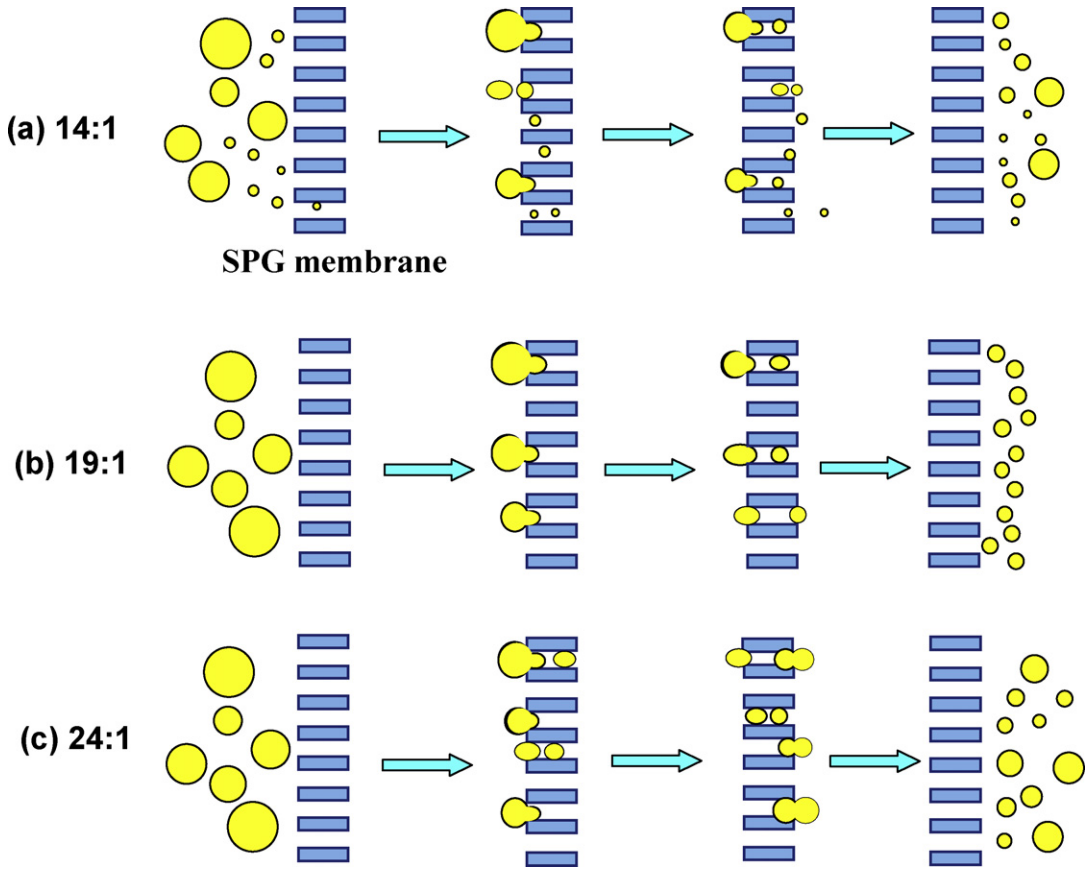

Fig. 2. Schematic illustration of the formation of droplets prepared with different mass ratios of PLA/mPEG: (a) 14:1; (b) 19:1; (c) 24:1.

or acetate containing PELA by homogenizing for 30 s in ice bath to form primary emulsion. This W/O emulsion was further emulsified into external aqueous phase containing PVA by magnetic stirring for 60 s at 300 rpm to prepare coarse double emulsions. The coarse double emulsions were then poured into the premix reservoir. Secondly, double emulsions with smaller and relatively uniform size were achieved by extruding the coarse double emulsions through the SPG membrane with a high pressure. After that, the emulsions were solidified into uniform microcapsules. When dichloromethane was used as oil phase, the double uniform emulsions were stirred overnight to evaporate organic solvent. When acetate was used as oil phase, the obtained uniform double emulsions were poured quickly into 800 mL solution containing 0.9% (w/v) NaCl (solidification solution) under magnetic stirring for 2 h to solidify the microcapsules [5,18]. The obtained microcapsules were collected by centrifugation and washed with distilled water for three times.

2.3. Characterization of microcapsules

The shape and surface morphology of PELA microcapsules were observed by a JSM-6700F (JEOL, Japan) scanning electron microscope (SEM). The particle size distribution of PELA microcapsules was measured by laser diffractometry. All analyses were carried out in triplicate. Microcapsules were dispersed in distilled water and analyzed by laser diffractometry using Ls230 Coulter (Coulter Co., USA). The uniformity of microcapsules was expressed as a coefficient of variation (CV) value and the smaller value of CV means the more narrow size distribution of microcapsules. CV value is defined as:

$$\mathrm{CV} = \left(\sum_{i=1}^{n} \frac{(d_i - \bar{d})^2}{N} \right)^{1/2} / \bar{d} \quad (1)$$

where d_i is the diameter of the ith particle, $\bar{d}$ is the number-average diameter and N is the total number of particles counted.

2.4. Determination of viscosity

The viscosities of double emulsions were determined by a rotational rheometer (L-90, Mechanical and Electric Plant), by measuring the torques on a rotor in Couette flow at 25 °C. Double emulsions were measured under the condition of a No. 1 rotor.

2.5. Determination of interfacial tension

Interfacial tension between external water phase and oil phase was measured by hanging drop method under the principle of the contact angle measurement. Under the effect of micro-flow pump, oil was injected slowly into the aqueous phase containing PVA with different concentrations. The CCD recording system recorded the process, and then calculated the values of interfacial tension (flow rate was 0.1 μL/s).

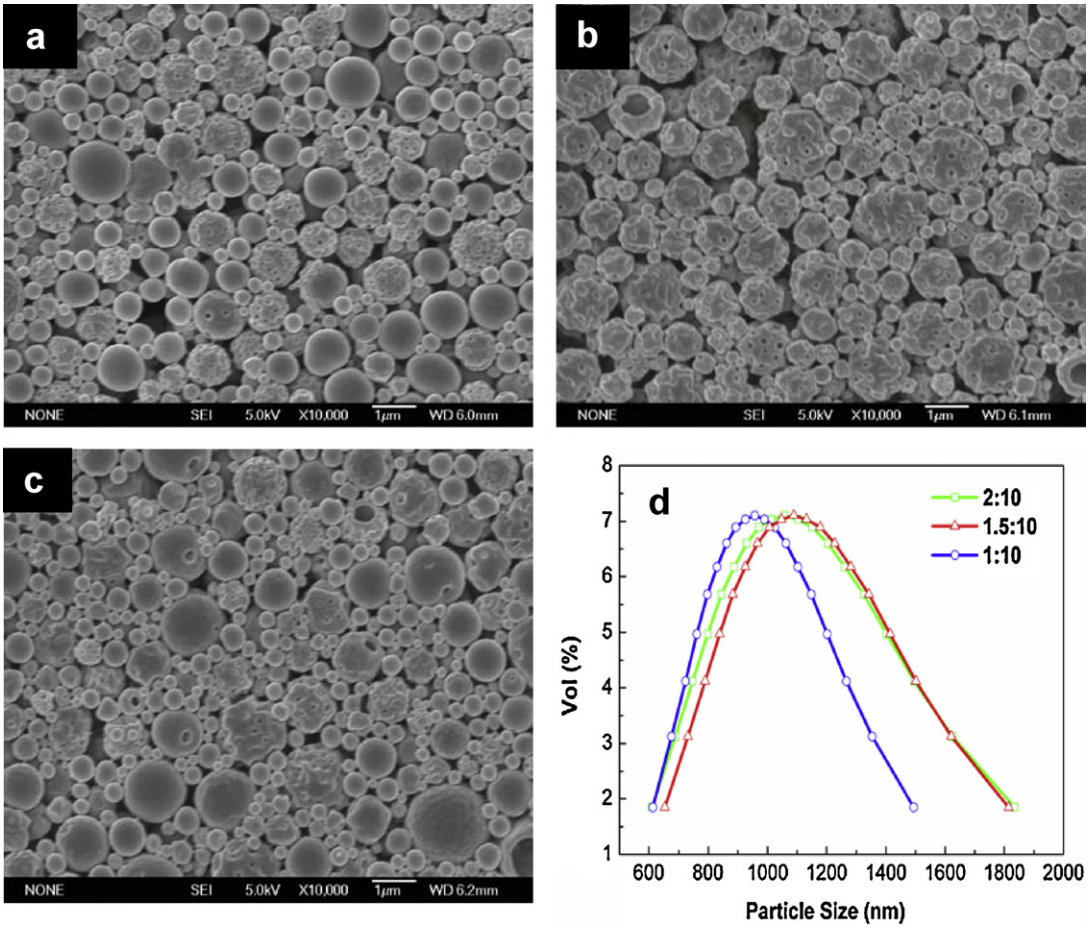

Fig. 3. SEM micrographs (a–c) and size distributions (d) of microcapsules prepared with different volume ratios of internal water phase to oil phase: (a) 1:10; (b) 1.5:10; (c) 2:10.

3. Results and discussion

3.1. Effect of mass ratio of PLA/mPEG on preparation of microcapsules

Increasing the mass ratio of hydrophilic mPEG in PELA will enhance matrix erosion and release rate of drug encapsulated, which facilitate the drug incomplete release. However, increasing the mass ratio of hydrophilic mPEG will decrease polymer viscosity, and which further resulted in decreasing viscosity of double emulsions [9]. Polymer viscosity has great influence on the uniformity of microcapsules, because polymer viscosity not only affects process parameters of membrane emulsification technique but also has important effect on solidification process of microcapsules. Here we systematically studied the effect of different polymer viscosities on the uniformity and surface morphology of the microcapsules. The polymers with different mass ratios of PLA/mPEG 14:1, 19:1 and 24:1 were used and their intrinsic viscosities were 0.510, 0.580 and 0.655 dL/g, respectively (data determined by Ubbelhode viscometer at $30.0 \pm 0.1\,^{\circ}C$ were supplied by company). Dichloromethane was employed as oil phase because it can dissolve polymer well and easily be removed from microcapsules by evaporation. The SEM micrographs and size distribution of microcapsules by polymers with different mass ratio are shown in Fig. 1. When polymer intrinsic viscosities were 0.510, 0.580 and 0.655 dL/g, the CV values of PELA microcapsules were 49.41%, 34.10% and 47.28%, respectively. This observation can be explained by the effect of the viscosity on double emulsion. Under the same weight concentration, an increase of polymer viscosity led to an increase of the double emulsion viscosity. For the above three polymers, the viscosities of double emulsions were 4.85, 17.4 and 36.5 mPa s, respectively. With the sharp increase of double emulsion viscosity (mass ratios of PLA/mPEG 24:1), the droplets formed on the opening of the membrane pore, adhered there for a longer time, causing the coalescence between the droplets [11], and thus resulting in droplets with relatively large size distributions (Fig. 2c). In contrast, when the polymer Mw was too low (mass ratios of PLA/mPEG 14:1), the viscosity of double emulsion was became lower. Then, the large sized droplets were easy to be broken into smaller droplets, and thus leading to broad size distribution (Fig. 2a). In addition, many smaller-sized droplets were formed during the preparation of coarse double emulsion due to lower viscosity of polymer, and these smaller droplets would directly go through the membrane pores, which further led to broad size distribution of microcapsules. The best result was obtained when the mass ratio of PLA/mPEG was 19:1, the polymer intrinsic viscosity was 0.580 dL/g and the viscosity of double emulsion was 17.4 mPa s (Fig. 2b). Therefore, the polymer with mass ratio of PLA/mPEG 19:1, was employed as encapsulation material in the following experiments.

3.2. Effect of volume ratio (W_1/O) on preparation of microcapsules

The volume of internal water phase played important role on the stability of primary emulsions, which also affected unifor-

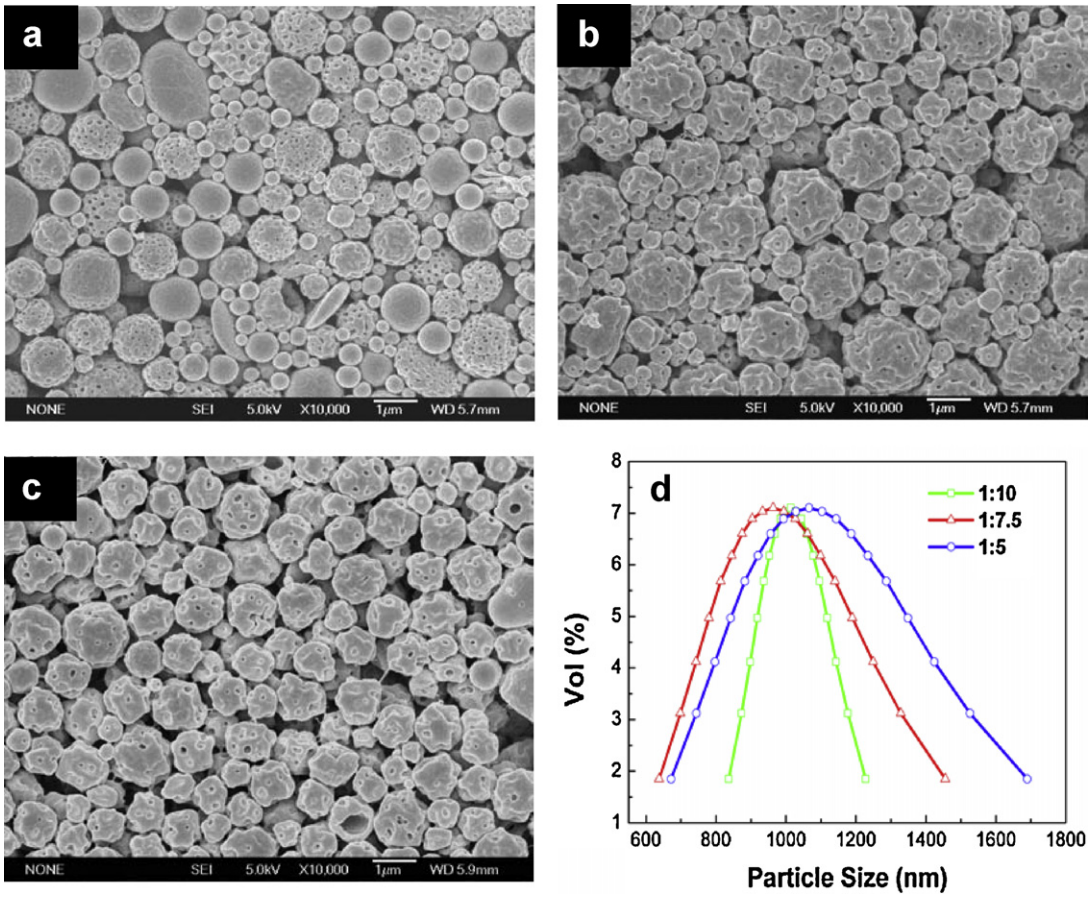

Fig. 4. SEM micrographs (a–c) and size distributions (d) of microcapsules prepared with different volume ratios of oil phase to external water phase. (a) 1:5; (b) 1:7.5; (c) 1:10.

mity of emulsions [15]. In order to optimize W_1/O ratio, three different W_1/O ratio values (1:10, 1.5:10 and 2:10 (v/v)) were used to prepare PELA microcapsules. The SEM micrographs and size distribution of the prepared microcapsules are shown in Fig. 3. When W_1/O ratio was 1:10, 1.5:10 and 2:10, respectively, the CV value of PELA microcapsules was 28.28%, 43.85% and 46.00%, respectively. When the internal water phase increased, the stability of primary emulsion reduced, resulting in coalesce of internal and external water phases more easily. It was found that when the volume of internal water phase was too high the microcapsules tended to break due to the thin oil film between internal and external water phases, and this further led to broad size distribution of prepared PELA microcapsules (Fig. 3b and c). When W_1/O was 1:10, the polymer of oil phase can encapsulate the internal water phase well, which led to more stable primary emulsion and narrow size distribution of microcapsules. If the volume of internal was too low, the loading content would be lower. Hence we chose W_1/O 1:10 in the following experiment.

3.3. Effect of volume ratio of oil phase to external water phase (O/W_2)

The volume of external water phase affects the viscosity of double emulsions, which will further affect the droplets uniformity when they are pressed through SPG membrane [18]. In addition, volume of external water phase affects evaporation rate of dichloromethane during solidification of microcapsules, which further affects the morphology of microcapsules. O/W_2 volume ratio 1:5, 1:7.5, and 1:10 were used in experiments to investigate the effect of O/W_2 on size distribution and morphology of microcapsules. The SEM micrographs and size distribution of the microcapsules are shown in Fig. 4. When O/W_2 ratio was 1:5, 1:7.5 and 1:10, respectively, the CV value of PELA microcapsules was 44.05%, 40.20% and 26.65%, respectively. However, it was found that the morphology of the prepared microcapsules became irregular when the volume of external phase increased (Fig. 4c). The possible reason was that when the volume of the external phase increased, the diffusion rate of methylene chloride into external water phase increased largely and thus resulted in its quick evaporation rate. On the other hand, the surface morphology of microcapsules exhibited much smoother when the volume of the external phase decreased as a result of the low evaporation rate (Fig. 4a). As related to the uniformity, microcapsules prepared by larger volume of external water phase were better than the others due to suitable viscosity of coarse emulsion. Otherwise, when the volume of external phase decreased, the viscosity of whole system became higher, and this resulted in that the coarse emulsion with large size were difficult to break into smaller and uniform one, exhibiting poor uniformity. Although the uniformity was better when the ratio of O/W_2 was 1:10, the pores of microcapsules may lead to leaking of loaded drug during process of washing and drying. So after comprehensive taking account of the uniformity and surface morphology, the ratio of O/W_2 1:5 was chosen for continual optimization of preparation conditions.

3.4. Effect of PVA concentration in external water phase

Stabilizers in the external water phase played crucial roles in preparation of individual spherical microcapsules [20], because

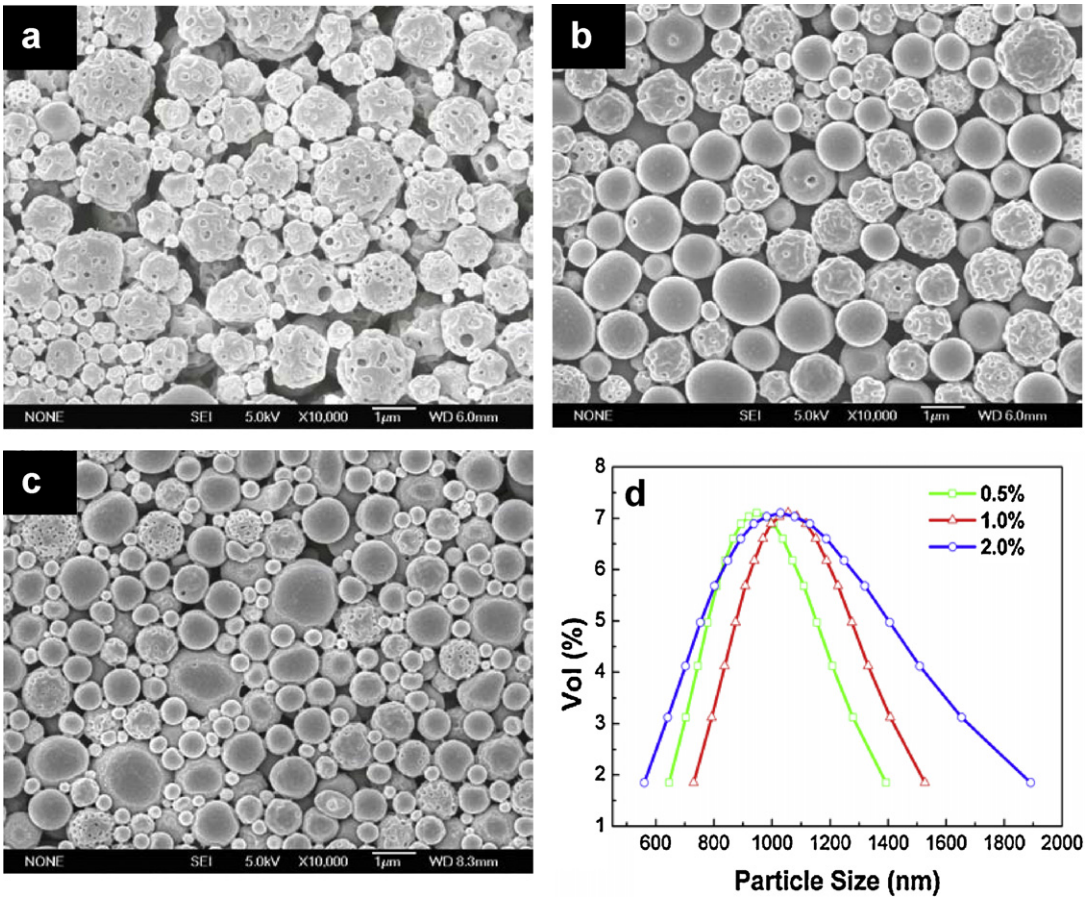

Fig. 5. SEM micrographs (a–c) and size distributions (d) of microcapsules prepared with different PVA concentrations in external water phase: (a) 0.5%; (b) 1.0%; (c) 2.0% (w/v).

the stabilizers can prevent the coalescence of droplets during emulsification and solvent removal process. PVA is the most commonly used stabilizer due to its low toxicity, good solubility in water and its availability in a wide range of molecular weights [21]. Therefore PVA was employed as the stabilizer and its effect on uniformity of the microcapsules was investigated. When concentration of PVA was 0.5%, 1.0% and 2.0%, respectively, the CV values of PELA microcapsules were 48.03%, 28.00% and 46.30%, respectively. It was found that when the PVA concentration was below 1.0% (Fig. 5a), the microcapsules showed not only pores on the surface but also non-uniformity. When the concentration of PVA reached 1.5% (Fig. 5c), the uniformity of emulsion droplets was not satisfied either. Further study was carried out to investigate the effects of PVA concentrations on oil–water interfacial tension to explain the above results. It was found that interfacial tension decreased with increase of PVA concentration (Fig. 6). When PVA concentrations were lower than 1% the interfacial tensions were still relatively high, thus led to coalescence between droplets easily. The interfacial tension did not change when PVA concentration reached a critical value of 1.0%, and then emulsion droplets can maintain a good uniformity. However, when concentration of PVA exceeded 1.0%, the microcapsules exhibited broad size distribution. This was probably because that when PVA concentration increased, the viscosity of double emulsion became higher. As a result, the droplets with large size were hard to be broken, and then resulted in broad size distribution. Thus the uniformity of the microcapsules was better and their surface morphology was relatively glossy when PVA concentration (w/v) was 1.0%.

3.5. *Effect of stirring rate during solidification*

It was found the stirring solidification rate during solidification has a pronounced effect on the surface morphology and size dis-

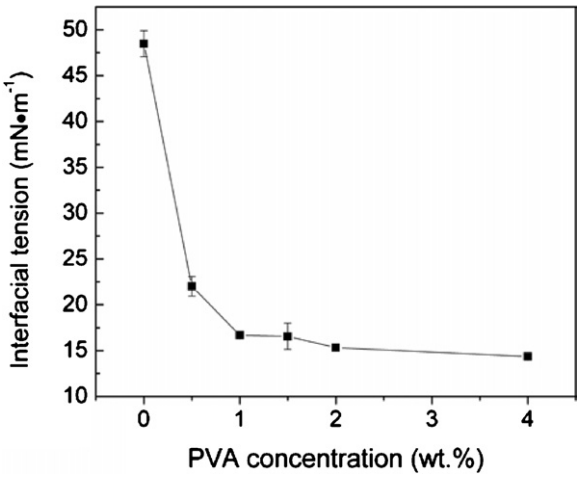

Fig. 6. Interfacial tension of oil–water phase at different PVA concentrations.

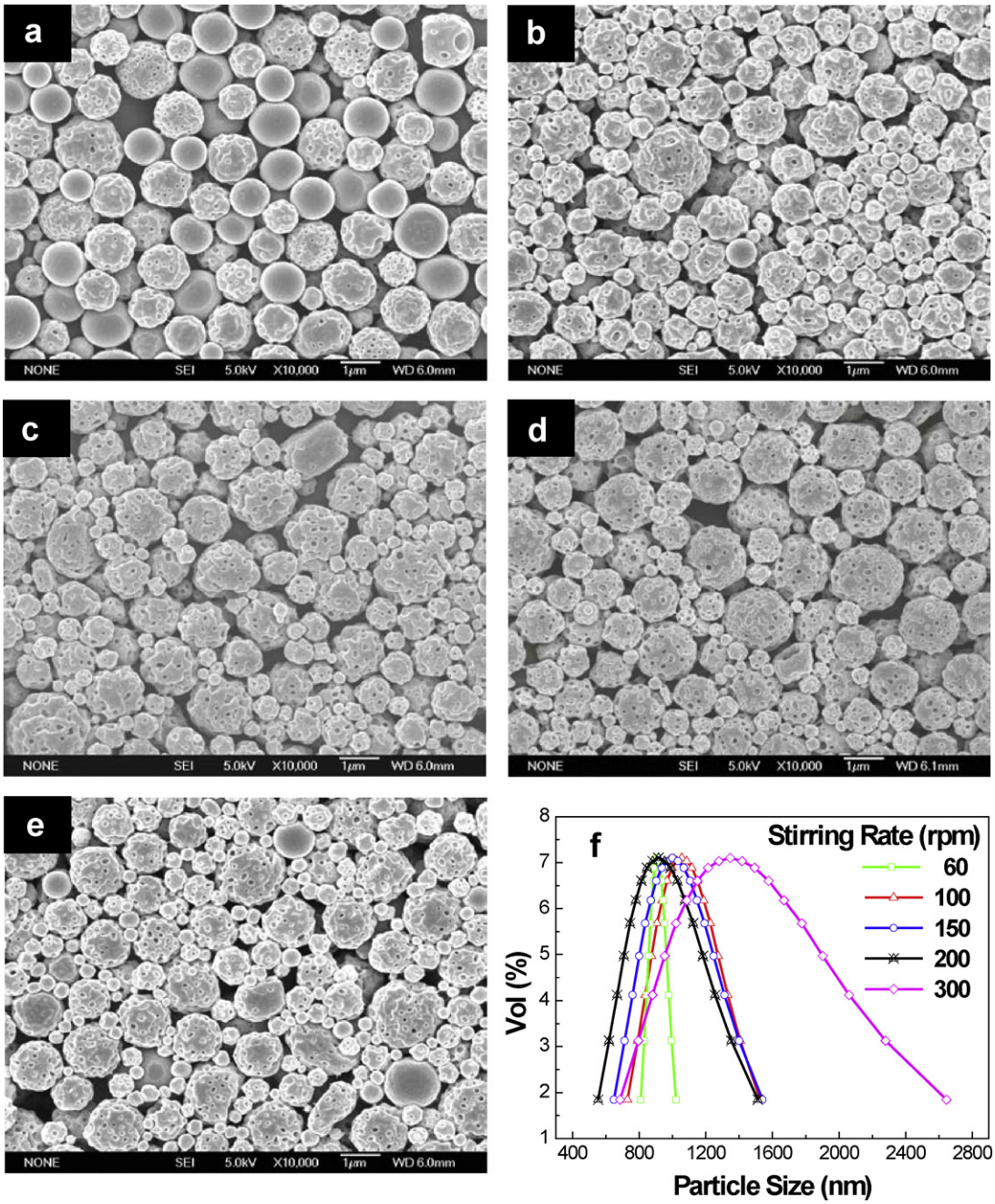

Fig. 7. SEM micrographs (a–e) and size distributions (f) of microcapsules prepared with different magnetic stirring rates (a) 60 rpm; (b) 100 rpm; (c) 150 rpm; (d) 200 rpm; (e) 300 rpm.

tribution of microcapsules due to different shear stress [18]. The experiment was carried out using different stirring rate of 60, 100, 150, 200 and 300 rpm of magnetic bar, respectively. The corresponding SEM micrographs are shown in Fig. 7. When stirring rates were 60, 100, 150, 200 and 300 rpm, respectively, the CV values of PELA microcapsules were 16.23%, 30.35%, 40.67%, 41.01% and 41.80%, respectively. The results showed that microcapsules prepared with low stirring rate appeared spherical and smooth surface morphology and relatively uniform size. The uniformity and morphology were better than the others when the stirring rate was 60 rpm. In contrast, the microcapsules prepared with high stirring rate showed macropores on the surface and the size distributions were broad. We proposed that with minimizing the magnetic stirring rate, the solidification rate of the double emulsion decreased, as well as the local diffusion rate of methylene dichloride decreased which led to smooth surface. Furthermore, the slower stirring rate could minimize the breakup of coalesce of the emulsion. When the stirring rate was high, large-sized droplets were easy to be broken into smaller droplets. In addition, the droplets also easily gathered to form larger droplets since the viscosity of oil phase increased with rapid evaporation of methylene dichloride at high stirring rate. So, the uniformity and morphology were both improved at the slower magnetic stirring rate (60 rpm).

3.6. *Effect of type of oil phase*

Based on the above results, the PELA microcapsules with relatively narrow size distribution were obtained (Fig. 7a), but the surface morphology was macropores. The result was quite different from those of PLA and PLGA systems which showed smooth sur-

face morphology using methylene dichloride as oil phase [19,22]. One reason was that the hydrophilic PEG segments extended on the surface of microcapsules with the evaporation of methylene chloride during solidification in aqueous phase. These hydrophilic PEG segments fully swelled and absorbed a large amount of water. The water adsorbed around PEG segments on the surface of the microcapsules would evaporate when microcapsules were dried, resulting in macropores. The other reason was the property of solvent used in oil phase. There are two steps during the solidification of microcapsules, solvent extraction followed by solvent evaporation. The rate of solvent extraction is decided by its solubility in water and the rate of solvent evaporation depends on its boiling point. In the second step (Fig. 9a), the fast rate of solvent evaporation due to low boiling point easily leads to local explosion of microcapsules, and thus resulting in the surface morphology with macropores [10]. Hence, the surface morphology of PELA microcapsules using methylene dichloride as oil phase exhibited macropores due to its low boiling point. However, as drug carriers the surface morphology with macropores was not satisfied, since the macropores were easy to produce burst initial release, brought about surplus drug concentration in vivo and decreased drug efficacy in later period. So, methylene dichloride was not suitable as oil phase in preparation of PELA microcapsules. In order to prepare microcapsules with smooth surface, we attempted to use another solvent, ethyl acetate as oil phase, because its boiling point is higher than that of methylene dichloride and its toxicity is low [23]. As shown in Fig. 8, it was exciting that the microcapsules with smooth surface were successfully prepared by using ethyl acetate as oil phase. It can be explained by the relatively high boiling point of ethyl acetate, which avoided the local explosion on the surface of microcapsules (Fig. 9b). Therefore, in the following study, ethyl acetate was used as oil phase in place of methylene dichloride.

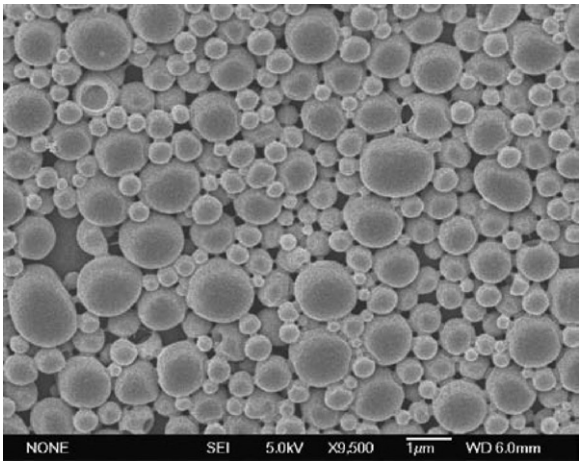

Fig. 8. SEM micrographs of microcapsules prepared by ethyl acetate as oil phase.

3.7. Effect of transmembrane pressure

Transmembrane pressure (P_{tm}) played a key role in the premix membrane emulsification process [16]. In order to select an adequate P_{tm}, the primary double emulsions were pressed through SPG membrane with different P_{tm}, and the relationship between the P_{tm} and the size distribution of microcapsules for the membrane with pore size of 2.8 μm was investigated. As shown in Fig. 10, when the P_{tm} was 300 kPa, the size distribution of microcapsules was rather narrow (CV value was 14.26%). However, when the P_{tm} was too high (400 kPa), primary double emulsions impacted surface of membrane at high speed and then produced acute friction with wall of membrane pores. The processes led to a quite high shear stress, which easily broke primary double emulsions into

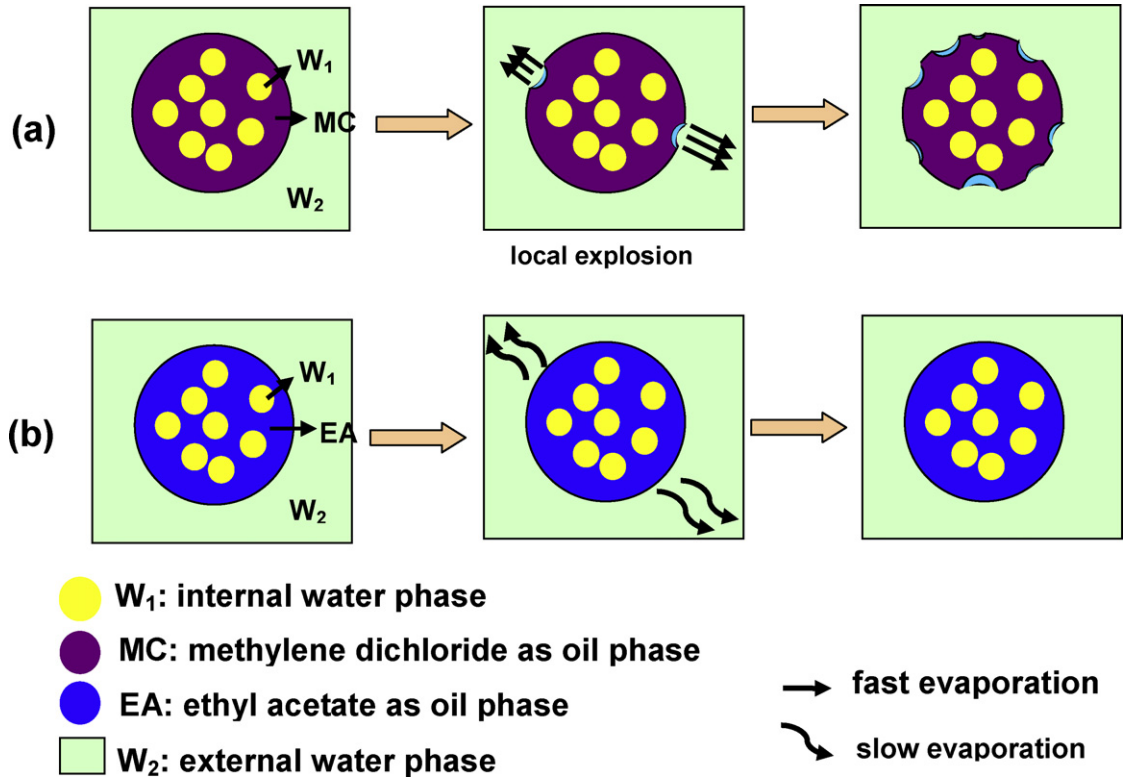

Fig. 9. Schematic illustration of the effect of oil phase on microcapsules surface morphology during the second step of solidification: (a) MC: methylene dichloride; (b) EA: ethyl acetate.

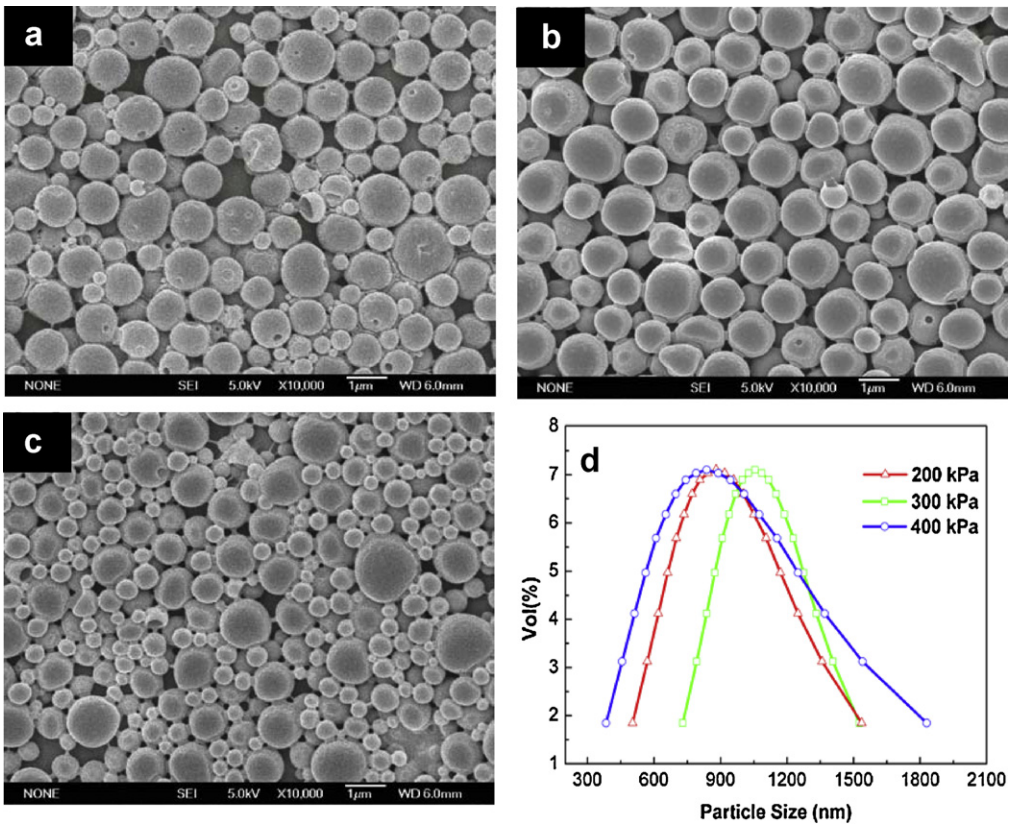

Fig. 10. SEM micrographs (a–c) and size distributions (d) of microcapsules prepared by different transmembrane pressures (membrane pore size: 2.8 μm): (a) 200 kPa; (b) 300 kPa; (c) 400 kPa.

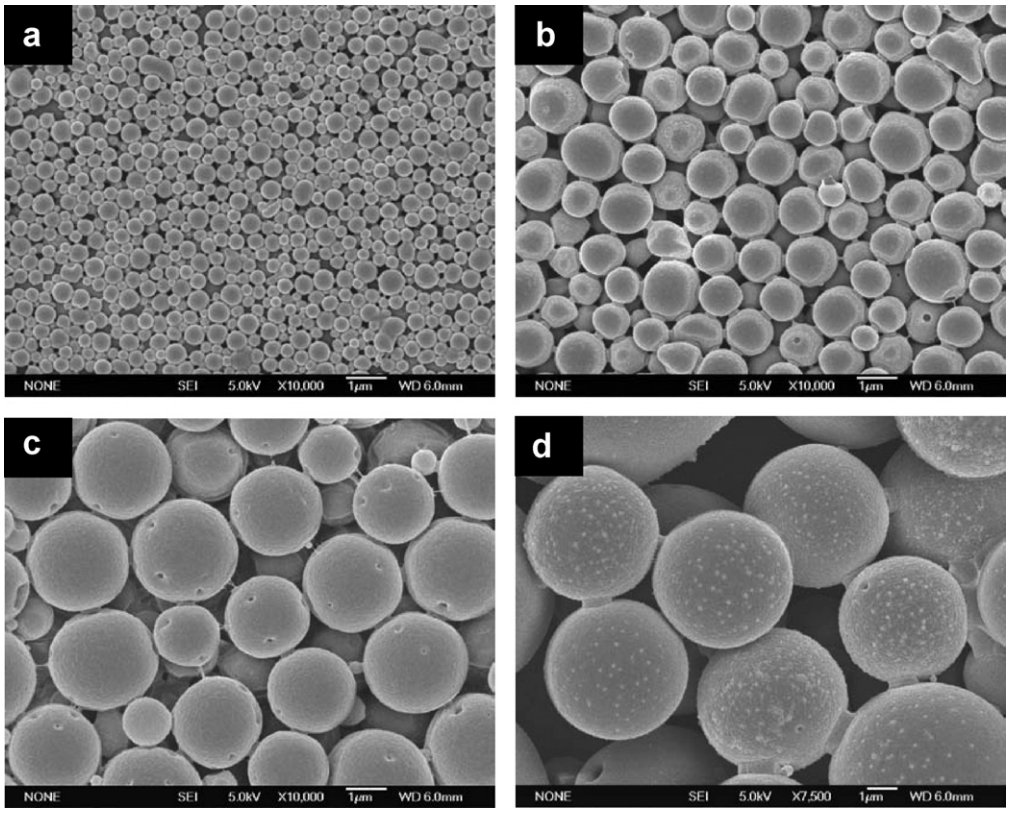

Fig. 11. SEM micrographs of microcapsules prepared by different membrane pore sizes: (a) 1.4 μm; (b) 2.8 μm; (c) 5.2 μm; (d) 18 μm.

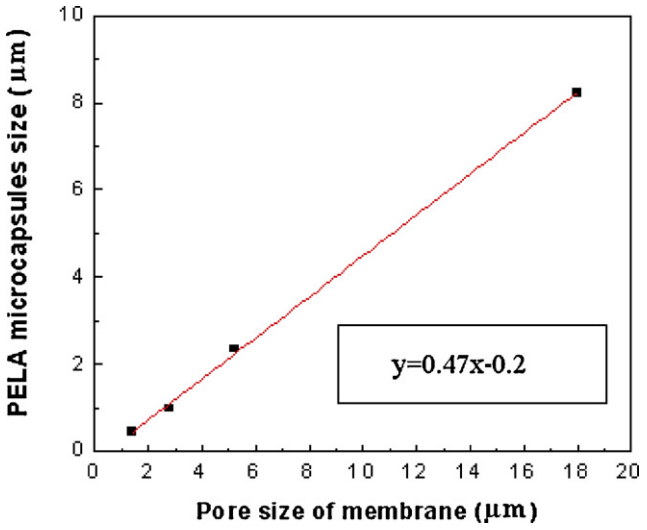

Fig. 12. Relationship between the mean sizes of PELA microcapsules and the pore sizes of membranes.

small size droplets, which contributed to broad sized distribution. On the other hand, if the P_{tm} was too low (200 kPa), droplets with large size were hard to be broken because they could pass through the membrane pores slowly by changing the droplet shape, thus resulted in broad size distribution (CV value was 29.62%). Therefore, 300 kPa was chosen as the suitable transmembrane pressure to prepare uniform PELA microcapsule for the membrane with pore size of 2.8 μm. The optimal preparation condition of PELA microcapsule was as follows: for the membrane with pore size of 2.8 μm, the intrinsic viscosity of polymer was 0.580 dL/g, volume ratio of W_1/O was 1:10 and O/W_2 was 1:5, PVA concentration (w/v) was 1.0%, magnetic stirring rate during solidification was 60 rpm and 300 kPa was chosen as transmembrane pressure.

3.8. The relationship between membrane pore size and particle size

It is important to control the size of PELA microcapsules for the application of drug delivery system since the bioavailability and release rate of the drug to a large extent correlated to the size of microcapsules. The PELA microcapsules with different size were prepared under the above optimized conditions by premix membranes emulsification technique using membrane with different pore sizes. The CV values of PELA microcapsules were 13.38%, 14.26%, 13.53% and 14.81%, respectively when pore sizes of membrane were 1.4, 2.8, 5.2 and 18 μm. As shown in Fig. 11, the PELA microcapsules showed narrow size distributions and their CV values were all under the 15%. The relationship between the pore size of membrane and the particle size of the microcapsules is shown in Fig. 12. The particle size of the PELA microcapsules mainly depended on the pore size of the membrane and a linear relationship was observed. Therefore, the membrane emulsification is an ideal method to prepare size-controllable droplets and particles with narrow size distribution. By choosing the adequate pore size of the membrane, the required size of the microcapsules can be obtained.

4. Conclusions

PELA microcapsules with narrow size distribution and smooth morphology were successfully prepared by combining premix membrane emulsification technique and double emulsion-solvent extraction method. It was found that the preparation results were quite different in terms of structure and size distribution between PELA and PLGA or PLA systems. The structure and size distribution of the microcapsules were difficult to control because of employing the amphiphilic polymer PELA. It was showed that internal water phase with less volume, slower stirring rate during solidification and using ethyl acetate as oil phase were favorable for preparing smooth-surface microcapsules. Especially, the choice of oil phase played significant role on surface morphology of microcapsules. The microcapsules surface transferred from macropores to smooth surface when using ethyl acetate as oil phase instead of methylene dichloride. It can be explained by the relatively high boiling point of ethyl acetate, which avoided the local explosion on the surface of microcapsules. It was also found that intrinsic viscosity of polymer, PVA concentration in external water phase and transmembrane pressure significantly affected size distribution of microcapsules. The optimal conditions were established as follows: for the membrane with pore size of 2.8 μm, the mass ratio of PLA/mPEG was 19:1, volume ratio of W_1/O was 1:10 and O/W_2 was 1:5, PVA concentration (w/v) was 1.0%, magnetic stirring rate during solidification was 60 rpm and 300 kPa was chosen as transmembrane pressures. By manipulating the preparation conditions, PELA microcapsules with CV value under 15% were obtained. Smooth-surface microcapsules with different sizes were obtained successfully under the optimized conditions by using membrane with different pore sizes. The research would motivate the investigating of other amphiphilic polymer formulations when premix membrane emulsification technique is employed.

Acknowledgements

We thank the financial support of National Natural Science Foundation of China (20706053), the Funds for Creative Research Groups of China (112022160303), Major International Joint Research Program of China (20820102036) and Significant Creation of New Drugs (20092x09503-027).

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