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Preparation of uniform-sized PLA microcapsules by combining Shirasu Porous Glass membrane emulsification technique and multiple emulsion-solvent evaporation method

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Abstract

Relatively Uniform-sized biodegradable poly(lactide) (PLA) microcapsules were successfully prepared by combining a Shirasu Porous Glass (SPG) membrane emulsification technique and multiple emulsion-solvent evaporation method. An aqueous phase containing lysozyme was used as the internal water phase (w_1), and PLA and Arlacel 83 were dissolved in a mixture solvent of dichloromethane (DCM) and toluene which was used as the oil phase (o). These two solutions were emulsified by a homogenizer to form a w_1/o primary emulsion. The primary emulsion was permeated through the uniform pores (5.25 μm) of an SPG membrane into the external water phase by the pressure of nitrogen gas to form the uniform $w_1/o/w_2$ droplets. Then, the solid polymer microcapsules were obtained by simply evaporating the solvent. It is necessary to avoid the phase separation of primary emulsion during the SPG membrane emulsification. It was found that when the density difference of the internal water phase and oil phase was reduced to nearly zero and Arlacel 83 was used as the oil emulsifier, the phase separation was not observed within 24 h. The $w_1/o/w_2$ emulsion with uniform diameter was obtained only when Arlaecl 83 concentration was limited below 2.5 wt.% based on oil phase. The drug encapsulation efficiency was found to be related to several factors including PLA molecular weight, additive type and its concentration in the internal water phase, the emulsifier type and concentration in the oil phase, the NaCl concentration and the pH value in the external water phase. Comparing with the stirring method, it was found that the size was more uniform and the drug encapsulation efficiency was much higher when the microcapsules were prepared by SPG membrane emulsification technique and the highest drug encapsulation efficiency of 92.20% was obtained. This is the first study to prepare PLA microcapsules by combining an SPG membrane emulsification technique and multiple emulsion-solvent evaporation method.

Keywords: SPG membrane emulsification; Multiple emulsion-solvent evaporation; Poly(lactide); Encapsulation efficiency

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1. Introduction

With the rapid development of DNA-recombinant techniques and other modern biotechnology, more and more protein and peptide drugs, which are becoming a very important class of therapeutic agents, can be produced on a large scale in recent years [1]. However, available protein and peptide drugs are generally characterized by a short biological half-life time. They are easily degraded by enzymes in vivo and most protein and peptide drugs poorly pass through biological barriers due to their poor diffusivity, which is unfavorable to diffuse in lipid membrane [2]. For these reasons, the entrapment of such drugs within microparticulate drug delivery systems, using various kinds of biodegradable polymers, has been studied extensively during the past two decades [3–8]. Microcapsules composed of biodegradable polymers are able to protect these drugs against degradation, to control their release from the site of administration and in some particular cases, to improve their passage through biological barriers. Furthermore, the biodegradable polymers do not accumulate in a living body so that they will not inflict any harm on the body. Until now, the most popular method to prepare microcapsules as drug delivery system is multiple emulsion water-in-oil-in-water (w/o/w)-solvent evaporation method, which was patented in 1970 by Vrancken and Claeys [9]. Ogawa et al. [10] first used the double emulsion-solvent technique to prepare an injectable poly(lactide-co-glycolide) microparticulate for the sustained release of leuprolide acetate. Until now, most of microcapsules are prepared by the conventional mechanical stirring, homogenization or ultrasonication method, the size distribution of microcapsules obtained is very broad, which is unfavorable for efficient absorption of microcapsules containing protein drugs in vivo. Le Fevre et al. [11] evaluated the effect of particle size on the uptake of polystyrene microparticles in mice. They showed that particles were taken up into the Peyer's patches more easily when the particle size was below 5 μm. Therefore, if microcapsules containing protein drugs are uniform, and their sizes are controlled under a limited value, the bioavailability of the protein drugs would be enhanced largely. Furthermore, the microcapsules with a narrow size distribution are necessary in the drug delivery system in order to decrease the side effects of the drugs, especially anti-cancer agents, because the accumulated locations of the microcapsules containing anti-cancer agents also depend on the size of the microcapsules [12]. On the other hand, the practical and theoretical evaluation such as release rate will become simple and precise if the microcapsules are uniform, and one can select one kind of microcapsules with the uniform diameter or mix several kinds of microcapsules with the designed diameter according to the desired controlled release rate. Therefore, developing a method which can provide the uniform microcapsules composed of biodegradable polymers is very important.

The SPG (Shirasu Porous Glass) membrane emulsification technique is a promising technique which was first proposed by Nakashima et al. [13] to prepare uniform-sized emulsion and later developed by Omi and Ma et al. [12,14–17] to prepare uniform microspheres by polymerizing uniform monomer droplets. However, the SPG membrane emulsification technique has not been used to prepare monodispersed polymer microcapsules by multiple emulsion-solvent evaporation method. In this study, we tried to prepare uniform-sized poly(lactide) (PLA) microcapsules containing lysozyme by pressing w_1/o primary emulsion through the uniform pores of the SPG membrane to obtain uniform $w_1/o/w_2$ double emulsion, followed by an evaporation process of solvent. Selecting SPG membrane emulsification technique to prepare PLA microcapsules has other advantages besides narrow size distribution: (1) the diameter of double emulsion and resultant microcapsules can be controlled easily by adopting the membrane with required pore size; (2) because of the narrow size distribution of the droplets, break-up and coalescence between droplets rarely occur during the emulsification and solidification processes, and therefore the drug would not escape out of the droplets by coalescence and break-up of the droplets, a much higher drug encapsulation efficiency can be expected [18]; (3) emulsification is carried out with low shear, and it is suitable for the encapsulation of protein and peptide drugs which may lose their bio-activities under violent stirring.

However, comparing with the conventional emulsification methods of microcapsules, such as the stirring method, the production of microcapsules by combining SPG membrane emulsification technique and multiple emulsion-solvent evaporation method

may face more difficulties, such as the stability of primary emulsion, because the emulsion system is thermodynamically unstable, which easily separate into water and oil phases. In general, it takes 1–5 h to prepare double emulsion by SPG membrane emulsification technique; therefore, the primary emulsion should be stable during SPG membrane emulsification. Moreover, the instability of primary emulsion would have influence on the inner structure and drug encapsulation efficiency of the microcapsules prepared.

In this study, we investigated factors influencing the stability of primary emulsion and found an appropriate condition where the primary emulsion can be maintained stable more than 24 h, and then we established a new method to prepare uniform-sized PLA microcapsules by combining the SPG membrane emulsification technique and multiple emulsion-solvent evaporation method. Poly(lactide) was used as polymer material because PLA is a nontoxic, biocompatible and biodegradable polymer approved by the Food and Drug Administration for human use [19]. The lysozyme was chosen as a model protein drug. The purpose of this study is to find an adequate condition to obtain uniform-sized $w_1/o/w_2$ double emulsion and microcapsules and to investigate the main factors influencing the size distribution and drug encapsulation efficiency of microcapsules systemically.

2. Materials and methods

2.1. Materials

Poly(lactide) (PLA) was purchased from the Institute of Medical instrument (SanDong, China), the average molecular weight (M_w) of which was 10 kDa, 20 kDa, 100 kDa and 300 kDa, respectively. Lysozyme was used as model protein drug and purchased from BioBasic (Canada). Dichloromethane (DCM) and toluene of analytical grade were purchased from Beijing Chemical Reagents Company (Beijing, China). Sorbitan Sesquioleate (Arlacel 83) of biochemical grade was purchased from Sigma (St. Louis, USA) and used as an emulsifier in the oil phase. Sodium dodecyl sulfate (SDS) of biochemical grade was purchased from Merck (Darmstacit, Germany) and was used as a surfactant in the external water phase. Poly(vinyl alcohol) (PVA-217, degreee of polymerization 1700, degreee of hydrolysis 88.5%) was provided by Kuraray (Tokyo, Japan) and was used as a stabilizer in the external water phase. Phosphatidylcholine of biochemical grade was purchased from Sigma (St. Louis, USA). Polyoxyethylene 10 hydrogenated castor oil (HCO 10), Polyoxyethylene 20 hydrogenated castor oil (HCO 20), Polyoxyethylene 40 hydrogenated castor oil (HCO 40) were all provided by Nikkol (Tokyo, Japan) and used as an emulsifier in the oil phase. Polyvinylpyrrolidone (PVP), gelatin and Tween 20 were purchased from Beijing Chemical Reagents Company (Beijing, China). Polyyoxyethylene 40 stearate (Myrj 52) was purchased from Sigma (St. Louis, USA). All other reagents were of reagent grade and used as received.

2.2. Apparatus

A miniature kit for emulsification with an MPG module (microporous glass, a brand name of SPG) installed was purchased from Ise Chemical Co. A schematic diagram of this kit is shown in Fig. 1. The

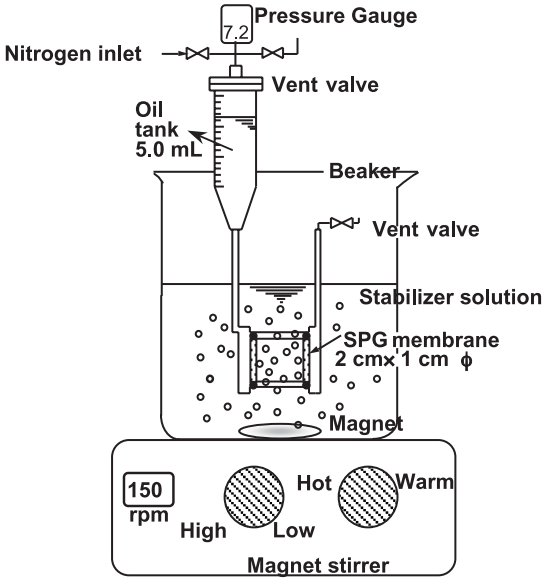

Fig. 1. Schematic diagram of a miniature kit for SPG membrane emulsification.

size of SPG membrane was 2 cm (L)×1 cm (Φ). The primary emulsion (w_1/o phase) was stored in a Teflon tank (20 ml) which was connected to a nitrogen gas inlet, the continuous phase (external aqueous phase) was stirred gently (150 rpm) with a magnet bar in a beaker (150 ml) to prevent the creaming of the droplets. By applying an adequate pressure of nitrogen gas, the w_1/o phase will permeate through the uniform pores of the membrane into the external water phase to form the droplets, then be stabilized by PVA and SDS dissolved in the external water phase.

2.3. Measurement of stability of w_1/o primary emulsion

1.5 ml water was added into 5 ml organic solvent dissolving emulsifier and PLA which was used as oil phase, and these two solutions were mixed and emulsified by a homogenizer at a rate of 9800 rpm for 1 min to form a w_1/o primary emulsion. Then, the primary emulsion was poured in a test tube and kept still to observe whether the phase separation occurred or not. The stability was expressed by the elapsed time until the phase separation was observed. The effect of solvent density and emulsifier type and concentration in oil phase was investigated.

2.4. Preparation of PLA microcapsules

An SPG membrane with an average pore size of 5.25 μm was used. A standard recipe is shown in Table 1. 10 mg lysozyme was dissolved in 1.5 g water and was used as the internal water phase (w_1). 0.25 g PLA and Arlacel 83 were dissolved in a 5.0 g mixture solvent of DCM and toluene, which was used as the oil phase (o). These two solutions were mixed and emulsified by a homogenizer at a rate of 9800 rpm for 1 min to form a w_1/o primary emulsion. The aqueous phase where PVA and SDS were dissolved was used as the external water phase (w_2). The primary emulsion was permeated through the uniform pores of an SPG membrane into the external water phase by the pressure of nitrogen gas to form the uniform-sized droplets. Then, DCM and toluene were evaporated at room temperature for 24 h under a gentle stirring at a rate of 150 rpm. A pressure slightly above the critical pressure, which is defined as a minimum pressure at which the primary emulsion begins to permeate through the membrane into the external water phase, was applied. The critical pressure usually was 5.0–10.0 kPa depending on the component of the w_1/o emulsion in this study. After DCM and toluene were evaporated, the hardened PLA microcapsules were collected by centrifugation and washed with distilled water three times and then vacuum dried for 48 h. To compare the results in this study and those obtained by conventional stirring method, the microcapsules were also prepared by the conventional stirring method. The procedure for preparing microcapsules by the stirring method was similar with the process mentioned above except that the w_1/o/w_2 double emulsion droplets were yielded by stirring method at a rate of 1000 rpm for 40 s.

Table 1
Standard recipe in the preparation of microcapsules

Phase/ingredient	Amount (g)
(w_1) Internal water phase	
Water	1.5
Lysozyme	0.010
Additives (PVA, PVP, gelatin, Tween20, Myrj52)	0–0.03 (maximum 2.0 wt.% of water)
(o) Oil phase	
Mixed solvent (DCM+toluene)	5.0 (DCM/toluene (v/v):21/79)
Oil-soluble emulsifier Arlacel 83	0.0125–0.375 (max 7.5 wt.% of mixed solvent)
PLA (10 kDa, 20 kDa, 100 kDa, 300 kDa)	0.25
(w_2) External water phase	
Water	100
PVA	1.0
SDS	0.067
Sodium chloride	0–2.0 (max 2.0 wt.% of water)

2.5. Measurement of the drug encapsulation efficiency

Total lysozyme loaded was measured using the Lowry assay [20] after disruption of the microcapsules with 2.0 % SDS/0.1 M NaOH solution. Sodium hydroxide catalyzes the hydrolysis of PLA, and SDS ensures the complete solubilization of the lysozyme during polymer hydrolysis. The resulting solution was then neutralized by stepwise addition of

1 M HCl. The drug encapsulation efficiency was calculated from the following formula:

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Total amount of lysozyme loaded}}{\text{Total amount of lysozyme}} \times 100$$

2.6. Measurement of size distribution of the microcapsules

The dried PLA microcapsules were redispersed in distilled water and measured by laser diffraction using a Coulter LS 230 (Coulter Electronics, USA). The particle size distribution was expressed as volume-mean diameter, and the CV (coefficient variation) value, defined as follows, was used to characterize the size distribution.

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

Where, di is the diameter of the ith diameter, $\bar{d}$ is the volume mean diameter, and N is the total number of microcapsules.

2.7. Observation of surface morphology of the microcapsules

The diameter and surface morphology of PLA microcapsules were observed by a JSM-6700F (JEOL, Japan) scanning electron microscope (SEM). The specimens for SEM observation were prepared by mounting sample on metal stubs with double-sided conductive adhesive tape and coating a thin gold film (approx. 60 nm in thickness) on sample under a reduced pressure below 5 Pa with a JFC-1600 fine coater (JEOL, Japan).

3. Results and discussion

3.1. Stability of primary w_1/o emulsion

The necessary conditions for preparation of $w_1/o/w_2$ double emulsion with narrow size distribution by SPG membrane emulsification technique are that the w_1/o primary emulsion should be stable during the emulsification, and the interfacial tension between the w_1/o primary emulsion and the pores of the membrane should be high. Although adding a high amount of emulsifier in the oil phase could improve the stability of w_1/o primary emulsion, that is, to retard the phase separation between the inner water phase and oil phase, it will decrease the interfacial tension between the w_1/o emulsion and the SPG membrane, and spoil the monodispersity of $w_1/o/w_2$ droplets. Therefore, it is necessary to find a condition where the w_1/o primary emulsion can be maintained at a lower level of emulsifier. DCM is usually used to dissolve PLA because DCM has low boiling point and is relatively easy to remove by evaporation. However, because the density of DCM is higher than that of the inner water phase, the primary emulsion separated into the inner water phase and oil phase within 1 h. At first, various emulsifiers were used to enhance the stability of primary emulsion. 2.5 wt.% of phosphatidylcholine, HCO 10, HCO 20, HCO 40, Arlacel 83 and Span 80 were added in the oil phase, respectively, to test their ability for enhancing the stability of primary emulsion. As shown in Fig. 2, the phase separation was observed within 1 h with all of the emulsifiers except Arlacel 83, with which the stability of primary emulsion was maintained for 3 h. Next , we attempted to reduce the difference of the density between internal water phase and oil phase by adding toluene (its density is 0.866 g/ml) to DCM solvent. The result is shown in Fig. 3 when 2.5 wt% of Arlacel 83 was used as the oil emulsifier.

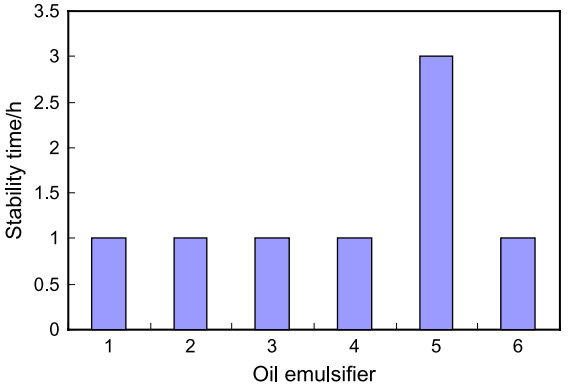

Fig. 2. Effect of the type of oil-soluble emulsifier on the stability of primary emulsion 1: phosphatidylcholine; 2: HCO 10; 3: HCO 20; 4: HCO 40; 5: Arlacel 83; 6: Span 80.

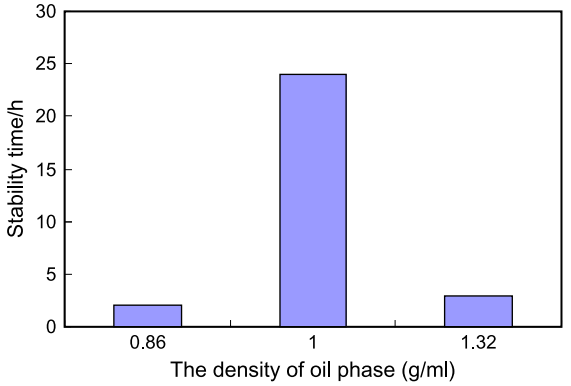

Fig. 3. Effect of the density of oil phase on the stability of primary emulsion.

Apparently, when the difference of the density between internal water phase and oil phase was reduced to nearly zero, the primary emulsion can maintain its stability for more than 24 h. Therefore, a mixture solvent (DCM: toluene=21/79 ml/ml) was accepted in the following SPG membrane emulsification process.

3.2. Microcapsules size and surface morphology prepared by different methods

Figs. 4 and 5 show the SEM photographs and size distribution of lysozyme-loaded microcapsules prepared by SPG membrane emulsification technique and stirring method, where PLA with molecular weight of 300 kDa was used, the concentration of Arlacel 83 was 0.25 wt.% based on oil phase. In this condition, the microcapsules with narrow size distribution can be obtained by SPG membrane emulsification technique, while those prepared by stirring method showed very broad size distribution. The volume-mean diameter and size distribution of the microcapsules (CV value) were 8 μm and 14.7%, respectively, in the former case, and those were 10.2 μm and 75.9% in the latter case. Although the microcapsules prepared by both methods were spherical shape with a smooth surface, those prepared by SPG membrane emulsification technique showed good dispersity, that is, the microcapsules did not adhere together. This is because that $w_1/o/w_2$ emulsion prepared by SPG membrane emulsification technique was very uniform, the surface energy of every droplet was almost the same with each other, and less coalescence and break-up of droplets occurred during the emulsification and solidification processes; therefore, the adhesion between microcapsules can be avoided.

3.3. Effect of oil-soluble emulsifier concentration on drug encapsulation efficiency

There are three main factors which are considered to affect the drug encapsulation efficiency during emulsification and solidification of double emulsion: (1) coalescence between internal water phase and external water phase, leading to leakage of drug into the external water phase; (2) coalescence and break-up of double emulsion droplets also leading to leakage of drug; (3) diffusion of drug from internal to external water phase through oil phase.

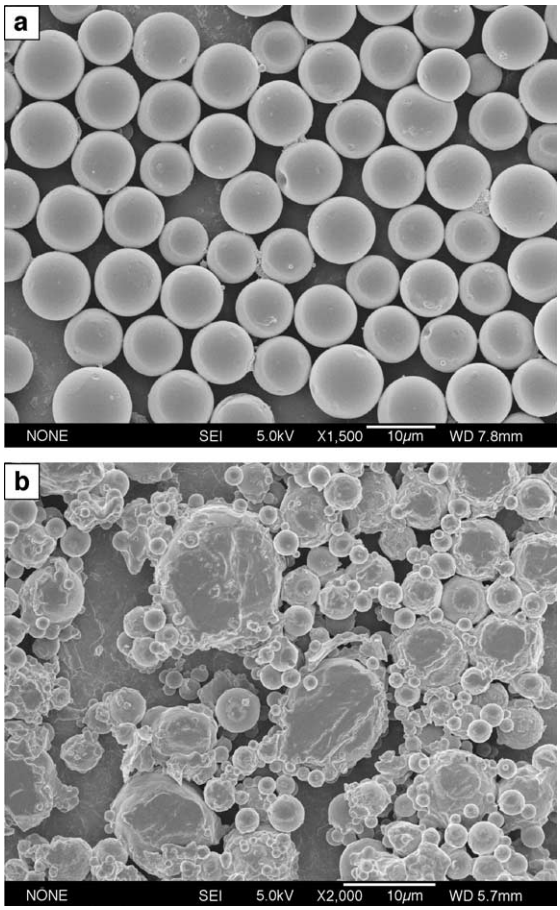

Fig. 4. SEM photographs of PLA microcapsules prepared by SPG membrane emulsification (a) and stirring method (b).

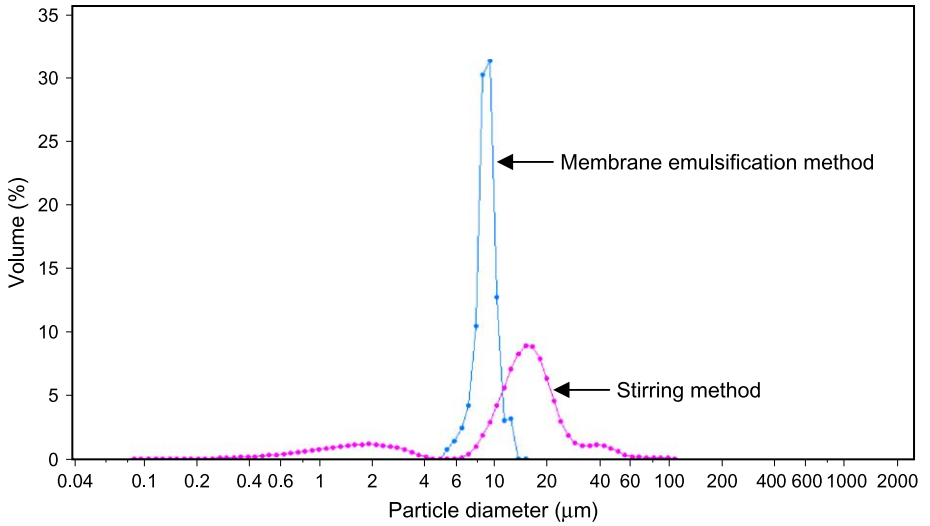

Fig. 5. Size distributions of PLA microcapsules prepared by SPG membrane emulsification and stirring method, respectively (measured by Coulter LA 230).

Oil-soluble emulsifier plays an important role in the formation of primary emulsion and the maintenance of its stability, the coalescence between internal water phase and external water phase can be avoided or retarded [21]. Therefore, the drug encapsulation efficiency usually can be improved by increasing the concentration of oil-soluble emulsifier in the oil phase. The effect of oil-soluble emulsifier concentration on the drug encapsulation efficiency was investigated for the SPG membrane emulsification technique and stirring method, and the results are shown in Fig. 6 where Arlacel 83 and PLA with molecular weight of 300 kDa were used. As shown in Fig. 6, the drug encapsulation efficiency increased gradually with the increase of Arlacel 83 concentration as predicted when the microcapsules were prepared by stirring method. However, the different trend was observed when the microcapsules were prepared by SPG membrane emulsification technique, the drug encapsulation efficiency decreased from 77.83% to 56.24% by increasing the concentration of Arlacel 83 from 2.5 to 7.5 wt.%. This is because that SPG membrane emulsification technique, as a new method to prepare emulsions, has its specificity. The dispersed phase should not wet the membrane pores [22]. In this study, with increasing the concentration of Arlacel 83, the hydrophilic ends of Arlacel 83 molecules had more chance to contact and wet the pore surface of the membrane and thus led to a jet-like steam of the dispersed phase to external water phase, forming polydispersed double emulsion. As a result, the coalescence and break-up between the polydispersed droplets would occur more frequently, and the drug would escape out of the droplets during the emulsification and solidification processes. The SEM photographs showing the effect of Arlacel 83 concentration are shown in Fig. 7, the size distribution became very broad when the concentration of Arlacel 83 increased from 2.5 to 7.5 wt.%. This result

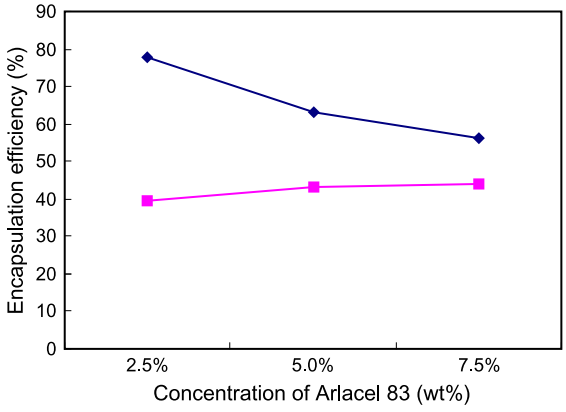

Fig. 6. Effect of the emulsifier concentration in the oil phase on the drug encapsulation efficiency by different preparation methods. –◆–: SPG membrane emulsification; –■–: stirring method.

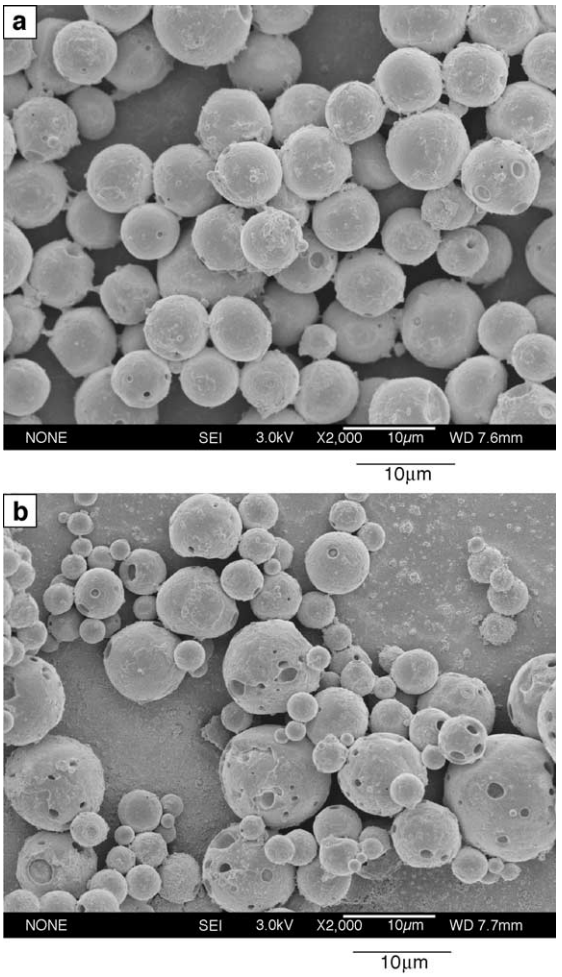

Fig. 7. SEM photographs of PLA microcapsules when different concentration of Arlacel 83 was used as emulsifier. Arlacel 83 concentration (wt.%): (a) 2.5; (b) 7.5.

confirmed the above explanation about the effect of Arlacel 83 concentration on drug encapsulation efficiency. Therefore, it is necessary to limit the oil-soluble emulsifier at a lower level to guarantee the uniform size and high drug encapsulation efficiency of microcapsules. It should be noticed that although the size distribution of microcapsules was broad and drug encapsulation efficiency decreased when higher concentration of Arlacel 83 was used, the drug encapsulation efficiency was still higher than that obtained by the stirring method. This result showed that SPG membrane emulsification process is a mild process without violent stirring during the preparation of double emulsion; less coalescence and break-up of droplets occurred, resulting in less drug being leaked to the external water phase. Therefore, SPG membrane emulsification is a potential method to prepare drug-loaded microcapsules.

3.4. Effect of PLA molecular weight on drug encapsulation efficiency

PLA molecular weight is known to be a key factor to influence the drug encapsulation efficiency. Here, PLAs with different molecular weight of 10 kDa, 20 kDa, 100 kDa and 300 kDa were used to prepare lysozyme-loaded microcapsules by the SPG membrane emulsification technique and stirring method, respectively. The concentration of Arlacel 83 was kept at 2.5 wt.%. Again, as shown in Fig. 8, the drug encapsulation efficiency was always much higher when microcapsules were prepared by SPG membrane emulsification technique than those by the stirring method irrespective of molecular weight of PLA. This result confirmed that less drug escaped out of the double emulsion droplets during the SPG membrane emulsification and solidification processes, because of the uniform size and mild emulsification condition of SPG membrane emulsification technique. For microcapsules prepared by SPG membrane emulsification technique, the drug encapsulation efficiency increased gradually with the increase of PLA molecular weight. When micro-

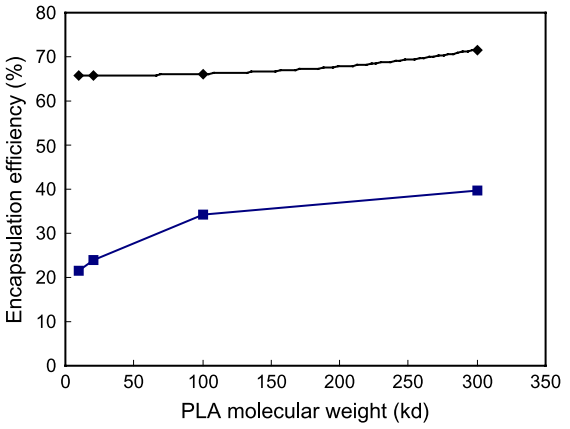

Fig. 8. Effect of PLA molecular weight on the drug encapsulation efficiency by different preparation methods. –◆–: SPG membrane emulsification; –■–: stirring method.

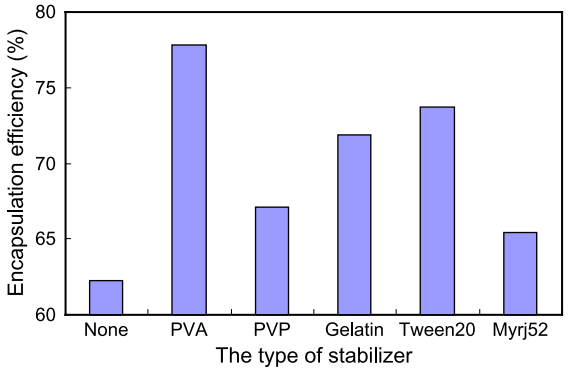

Fig. 9. Effect of the additive type in the internal water phase on the drug encapsulation efficiency by SPG membrane emulsification.

capsules were prepared with PLA of 300 kDa, the highest encapsulation efficiency (71.63%) was obtained. For the microcapsules prepared by stirring method, the drug encapsulation efficiency increased more apparently as the PLA molecular weight increased. When higher molecular weight of PLA was used, more vicious primary emulsion was yielded; thus the primary emulsion stability was enhanced which was considered to be an important factor to improve the drug encapsulation efficiency as mentioned above. Furthermore, the double emulsion was not easy to be broken in the case of stirring method, and the drug was more difficult to diffuse into external water phase through the oil phase when the viscosity of oil phase increased, resulting in lower possibility of the leakage of drug. In the case of SPG membrane emulsification technique, because less coalescence and break-up of the double emulsion occurred even when the PLA molecular weight was lower, as a result, the effect of PLA molecular weight on drug encapsulation efficiency was not so apparent.

3.5. Effect of additive in internal water phase on drug encapsulation efficiency

The type and concentration of the additive in the internal water phase also affect the drug encapsulation efficiency, because it will interact with the drug and emulsifier in the interface. Here, different additives, PVA, PVP, gelatin, Tween 20 and Myrj 52, were added to the internal water phase separately to improve the drug encapsulation efficiency. Fig. 9 shows the effect of additive in the internal water phase on the drug encapsulation efficiency for SPG membrane emulsification technique, where PLA with molecular weigh of 300 kDa was used and Arlacel 83 concentration was kept at 2.5 wt.%. Comparing with the case where no additive was used, all of the additives used showed positive effect on the improvement of drug encapsulation efficiency. At the same concentration (1.0 wt.%), PVA increased the drug encapsulation efficiency more apparently, from 62.22% to 77.83%. There were three reasons responsible for the apparent effect of PVA: (1) PVA interacted with the oil-soluble emulsifier to enhance the intensity of the interfacial membrane formed between the oil phase and the internal water phase, which can reduce the possibility of coalescence between internal and external water phase [23]; (2) PVA might interact with the drug encapsulated to prevent the drug from diffusing into the external water phase; (3) PVA aqueous solution was quite viscous which retard the diffusion of the drug into the external water phase through the oil phase. After confirming that PVA can efficiently enhance the drug encapsulation efficiency, the effect of PVA concentration on drug encapsulation efficiency was investigated; the result is shown in Fig. 10. As shown in Fig. 10, the drug encapsulation efficiency increased with the increase of the PVA concentration. However, when the PVA concentration exceeded 1.0 wt.%, the effect of PVA concentration became less

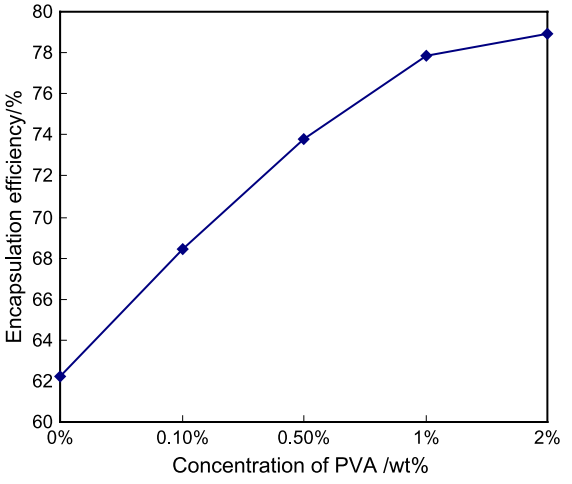

Fig. 10. Effect of the PVA concentration in the internal water phase on the drug encapsulation efficiency by SPG membrane emulsification.

apparent, probably because all of the drug and oil-soluble emulsifier have already interacted with PVA.

3.6. Effect of NaCl concentration in external water phase on drug encapsulation efficiency

It has been known that salt concentration in the external water phase affected the drug encapsulation efficiency apparently when the microcapsules were prepared by stirring method [24], because of the difference of osmotic pressure between internal and external water phase. Here, the effect of NaCl concentration in the external water phase on the drug encapsulation efficiency was also investigated for SPG membrane emulsification technique. The results are shown in Fig. 11 and compared with those by stirring method, where PLA with molecular weight of 300 kDa was used and Arlacel 83 concentration was 2.5 wt.%. From Fig. 11, it was evident that the drug encapsulation efficiency was also affected by NaCl concentration in the case of SPG membrane emulsification technique; it increased with the increase of NaCl concentration, which was similar with the results obtained by stirring method. When NaCl concentration exceeded 1.0 wt.%, its influence on drug encapsulation efficiency became less apparent; this is because that the osmotic pressure between the internal and the external water phase became close at 1.0 wt.% of NaCl. To elucidate the osmotic pressure

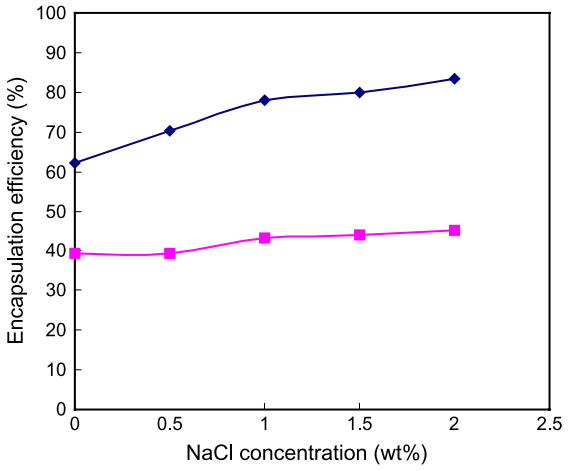

Fig. 11. Effect of NaCl concentration in the external water phase on the drug encapsulation efficiency by different preparation methods. –◆–: SPG membrane emulsification; –■–: stirring method.

effect, Kun Han et al. [25] used 9-aminoacridine as a influx tracer dissolved in the external water phase, and the result indicated that the different amount of phosphate in the external water phase induced different osmotic pressure between the internal water phase and the external water phase. When the osmotic pressure of the internal water phase was higher than that of the external water phase, due to the influx of water from the external to internal water phase, this will lead to the thinner oil film and eventually yield larger microcapsules. On the contrary, when the osmotic pressure of the external water phase was higher, due to the influx of water from the internal to external water phase, this will lead to the thicker oil film and eventually yield smaller microcapsules which was considered to be favorable for the enhancement of the drug encapsulation efficiency. Fig. 12 shows the SEM photographs of microcapsules prepared by SPG membrane emulsification technique when 2.0 wt.% or no NaCl was added into the external water phase. It was found that the size of microcapsules was much smaller when the concentration of NaCl in the external water phase was 2.0 wt.%, which correspond to the explanation as mentioned above. Therefore, it is necessary to adjust osmotic pressure of the external water phase according to the amount of drug in the internal water phase, to prepare microcapsules with high drug encapsulation efficiency by SPG membrane emulsification technique.

From Fig. 12, it was also confirmed that the drug encapsulation efficiency was much higher when the microcapsules were prepared by SPG membrane emulsification technique than those by stirring method at each NaCl concentration.

3.7. Effect of pH in external water phase on drug encapsulation efficiency

It has been known that pH was also an important factor in the drug encapsulation process [2]. Therefore, the effect of pH on drug encapsulation efficiency was also investigated in this study for the SPG membrane emulsification technique. The pH values of external water phase was adjusted by 0.1 N HCl and/or 0.1 N NaOH at 3.0, 6.0, 9.0 and 11.0, respectively. The PLA with molecular weight of 300 kDa was used, Arlacel 83 concentration was 2.5 wt.%, and 1.0 wt.% PVA and 1.0 wt.% NaCl were added in

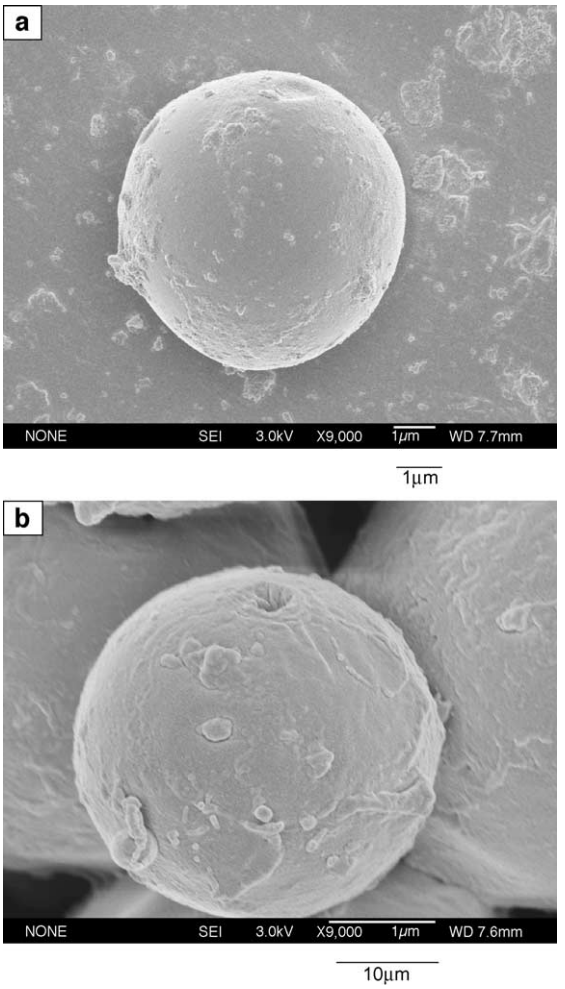

Fig. 12. SEM photographs of PLA microcapsules prepared by SPG membrane emulsification when 2.0 wt.% or no NaCl was added in the external water phase. NaCl concentration (wt.%): (a) 0; (b) 2.0.

the internal and external water phase, respectively. As shown in Fig. 13, when the pH value was 11.0 which is equal to the isoelectric point of lysozyme, the highest drug encapsulation efficiency was obtained for both cases of SPG membrane emulsification technique and stirring method. When pH value of the external water phase was close to the isoelectric point of the drug entrapped, the solubility of the drug in the external water phase was lowered; therefore, the drug diffusion into the external water phase was prevented. The highest drug encapsulation efficiency of 92.20% was obtained at pH 11.0 when the microcapsules were prepared by SPG membrane emulsification technique.

From all the above results, it can be concluded that the uniform-sized PLA microcapsules with high drug encapsulation efficiency can be prepared when the density of oil phase was adjusted close to the internal water phase and adequate oil-soluble emulsifier was used. The effects of PLA molecular weight, NaCl concentration and pH value in external water phase on drug encapsulation efficiency were similar with the case of the stirring method. The drug encapsulation efficiency was much higher when the microcapsules were prepared by SPG membrane emulsification technique than those by conventional stirring method. SPG membrane emulsification is a potential technique to prepare microcapsules containing protein and peptide drugs because it is a mild emulsification process, and it can provide uniform-sized microcapsules with high drug encapsulation efficiency. One concern in this study is that the residual amount of toluene after solidification because its residual amount should be limited at a low value in the drug delivery system. Although toluene was not detected by gas chromatography after evaporation of 24 h in this study, other solidification methods with short time should be studied in order to save the cost and maintain the bioactivity of protein drugs.

Controlled release is a desirable characteristic for drug delivery system. In a next study, we will investigate the controlled release behavior of protein drugs from these uniform-sized microcapsules. There are many factors affecting the controlled release

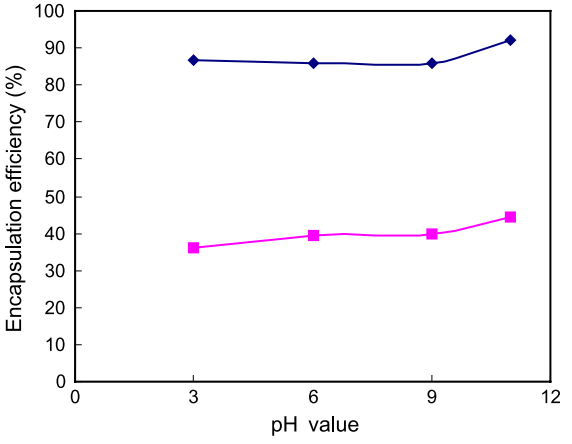

Fig.13. Effect of pH value in the external water phase on the drug encapsulation efficiency by different preparation methods. –◆–: SPG membrane emulsification; –■–: stirring method.

behavior of drugs encapsulated, including the size of the microcapsules, the degradation rate of polymer used, the additive type and concentration in the internal water phase, the porosity of the microcapsules and the amount of loaded drugs, etc. Comparing to the conventional mechanical stirring, homogenization or ultrasonication method, microcapsules prepared by SPG membrane emulsification technique are more uniform, and the results are more reproducible. Therefore, it is expected that a relationship between the release behavior of protein drugs and the size of microcapsules can be obtained steadily. By mixing microcapsules of different sizes, it is easy to obtain desirable controlled release behavior. Furthermore, in the process of SPG membrane emulsification, some procedure parameters on the release behavior of drugs, such as the removal rate of organic solvent, which is an important factor affecting the inner structure of microcapsules, also will be investigated.

4. Conclusion

Uniform-sized biodegradable poly(lactide) (PLA) microcapsules loading lysozyme were successfully prepared by combining an SPG membrane emulsification technique and a multiple emulsion-evaporation method, where PLA and Arlacel 83 were dissolved in a mixture organic solvent of DCM and toluene used as an oil phase. The size distribution of the microcapsules prepared by SPG membrane emulsification technique were more uniform than those prepared by stirring method. Various factors influencing the drug encapsulation efficiency were investigated. The results indicated that the drug encapsulation efficiency of microcapsules prepared by SPG membrane emulsification technique was much higher than those prepared by stirring method, and the drug encapsulation efficiency was affected by PLA molecular weight, additive type and amount in the internal water phase, emulsifier type and amount in the oil phase, and NaCl concentration and pH value in the external water phase. The highest drug encapsulation efficiency of 92.20% was obtained by optimizing the above factors. SPG membrane emulsification technique is a potential method for preparing uniform-sized microcapsules containing protein or peptide drugs although some difficulties should be solved in the near future.

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