Available online at www.sciencedirect.com
SCIENCE DIRECT
Colloids and Surfaces B: Biointerfaces 45 (2005) 144–153
COLLOIDS AND SURFACES B
www.elsevier.com/locate/colsurfb

Influence of process parameters on the size distribution of PLA microcapsules prepared by combining membrane emulsification technique and double emulsion-solvent evaporation method

Rong Liu, Guang-Hui Ma*, Yin-Hua Wan, Zhi-Guo Su

State Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, P.O. Box 353, Beijing 100080, China

Received 20 June 2005; received in revised form 21 July 2005; accepted 10 August 2005

Abstract

Relatively uniform-sized biodegradable poly(lactide) (PLA) microcapsules with various sizes were successfully prepared by combining a glass membrane emulsification technique and water-in-oil-in-water ($w_1/o/w_2$) double emulsion-solvent evaporation method. A water phase was used as the internal water phase, a mixture solvent of dichloromethane (DCM) and toluene dissolving PLA and Arlacel 83 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 of a glass membrane into the external water phase by the pressure of nitrogen gas to form the uniform $w_1/o/w_2$ double emulsion droplets. Then, the solid polymer microcapsules were obtained by simply evaporating solvent. The influence of process parameters on the size distribution of PLA microcapsules was investigated, with an emphasis on the effect of oil-soluble emulsifier. A unique phenomenon was found that a large part of emulsifier could adsorb on the interface of internal water phase and oil phase, which suppressed its adsorption on the surface of glass membrane, and led to the successful preparation of uniform-sized double emulsion. Finally, by optimizing the process parameters, PLA microcapsules with various sizes having coefficient of variation (CV) value under 14.0% were obtained. Recombinant human insulin (rhI), as a model protein, was encapsulated into the microcapsules with difference sizes, and its encapsulation efficiency and cumulative release were investigated. The result suggested that the release behavior could be simply adjusted just by changing precisely the diameters of microcapsule, benefited from the membrane emulsification technique.

Keywords: Membrane emulsification; Double emulsion-solvent evaporation; Poly(lactide); Size distribution

1. Introduction

Biodegradable poly(lactide) (PLA) microcapsules have received more and more attention for the sustained release of bioactive macromolecules such as polypeptide and protein drugs in recent years. Until now, the most commonly used method to prepare PLA microcapsules as drug delivery system is the water-in-oil-in-water ($w_1/o/w_2$) double emulsion process, also called the in-water drying method, which was patented by Vrancken and Claeys [1] in USA in 1970 and by Dejaeger and Tavernier in UK in 1971 [2]. Further, Kitajima and Kondo showed that this process could be used to immobilize highly labile molecules such as enzymes [3]. Ogawa et al. [4] first used the double emulsion-solvent evaporation method to prepare an injectable poly(lactide-co-glycolide) microparticle for the sustained release of leuprolide acetate. In this process, an active water-soluble drug is first dissolved in an aqueous solution, which is then emulsified in an organic solvent containing biodegradable polymer to make a primary w_1/o emulsion. Then, this primary emulsion is added to an emulsifier-containing aqueous solution to form a $w_1/o/w_2$ double emulsion. After removing the organic solvent, the solid microcapsules are left in the aqueous continuous phase and can be washed, centrifuged and lyophilized.

However, in the conventional process, the double emulsion is usually prepared by the mechanical stirring, homogenization or ultrasonication method, the size distribution of microcapsules obtained is very broad, which is unfavorable to efficient absorption in vivo. Le Fevre et al. [5] evaluated the effect of particle size on the uptake of polystyrene microparticles in mice when microsphere was administrated orally. They showed that the particles

* Corresponding author. Tel.: +86 1082627072; fax: +86 1082627072.
E-mail address: ghma@home.ipe.ac.cn (G.-H. Ma).

0927-7765/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.colsurfb.2005.08.004

were taken up into the Peyer's patches more easily when the particle size was below 5 μm. Therefore, if microcapsules containing drugs are uniform, and their sizes are controlled under a limited value, the bioavailability of the protein drugs would be enhanced largely. Moreover, the microcapsules with a narrow size distribution are necessary in the drug delivery system in order to decrease 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 [6]. 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 the several kinds of microcapsules with the designed diameters according to the desired controlled release rate. Therefore, developing a method that can provide the uniform-sized microcapsules composed of biodegradable polymers is very important.

The glass membrane emulsification technique is a promising technique. It was firstly proposed by Nakashima et al. [7] to prepare uniform o/w emulsion, and later developed by Omi and Ma et al. [8–12] to prepare uniform microspheres by polymerizing uniform monomer droplets. It is a big challenge to prepare uniform-sized PLA microcapsules by combining the membrane emulsification technique and $w_1/o/w_2$ emulsion method. However, comparing with single emulsion such as o/w or w/o emulsion, it is more difficult to prepare uniform $w_1/o/w_2$ double emulsion because of following reasons: (1) the double emulsion system is more complex, there exists more factors influencing the size distribution of the double emulsion obtained. Although the influence of some process parameters on size distribution of particle for o/w or w/o emulsion had been investigated, those for $w_1/o/w_2$ emulsion, especially for PLA microcapsules had never been studied; (2) the double emulsion was prepared by pressing w_1/o primary emulsion through the pores of the membrane into the external phase (w_2), the primary emulsion should be stable during the emulsification process, which had never been encountered in the preparation of o/w or w/o emulsion. Although adding oil-soluble emulsifier can increase stability of primary w_1/o emulsion, it was concerned that it would wet the membrane, leading to polydispersed droplets. So, the optimum adding amount should be defined.

In a previous study, we found that the difficulty of preparing PLA microcapsule by membrane emulsification technique was the unstability of primary w_1/o emulsion during the emulsification process of $w_1/o/w_2$ double emulsion [13]. By using Arlacel 83 as the oil-soluble emulsifier, and dichloromethane (DCM)/toluene as mixture solvent to adjust the density of the oil phase to the same as the water phase, the relatively stable w_1/o emulsion, and relatively uniform $w_1/o/w_2$ double emulsion and microcapsules with higher encapsulation efficiency of drug were obtained, comparing with conventional stirring method. Poly(lactide) was used as the polymer material because PLA is a non-toxic, biocompatible and biodegradable polymer approved by the Food and Drug Administration for human use [14]. Toluene was removed completely under vacuum after washing, and no toluene was detected by gas chromatography. However, the microcapsules are not uniform enough. Therefore, in this study, we investigated the influence of the process parameters on the size distribution of the $w_1/o/w_2$ double emulsion and tried to prepare uniform-sized PLA microcapsules by combining the membrane emulsification technique and double emulsion-solvent evaporation method, with an emphasis on the effect of oil-soluble emulsifier because it had not been encountered in o/w or w/o single emulsion. In the study of effect of oil-emulsifier, a unique phenomenon was found. The membrane was not wetted as expected because a large part of emulsifier adsorbed on the interface of internal water phase and oil phase, which suppressed its adsorption on the surface of glass membrane. This important phenomenon allowed membrane emulsification technique successful for preparation of double emulsion.

We also encapsulated the recombinant human insulin (rhI), a model protein drug, by this special technique, and investigated the effect of microcapsule size on release behavior, taking the advantage that the microcapsule size can be controlled precisely by pore size of the membrane.

2. Materials and methods

2.1. Materials

Poly(D,L-lactide) (PLA) (the average molecular weight, M_w, 300 kDa) was purchased from the Institute of Medical instrument (SanDong, China). Dichloromethane and toluene were purchased from Beijing Chemical Reagents Company (Beijing, China). Recombinant human insulin was provided by G.L. Biotechnical Company (Beijing, China). Sorbitan Sesquioleate (Arlacel 83) was purchased from Sigma (St. Louis, USA), Polyoxyethylene 40 hydrogenated castor oil (HCO40) was provided by Nikkol (Tokyo, Japan), Span 85 was purchased from Beijing Chemical Reagents Company, and they were used as the emulsifier in the oil phase, respectively. Poly(vinyl alcohol) (PVA-217, degree of polymerization 1700, degree of hydrolysis 88.5%) was provided by Kuraray (Tokyo, Japan) and was used as a stabilizer in the external water phase. 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 the glass membrane) installed was purchased from Ise Chemical Company. A schematic diagram of this kit and the detailed emulsification process were given in a previous paper [13]. Membranes with pore sizes of 1.4, 2.8, 5.2 and 10.2 μm were used, respectively, in this study.

2.3. Preparation of blank and drug-loaded PLA microcapsules

The blank PLA microcapsules were prepared by combining a glass membrane emulsification technique and double emulsion-solvent evaporation method as described previously [13]. A standard recipe is shown in Table 1. Briefly, 0.25 g water was used as the internal water phase (w_1), and 0.125 g PLA and

Table 1
Standard recipe in the preparation of microcapsules

Phase/ingredient	Amount (g)
Internal water phase (w_1)	
Water (or adding rhI)	0.25 (or adding 2.5 mg rhI)
Oil phase (o)	
Mixed solvent (DCM + toluene)	2.5 (DCM/toluene: 21/79, v/v)
Oil-soluble emulsifier (Arlacel 83)	0.0025–0.0200 (max. 2.0 wt.% of mixed solvent)
PLA (300 kDa)	0.125
External water phase (w_2)	
Water	100
PVA	0.1–2.0 (max. 2.0 wt.% of water)

Arlacel 83 were dissolved in a 2.5 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 50 s to form a w_1/o primary emulsion. The aqueous phase where PVA were dissolved was used as the external water phase (w_2). The primary emulsion was permeated through the uniform pores of the glass membrane into the external water phase by the pressure of nitrogen gas to form the uniform-sized droplets. 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. Then, DCM and toluene were evaporated at room temperature for 24 h under a gentle stirring at a rate of 150 rpm. 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. The procedure for preparing drug-loaded PLA microcapsules was similar with the process mentioned above except that 2.5 mg was dissolved into 250 μl 1.0 wt.% acetic acid (pH 2.9) as the internal water phase. 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 prepared by stirring method at a rate of 1000 rpm for 40 s.

2.4. Measurement of size distribution of the microcapsules

The dried PLA microcapsules were redispersed in distilled water and observed with an optical microscope. Two hundred particles were picked up randomly from OM photographs and their diameters were measured to calculate the average diameter and size distribution. The particle size distribution was expressed by a coefficient of variation (CV) value, which is defined as

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

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

2.5. SEM observation

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 platinum film (approximately 60 nm in thickness) on sample under a reduced pressure below 5 Pa with a JFC-1600 fine coater (JEOL, Japan).

2.6. Measurement of the drug encapsulation efficiency

Total rhI loaded was measured using the Peterson–Lowry method [20] after disruption of the microcapsules with 2.0% SDS/0.1 M NaOH solution. Sodium hydroxide catalyzes the hydrolysis of PLA/PLGA, and SDS ensures the complete solubilization of the human insulin during polymer hydrolysis. The resulting solution was then neutralized by stepwise addition of 1 M HCl. The encapsulation efficiency was calculated from the following formula:

$$\text{Encapsulation efficiency (\%)} = \frac{\text{total amount of human insulin loaded}}{\text{total amount of human insulin}} \times 100$$

2.7. In vitro protein release studies

Twenty milligrams of freeze-dried microcapsules, accurately weighted, were placed in a test tube and incubated in 1.5 ml of PBS buffer, pH 7.4 (8 g NaCl, 0.2 g KCl, 0.24 g KH_2PO_4, 1.81 g $Na_2HPO_4{\cdot}H_2O$, 0.5 g NaN_3, 0.1 g Tween 20 and 1000 ml distilled water). The samples were agitated in a 37 °C incubator-shaker at 120 rpm. At defined time intervals, 1.0 ml of supernatant was collected by centrifugation at 8000 rpm for 5 min and 1.0 ml of fresh PBS buffer was added back. The amount of human insulin released was determined by measuring protein concentration in the supernatant using the Peterson–Lowry method mentioned above. Each sample was assayed in duplicate.

3. Results and discussion

3.1. Effect of oil-soluble emulsifier on the size distribution of microcapsules

The necessary conditions for preparing w_1/o/w_2 double emulsion with narrow size distribution by membrane emulsification technique are that the w_1/o primary emulsion should be stable during the emulsification process, 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 primary emulsion and the glass membrane, and spoil the monodispersity of w_1/o/w_2 double emulsion droplets. In a previous study [13], the stable w_1/o primary emulsion was obtained successfully by adjusting the density of the organic solvent and choosing Arlacel 83 as the oil-soluble emulsifier. However, the microcapsules obtained are still not uniform enough though they are much more uniform than those prepared by stirring method. The effect of various

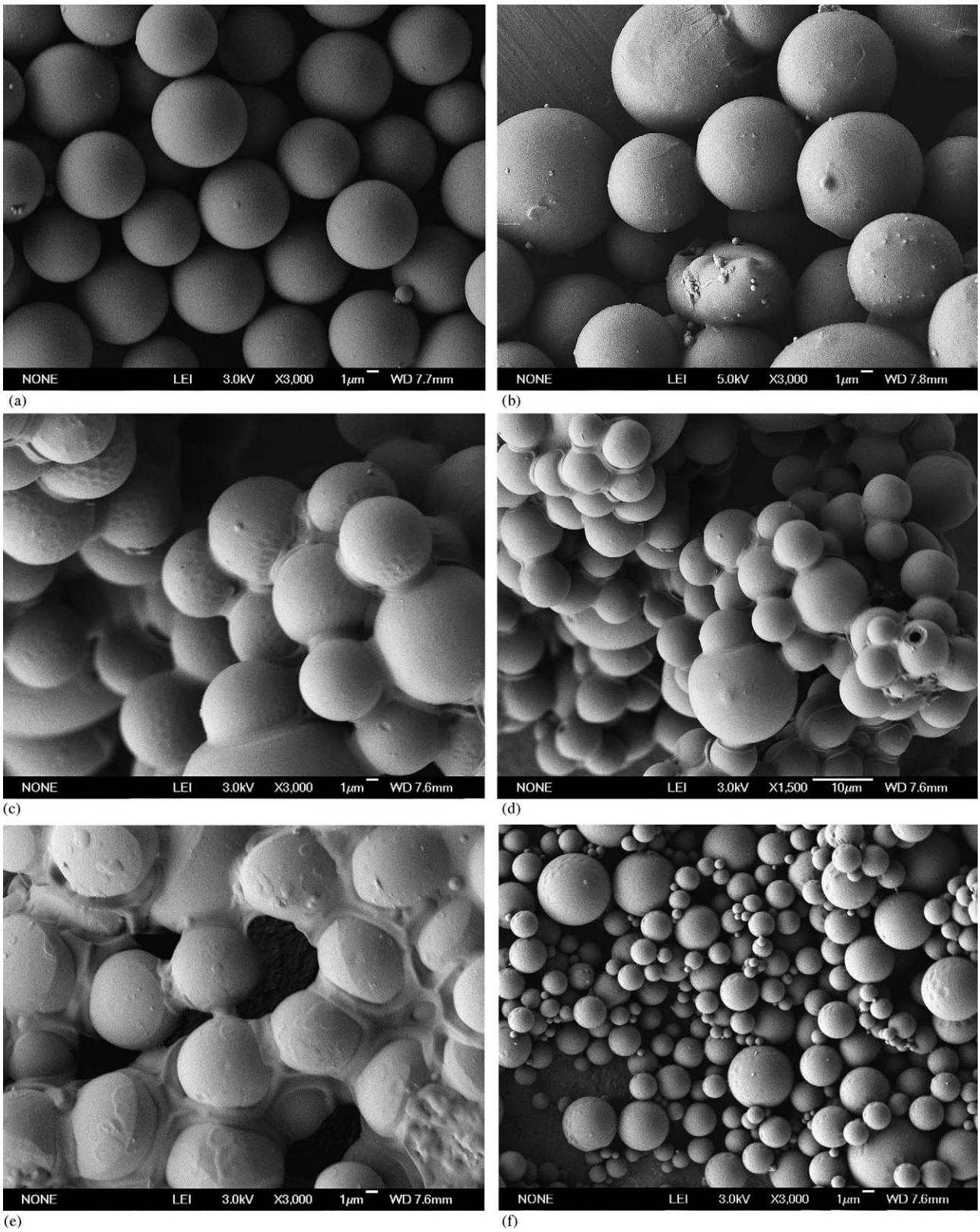

Fig. 1. SEM photographs of PLA microspheres prepared by combining SPG membrane emulsification and o/w single emulsion evaporation method, the emulsifier in the oil phase was: (a) no emulsifier; (b) 0.25 wt.% Arlacel 83; (c) 1.0 wt.% Arlacel 83; (d) 2.0 wt.% Arlacel 83; (e) 1.0 wt.% Span 85; (f) 1.0 wt.% HCO 40.

parameters on size and size distribution of PLA microcapsules should be optimized further. Here, the effect of oil-soluble emulsifier concentration was studied firstly. In order to eliminate the effect of internal water phase, a simple system (o/w_2) was prepared by membrane emulsification process instead of $w_1/o/w_2$ system at the first, that is, 1.0 wt.% oil-soluble emulsifier such as Span 85, Arlacel 83, HCO 40, were added in the oil phase, respectively, then the oil phase was permeated into the external water phase to obtain o/w emulsion with oil-soluble emulsifier in oil phase. As shown in Fig. 1a, when no oil-soluble emulsifier was added in the oil phase, the CV value of microspheres obtained was 10.07%, which was much more uniform than those prepared when oil-soluble emulsifier was added in the oil phase, irrespective of emulsifier type. Comparing with other oil-soluble emulsifier, Arlacel 83 seemed to have less effect on the size distribution of microspheres, the CV values were 31.18, 35.87 and 50.49% when 1.0 wt.% of Arlacel 83, Span 85 or HCO 40 was used, respectively (Fig. 1c, e and f). The effect of the Arlacel 83 concentration was also investigated and the results are shown in Fig. 1b–d. The CV value increased from 28.67 to 50.49% with the increase of Arlacel 83 from 0.25 to 2.0 wt.%. The results suggested that a part of oil-soluble emulsifier was adsorbed on and wetted the pore surface of the membrane because it is an amphiphilic molecule, leading to a polydispersed droplets. In order to obtain uniform-sized emulsion, Arlacel 83 is more suitable than other emulsifiers, and its concentration should be kept as low as possible. The reason why Arlacel 83 was the most suitable emulsifier was still unclear, which was probably related to both of the structure and property of the emulsifier. The HLB values for Span 85, Arlacel 83 and HCO 40 were 1.8, 3.7 and 14.0, respectively. The results confirmed that emulsifiers with too low (1.8 for Span 85) or too high (14.0 for HCO 40) were unfavorable for the preparation of uniform-sized emulsion.

Next, the effect of the oil-soluble emulsifier concentration on the size distribution of microcapsules was investigated for the preparation of $w_1/o/w_2$ emulsion. The results are shown in Fig. 2, the microcapsules prepared were all spherical shape with a smooth surface. With increasing the amount of Arlacel 83, from

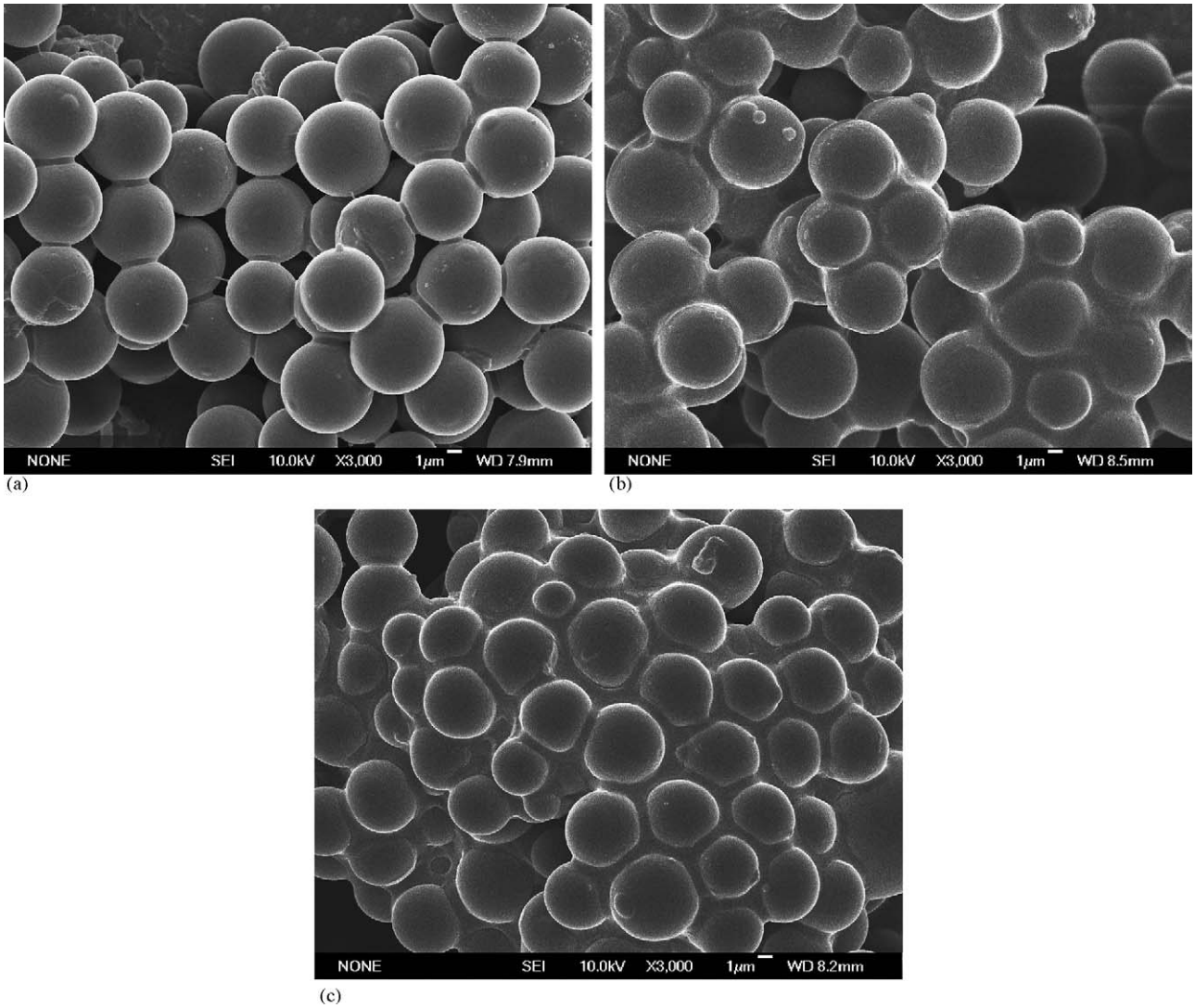

Fig. 2. SEM photographs of PLA microcapsules prepared by combining SPG membrane emulsification technique and $w_1/o/w_2$ double emulsion evaporation method. The concentration of Arlacel 83 in the oil phase: (a) 0.25 wt.%; (b) 1.0 wt.%; (c) 2.0 wt.%.

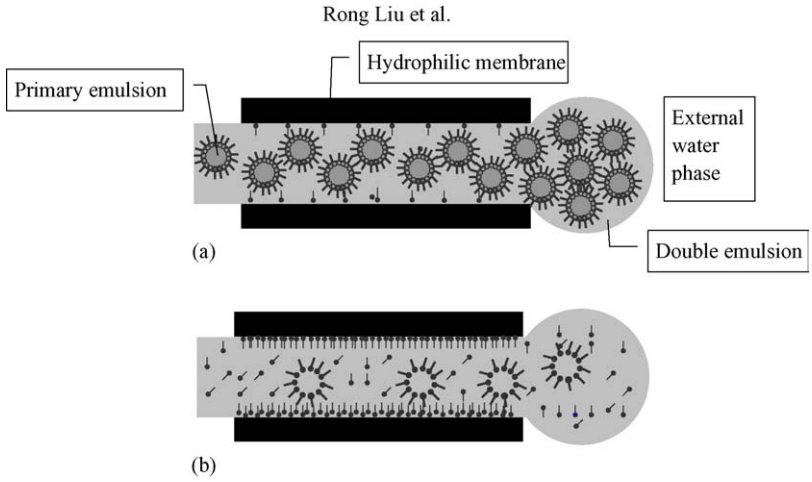

Fig. 3. Schematic diagram of membrane emulsification process. (a) The process of w_1/o primary emulsion permeation through the membrane into the external water phase; (b) the process of oil phase containing Arlacel 83 permeation through the membrane into the external water phase.

0.25 to 2.0 wt.%, the size distribution of microcapsules became broader, and the CV value increased from 13.90 to 23.41%. This result confirmed that lower concentration of oil-soluble emulsifier was favorable for the formation of more uniform-sized microcapsules prepared by membrane emulsification. However, a unique phenomenon was observed by comparing the cases of o/w_2 and $w_1/o/w_2$ emulsions. At the same concentration of Arlacel 83, the CV value of the microspheres was higher in o/w_2 system than in $w_1/o/w_2$ system. For example, the CV values were 13.90 and 28.67% for these two cases when 0.25 wt.% of Arlacel 83 was added. This phenomenon can be explained schematically as shown in Fig. 3. In o/w_2 system, a large part of oil-soluble emulsifier was adsorbed on and wetted the pore surface of the membrane (Fig. 3b), while in $w_1/o/w_2$ system, much smaller amount of oil-soluble emulsifier was adsorbed on the membrane because most of the oil emulsifier were used to keep the w_1/o primary emulsion stable (Fig. 3a). This unique phenomenon allowed the preparation of uniform-sized double emulsion successful.

3.2. *Effect of the PVA concentration in the external water phase*

The presence of a stabilizer in the external water phase is critical for the successful formation of individual spherical microspheres during the emulsification process [15]. The role of the stabilizer is to prevent the coagulation of microspheres during emulsification and solvent removal process. PVA is the most commonly used stabilizer mainly due to its low toxicity, good solubility in water and its availability in a range of molecular weights [16]. Here, PVA was chosen as the stabilizer in the external water phase and the effect of its concentration on the size distribution of microcapsules was investigated. As shown in Fig. 4, when PVA concentration was 0.1 wt.%, the CV value of microcapsules was rather high (39.50%), but with the increase of PVA concentration, the CV value of microcapsules decreased gradually from 39.20 to 10.72%. After PVA concentration exceeded 1.0 wt.%, the size distribution of microcapsules obtained was rather narrow, and the CV values were all below 14.0% and the decreasing trend became less apparent. For 1.0, 1.5 and 2.0 wt.% PVA concentrations, the CV values were 13.90, 11.59 and 10.72%, respectively. It was evident that lower PVA concentration such as 0.1 or 0.5 wt.% was not enough to prevent the coagulation of the emulsion droplets formed, which eventually led to the polydispersity of microcapsules.

From Fig. 5, it was found that the lower the PVA concentration was, the larger the mean diameters of microcapsules became, which confirmed that the polydispersity of microcapsules was caused mainly by the coalescence of emulsion droplets when PVA concentration was below 1.0 wt.%. Although the results mentioned above demonstrated that a higher concentration of PVA was favorable for the formation of more uniform microcapsules, it was better to use PVA as lower as possible because

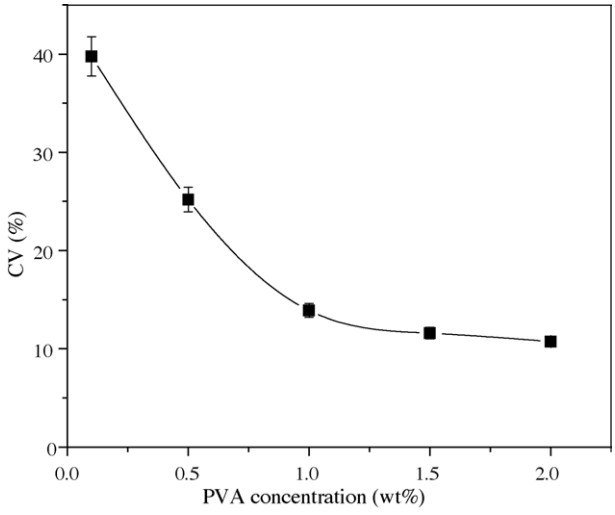

Fig. 4. Effect of PVA concentration in the external water phase on the size distribution of PLA microcapsules prepared by membrane emulsification technique. The data are presented as mean ± S.D. ($n = 2$).

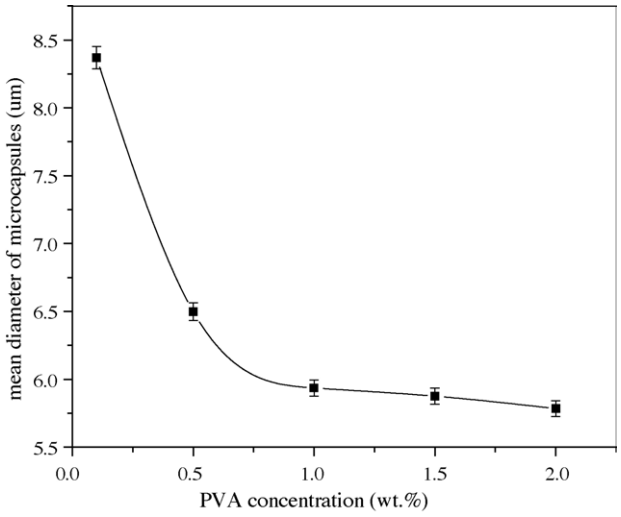

Fig. 5. Effect of PVA concentration in the external water phase on the mean diameters of microcapsules prepared by membrane emulsification technique. The data are presented as mean ± S.D. ($n = 2$).

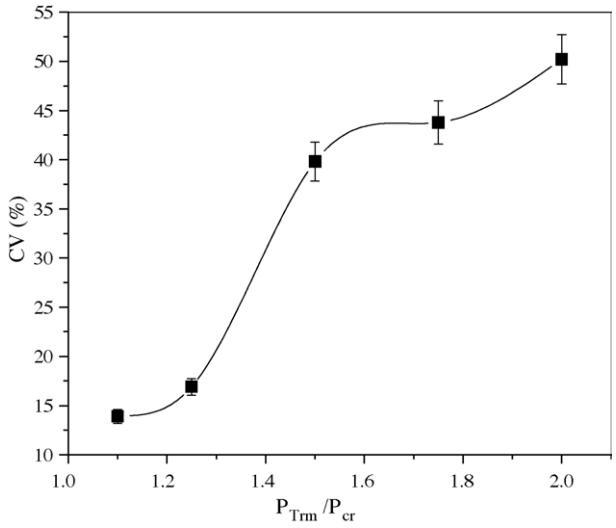

Fig. 6. Effect of P_{tm}/P_{cr} on the size distribution of PLA microcapsules prepared by membrane emulsification technique. The data are presented as mean ± S.D. ($n = 2$).

PVA is easily bound onto the microcapsules and difficult to be washed away, as reported by Singh and O'Hagan [16]. Therefore, by optimizing the conditions for preparing uniform-sized microcapsules, 1.0 wt.% PVA was chosen to be the stabilizer in the external water phase during the microcapsules preparation process.

3.3. Effect of transmembrane pressure on the size distribution of microcapsules

Transmembrane pressure (P_{tm}) plays an important role in the membrane emulsification process. Firstly, the P_{tm} must be kept constant during the whole emulsification process, otherwise the double emulsion droplets will be polydisperse. Secondly, a proper P_{tm} must be chosen, too high P_{tm} leads to jet-like steam of dispersed phase and very large droplets. On the other hand, if the P_{tm} is too low, the emulsification rate will be very slow, which results in low work efficiency. In order to select an adequate P_{tm}, in this study, the w_1/o primary was pressed into the external water phase 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. The PVA concentration in the external water phase was 1.0 wt.%. As shown in Fig. 6, the size distribution of microcapsules prepared was rather narrow (CV value was 13.90%) when P_{tm} was slightly higher than the critical pressure (P_{cr}, $P_{tm}/P_{cr} = 1.1$). However, when the P_{tm}/P_{cr} exceeded 1.25, the CV values increased dramatically from 16.90 to 50.20%. For the transmembrane pressure, there exist different results with different emulsification systems. Williams et al. [17] have suggested that between 2 and 10 times the minimum membrane pressure is probably usable range. The broader size distribution of microcapsules was mainly ascribed to the formation of larger emulsion droplets during the membrane emulsification process, which could be confirmed from Fig. 7, the mean diameter of microcapsules increased apparently with increasing P_{tm}/P_{cr}. The reason why higher P_{tm} led to larger emulsion droplets can be explained as follows. The interfacial tension between oil and external water phase, σ, is recognized to be the major retention force, that is, the force which keeps the droplets attached to the exit of pore [18]. Therefore, greater σ is expected to cause the production of larger emulsion droplets due to the long retention time of the droplets on the membrane surface. In this study, PVA is dissolved in the continuous phase to stabilize the formed emulsion droplets against coalescence. Since PVA has a finite rate of adsorption at the oil–water interface [19], the coverage of the droplets surface with adsorbed PVA molecules decreases when the frequency of droplets released from the membrane pores grows, which leads to the increase of the oil–water interfacial tension (σ), and produces larger emulsion droplets, and eventually leads to the formation of polydispersed microcapsules.

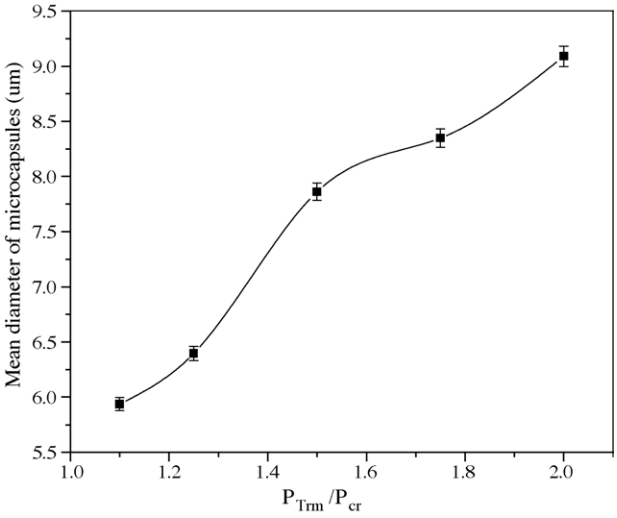

Fig. 7. Effect of P_{tm}/P_{cr} on the mean diameters of microcapsules prepared by membrane emulsification technique. The data are presented as mean ± S.D. ($n = 2$).

3.4. Preparation of uniform-sized microcapsules with different size

After investigating the process parameters influencing the size distribution of the microcapsules prepared by combining a membrane emulsification technique and double emulsion-solvent evaporation method, the optimized conditions were obtained. Next, the PLA microcapsules with different size were prepared in the optimized conditions by using the membrane with different pore size. PVA and Arlacel 83 concentrations in the external water phase and oil phase were fixed at 1.0 and 0.25 wt.%, respectively. The transmembrane pressure was slightly higher than the critical pressure (P_{tm}/P_{cr} was no more than 1.1) and was kept constant during the emulsification process. As shown in Fig. 8, the PLA microcapsules were rather uniform, and their CV values were all under 14.0%. The relationship between the pore size of membrane and the particle size of the microcapsules is shown in Fig. 9. A linear relationship was observed, similar to the result in the o/w_2 single emulsion system [20], That is, the particle size of the PLA microcapsules mainly

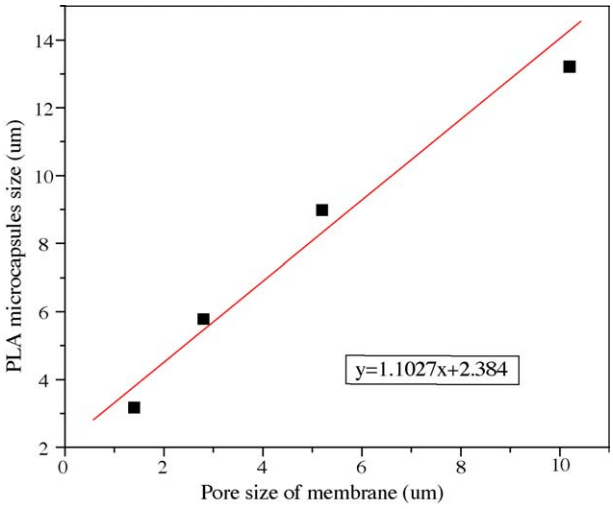

Fig. 9. Relationship between the mean size of PLA microcapsules and the pore size of membrane used.

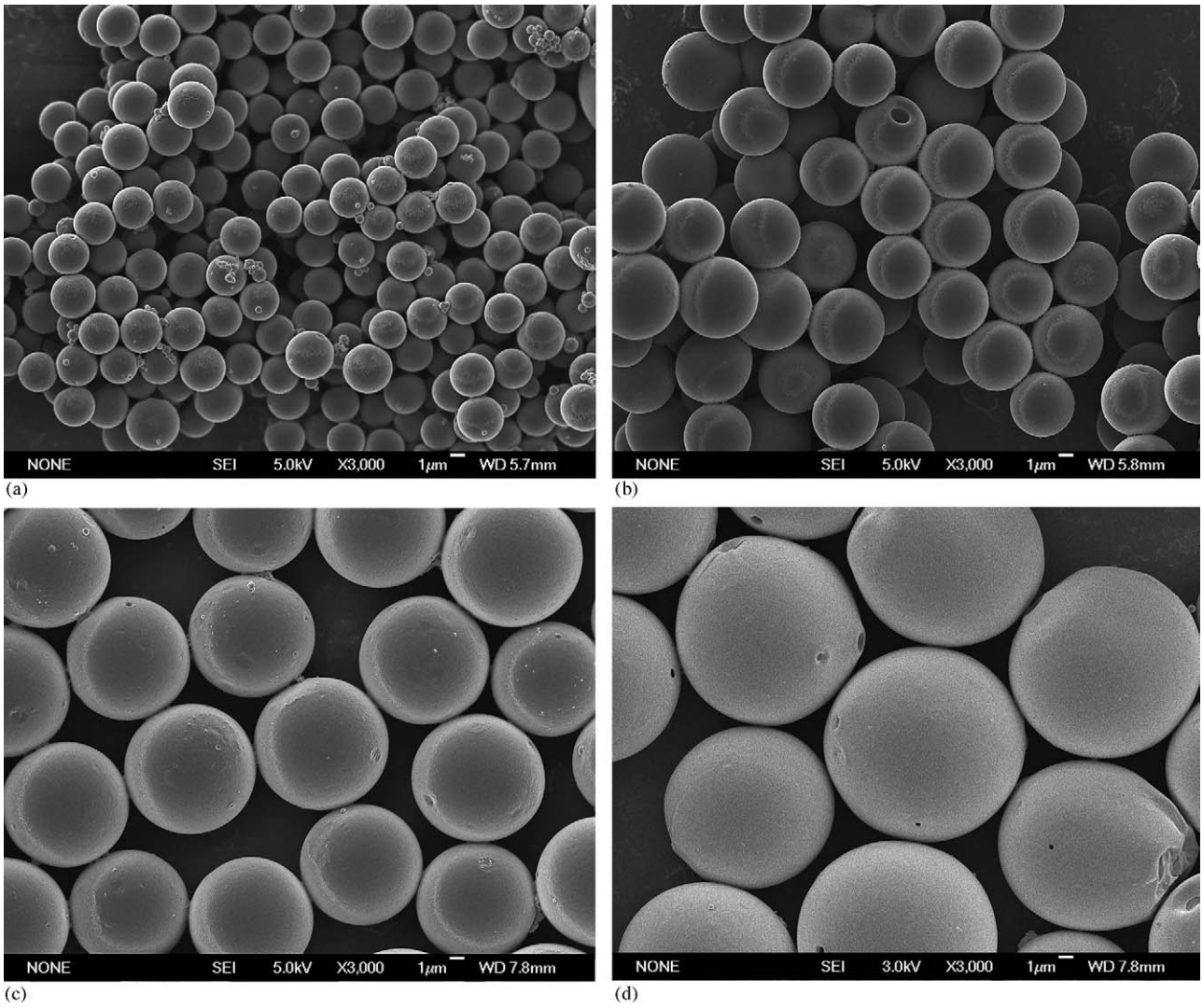

Fig. 8. SEM photographs of PLA microcapsules prepared by membrane with different pore sizes: (a) 1.4 μm; (b) 2.8 μm; (c) 5.2 μm; (d) 10.2 μm.

depended on the pore size of the membrane; PLA microcapsules with desired sizes could be prepared easily by choosing a membrane with proper pore size. It is important to control the diameter of PLA microcapsule for the application of drug delivery system, because the bioavailability and release rate of the drug depend on the diameter of the Microcapsules largely.

3.5. Encapsulation of recombinant human insulin and release in vitro

After preparing uniform-sized microcapsules successfully by optimizing the preparation conditions, rhI, as a model protein, was encapsulated, respectively, in the microcapsules with different sizes, and its encapsulation efficiency and release in vitro were investigated. As shown in Table 2, when the diameter of drug-loaded microcapsules increased from 3.2 to 9.0 μm, the drug encapsulation efficiency increased from 54.94 to 70.80% and the initial release on the first day decreased from 16.95 to 4.5%, respectively. To prepare drug-loaded microcapsules, $w_1/o/w_2$ double emulsion droplets were formed firstly, followed by the solvent removal process. However, during process of the double emulsion droplets formation and solvent removal, the drug dissolved in the internal water phase was ready to diffuse into the external water phase through the oil phase, which led to the loss of drug encapsulated, and thus affecting the drug encapsulation efficiency. When the size of drug-loaded microcapsules was smaller, the interfacial area between the emulsion droplets and the external water phase became larger, and the drug had more chance to diffuse into the outer water phase, then the drug encapsulation efficiency decreased accordingly. At the same condition, drug-loaded PLA microcapsules were also prepared by stirring method, and the drug encapsulation efficiency was compared with those prepared by membrane emulsification technique. It was found that the drug encapsulation efficiency was only 29.85% when microcapsules were prepared by stirring method, although the size of microcapsules was much larger than those prepared by membrane emulsification technique. This is because the results were conformed that membrane emulsification technique was more efficient for encapsulating drugs than stirring method. From Fig. 10, it illustrated that the rhI cumulative release was faster after initial release when the size of drug-loaded microcapsules was smaller. The smaller the size of microcapsules was, the shorter distance between the drug encapsulated and the surface of microcapsules, and it was easier for the drug to diffuse into the release medium through the water channel inside the microcapsules. In addition, the increase of interfacial area between the microcapsules and the release medium was another reason for the faster cumulative release of drugs encapsulated in smaller microcapsules. Furthermore, an interesting phenomenon was found that a apparent shoulder was observed in each curve after 10 days of release experiment, as shown in Fig. 10. Generally, there exists three mechanisms for drug release from the polymer microcapsules [21]: Fickian diffusion through the polymer matrix, diffusion through water-filled pores (aqueous channels) formed by water penetration into the matrix and liberation by erosion of the polymer matrix. In phosphate buffer, microcapsules often exhibited a triphasic release with a faster release phase called “burst effect”, followed by a slower one, and then a faster one when the polymer began to degrade. Therefore, the shoulders were probably due to the degradation of the outer thin membrane of microcapsules, leading to the fast release of the drugs located in the internal water phase near to the surface of the microcapsules. However, the cumulative release was still very slow due to the slow degradation rate of high molecular weight PLA applied in this study. Therefore, we are planning to add some polymer with faster degradation rate in the oil phase to increase the cumulative release of drugs in the near future research.

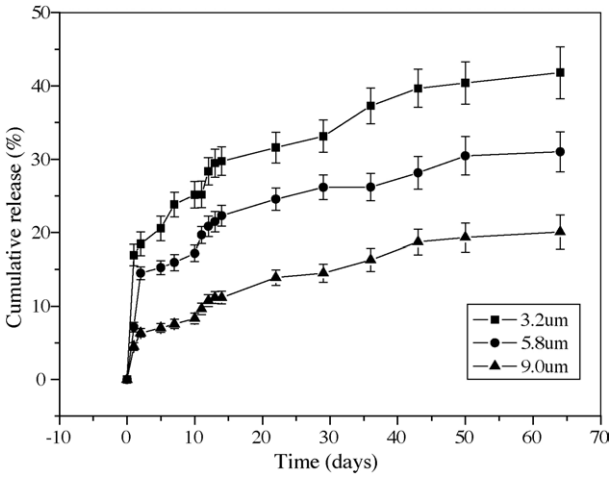

Fig. 10. Effect of microcapsules size on rhI cumulative release profile.

Table 2
Effect of the microcapsule sizes on the drug encapsulation efficiency and initial release

Emulsification technique	Mean size (μm)	Encapsulation efficiency (%)	Initial rhI burst[a] (%)
Membrane	3.2	54.94	16.94
	5.8	62.27	7.16
	9.0	70.80	4.50
Stirring	15.9	29.85	–

[a] rhI released during the first 24 h.

4. Conclusion

Uniform-sized biodegradable PLA microcapsules with various sizes were successfully prepared by combining a glass membrane emulsification technique and double emulsion-solvent evaporation method. Several factors influencing the size distribution of microcapsules were investigated. The results indicated the size distribution of microcapsules was affected by PVA concentration in the external water phase, the transmembrane pressure, and especially the concentration of oil-soluble emulsifier. By optimizing the factors examined, PLA microcapsules with CV value under 14.0% were obtained. An unique phenomenon was found that a large part of emulsifier could adsorb on the interface of internal water phase and oil phase, which suppressed its adsorption on the surface of glass membrane, and led

to successful preparation of uniform-sized double emulsion. The diameter of the microcapsules can be controlled easily just by using the membrane with proper pore size. rhI, as a model protein, was encapsulated, respectively, into the microcapsules with difference size, and its encapsulation efficiency and cumulative release were investigated. The results showed that the membrane emulsification technique also offered an advantage that the release rate can be simply adjusted just by changing precisely the diameter of microcapsules.

Acknowledgements

We would like to thank the financial support of the National Nature Science Foundation of China under Contract 20125616 and 20221603.

References

[1] M.N. Vrancken, D.A. Clays, Method for encapsulating water and compounds in aqueous phase by extraction, United States Patent Office 3,526,906 (1970).
[2] N.C. Jaeger, B.H. Trvernier, British Patent 1,405,108 (1971).
[3] M. Kitajima, A. Kondo, Fermentation without multiplication of cells using microparticles that contain zymase complex and muscle enzyme extract, Bull. Chem. Soc. Jpn. 44 (1971) 3201–3202.
[4] Y. Ogawa, M. Yamamoto, S. Takada, H. Okada, T. Shimamoto, Controlled-release of leuprolide acetate from polylactic acid or copoly(lactic/glycolic) acid microparticles: influence of molecular weight and copolymer ratio of polymer, Chem. Pharm. Bull. 36 (1988) 1502–1507.
[5] M.E. Le Fevre, D.C. Hancock, D.D. Joel, Intestinal barrier to large particulates in mice, J. Toxicol. Environ. Health 6 (1980) 691–704.
[6] G.H. Ma, M. Nagai, S. Omi, Preparation of uniform poly (lactide) microspheres by employing the Shirasu porous glass (SPG) emulsification technique, Colloids Surf. A: Physicochem. Eng. Aspects 153 (1999) 383–394.
[7] T. Nakashima, M. Shimizu, M. Kukizaki, Membrane emulsification by microporous glass, Key Eng. Mater. 61/62 (1991) 513–516.
[8] G.H. Ma, M. Nagai, S. Omi, Effect of lauryl alcohol on morphology of uniform polystyrene-poly (methyl methacrylate) composite microspheres prepared by porous glass membrane emulsification technique, J. Colloid Interface Sci. 219 (1999) 110–128.
[9] H. Yuyama, T. Watanabe, G.H. Ma, M. Nagai, S. Omi, Preparation and analysis of uniform emulsion droplets using SPG membrane emulsification technique, Colloids Surf. A: Physicochem. Eng. Aspects 168 (2000) 159–174.
[10] G.H. Ma, M. Nagai, S. Omi, Study on preparation and morphology of uniform artificial polystyrene-poly (methyl methacrylate) composite microspheres by employing the SPG (Shirasu porous Glass) membrane emulsification technique, J. Colloid Interface Sci. 214 (1999) 264–282.
[11] G.H. Ma, M. Nagai, S. Omi, Synthesis of uniform microspheres with higher content of 2-hydroxyethyl methacrylate by employing SPG (Shirasu porous glass) emulsification technique followed by swelling process of droplets, J. Appl. Polym. Sci. 66 (1997) 1325–1341.
[12] G.H. Ma, H. Sone, S. Omi, Preparation of uniform-sized polystyrene–polyacrylamide composite microspheres from a w/o/w emulsion by membrane emulsification technique and subsequent suspension polymerization, Macromolecules 37 (2004) 2954–2964.
[13] R. Liu, G.H. Ma, F.T. Meng, Z.G. Su, Preparation of uniform-sized PLA microcapsules by combining Shirasu porous glass (SPG) membrane emulsification technique and multiple emulsion-solvent evaporation method, J. Control. Release. 103 (2005) 31–43.
[14] N. Wakiyama, K. Juni, M. Nakano, Preparation and evaluation in vitro and in vivo of polylactic acid microspheres containing dibucaine, Chem. Pharm. Bull. 30 (1982) 3719–3727.
[15] B.V. Parikh, S.M. Upadrashta, S.H. Neau, N.O. Nuessle, Oestrone loaded poly(L-lactic acid) microspheres: preparation, evaluation and in vitro release kinetics, J. Microencapsul. 10 (1993) 141–153.
[16] M. Singh, D. O'Hagan, The preparation and characterization of polymeric antigen delivery systems for oral administration, Adv. Drug Del. Rev. 34 (1998) 285–304.
[17] R.A. Williams, S.J. Peng, D.A. Wheeler, N.C. Morley, D. Taylor, M. Whalley, D.W. Houldsworth, Controlled production of emulsions using a crossflow membrane. Part II. Industrial scale manufacture, Chem. Eng. Res. Des. 76A (8) (1998) 902–910.
[18] V. Schröder, O. Behrend, H. Schubert, Effect of dynamic interfacial tension on the emulsification process using microporous, ceramic membranes, J. Colloid Interface Sci. 202 (1998) 334–340.
[19] N.C. Christov, D.N. Ganchev, N.D. Denkov, K.D. Danov, P.A. Kralchevsky, Capillary mechanisms in membrane emulsification: oil-in-water emulsions stabilized by Tween 20 and milk proteins, Colloids Surf. A 209 (2002) 83–104.
[20] T. Nakashima, M. Shimizu, M. Kukizaki, Particle control of emulsion by membrane emulsification and its applications, Adv. Drug Del. Rev. 45 (2000) 47–56.
[21] K.A. Athanasiou, G.G. Niederauer, C.M. Agrawal, Sterilization, toxicity, biocompatibility and clinical applications of polylactic acid/polyglycolic acid copolymers, Biomaterials 17 (1996) 93–102.