Chinese Journal of Polymer Science Vol. 33, No. 7, (2015), 955–963

Chinese Journal of Polymer Science

A Detailed View of PLGA-mPEG Microsphere Formation by Double Emulsion Solvent Evaporation Method*

Shui-bin Feng[a†], De-hao Fu[b†], Lei Nie[a], Peng Zou[a] and Jin-ping Suo[a**]

[a] *State Key Laboratory of Material Processing and Die and Mould Technology, College of Materials Science and Engineering, Huazhong University of Science and Technology, Wuhan 430074, China*

[b] *Department of Orthopedics, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China*

Abstract PLGA, mPEG diblock copolymer was synthesized by bulk ring-opening polymerization method. The double emulsion solvent evaporation method was used to prepare bovine serum albumin (BSA)-loaded microspheres. Optical microscopy was used to observe the whole microsphere fabrication process. It is confirmed that the proportion of inner aqueous phase is one of the most critical factors that determines the morphology of microspheres. Double emulsion droplets which have appropriate amount of inner aqueous phase can form closed and dense microspheres, while, too much inner aqueous phase will cause a collapse of the double emulsion droplets, resulting in a loss of drug. The proportion of inner aqueous phase was varied to prepare microspheres of different morphology. The results show that with increasing the amount of inner aqueous phase, a higher percent of broken microspheres and lower encapsulation efficiency appeared, and also, a more severe initial burst release and faster release rate.

Keywords: Microspheres; PLGA-mPEG; Morphology; Encapsulation efficiency; Release profile.

INTRODUCTION

Polymeric microspheres have been widely utilized as a favorable tool in delivering various cytokines and proteins[1–5]. Biodegradable polyesters such as poly(lactide-*co*-glycolide) (PLGA), poly(lactic acid) (PLA) and poly(*ε*-caprolactone) (PCL) have been widely used as carriers in controlled-release delivery systems due to their biocompatibility and biodegradability[6–11]. Their degradation time can be varied from days to years by altering the type of polymer, polymer molecular weight, or the structure of the microspheres[12]. Nevertheless, PLGA and PLA microspheres showed a low encapsulation efficiency of hydrophilic peptides and proteins due to their hydrophobic nature. Furthermore, during the initial drug release, the hydrophobic PLGA or PLA prevents the penetration of water into the center of microspheres, forming an acidic environment due to the accumulated acidic breakdown products, which will cause a degeneration of peptides or proteins[13]. Poly(ethylene glycol) (PEG) segments are hydrophilic that can change the physicochemical properties of hydrophobic PLGA block segments. Diblock poly(lactic acid)-methoxypoly(ethyleneglycol) (PLA-mPEG) and poly(dl-lactide-*co*-glycolide)-methoxypoly(ethyleneglycol) (PLGA-mPEG) form more hydrophilic matrices than PLGA and are considered more suitable for the controlled delivery of proteins[14, 15]. Furthermore, hydrophilic and hydrophobic

* This work was financially supported by the Independent Innovation Foundation of Huazhong University of Science and Technology (No. 2013ZHYX008) and the National Natural Science Foundation of China (No. 81370980).

** Corresponding author: Jin-ping Suo (索进平), E-mail: jinpingsuo@hust.edu.cn

† These two authors contributed equally to this work.

Received October 23, 2014; Revised March 10, 2015; Accepted March 17, 2015
doi: 10.1007/s10118-015-1660-9

balance in PLGA-mPEG diblock copolymer was one of the key roles to maintain intrinsic properties of drugs.

Microspheres composed of these biodegradable polymers have been prepared by various methods such as phase separation[16], solvent evaporation[17], spray drying[18] and supercritical fluid precipitation[19]. Due to its simple instrument and operation, double emulsion solvent evaporation method ($W_1/O/W_2$) becomes the most common technique to fabricate microspheres encapsulating water-soluble drugs. Over the past few decades, many researches had been focused on the morphology, encapsulation efficiency and drug-release behavior of microspheres[17, 20, 21], and also, the application for treatment of different kinds of diseases[19, 22, 23]. However, few researches considered the microsphere formation process, which is actually, crucial for microspheres morphology and drug release behavior[6]. So, we would like to study the microsphere formation process in depth and find out the crucial factors that determine the microsphere properties.

In this study, we synthesized amphiphilic PLGA-mPEG diblock copolymer and prepared bovine serum albumin (BSA)-loaded PLGA-mPEG microspheres by double emulsion solvent evaporation method. The microsphere formation process was monitored by optical microscope. We expected to find the crucial factor that determine the morphology, encapsulation efficiency and release behavior of microspheres, thus optimizing the fabrication conditions.

EXPERIMENTAL

Materials

Glycolide (GA), DL-Lactide (LA) and monomethoxypoly(ethylene glycol) (mPEG, M_n = 5000) were purchased from Aldrich. LA and GA were recrystallized twice in ethyl acetate and sublimated before use. mPEG was lyophilized for 24 h in case of moisture. Stannous octoate ($Sn(Oct)_2$) and BSA were obtained from Sigma and used as received. Poly(vinyl alcohol) (PVA) from Sinopharm Chemical Reagent Co., Ltd, was used as a stabilizer in the emulsion. Methylene chloride and diethyl ether, as solvents, were of analytical grade and purchased from China National Medicines Corporation Ltd, and used without purification.

Synthesis and Characterization of PLGA-mPEG

PLGA-mPEG diblock copolymer was synthesized by bulk ring-opening polymerization method[24]. Before the synthesis, LA and GA were recrystallized twice in ethyl acetate and sublimated. mPEG was lyophilized for 24 h in case of moisture. All glasses were heated in vacuum before use. The typical process for the polymerization is as follows: mPEG-5000 (0.51 g) was stirred at 115 °C in a three-necked flask under the protection of nitrogen for 30 min. LA and GA were added and heated at 115 °C to make them melted, then, 0.04 g of $Sn(Oct)_2$ was added and the reaction mixture was further heated at 125 °C for 8 h under the protection of nitrogen. The synthesized polymer was purified by dissolving in dichloromethane and then precipitated in diethyl ether. The precipitate was lyophilized under vacuum for 24 h.

The properties of the copolymer were examined by gel permeation chromatography (GPC), infrared (IR) spectra and ^{1}H nuclear magnetic resonance (^{1}H-NMR) spectroscopy. Gel permeation chromatography in an Agilent 1100 apparatus with a differential refractometer as a detector was used to measure the molecular weight (M_w) of the polymer and molecular weight distribution (P.I. = polydispersity index). The IR spectra were taken in a Bruker VERTEX 70 infrared spectrometer over the range 4000–500 cm^{-1}. KBr discs, containing 1% (*W*/*W*) of the copolymers were used. ^{1}H-NMR (Bruker AV 400) spectrometer was used to characterize the chemical composition of the copolymer with deuterated chloroform ($CDCl_3$) as solvent and tetramethylsilane (TMS) as standard.

Preparation of Microspheres

BSA-loaded microspheres were prepared using the double emulsion solvent evaporation method[25]. First, 0.1 mL (0.2 mL, 0.4 mL) BSA aqueous solution (100 mg/mL, 1% *W*/*V* PVA, called W_1 phase) was added to 4 mL of dichloromethane containing 0.25 g PLGA-mPEG (called O phase), the mixture was homogenized at 14500 r/min for 1 min to obtain the primary W_1/O emulsion. One drop of the primary emulsion was poured on a microscope slide, sealed with cover glass and observed under the ternary photographic biological microscope

(XSP-2CAV, Shanghai). The primary emulsion was then, injected into 10 mL of 0.25% *W*/*V* PVA aqueous solution (called W_2 phase) and emulsified at 8500 r/min for 10 s, creating the $W_1/O/W_2$ double emulsion. One drop of the double emulsion was poured on a microscope slide, sealed with cover glass and observed under the microscope. Next, the double emulsion was dispersed into 120 mL of 0.25% *W*/*V* PVA solution under magnetic stirring at 600 r/min for 3 h to evaporate the dichloromethane. The microspheres were collected by centrifugation at 3000 r/min and washed three times with deionized water. Subsequently, the microspheres were lyophilized and stored at 4 °C.

Morphology and Size Distribution of Microspheres

The surface morphology of microspheres was observed using a scanning electron microscope (SEM, Quanta 200, Holland, FEI). Microspheres were mounted onto metal stubs using a double-sided adhesive tape. After vacuum-coated with a thin layer of gold, the microspheres were examined by SEM at 15 kV. For the mean size and size distribution analysis, three hundred microspheres in each group were randomly chosen from the SEM micrograph and the software (Nano Measurer 1. 2) was applied to counting the mean size and size distribution. In the mean time, we made a statistics of broken microspheres in these three hundred microspheres.

Determination of BSA Encapsulation Efficiency

The BSA encapsulation efficiency of the microspheres was measured by the BCA assay[17]. Briefly, 10 mg of dried microspheres was dissolved in 1 mL of methylene chloride under stirring and 3 mL of PBS (pH 7.4, 0.01% sodium azide, 0.02% Tween 80) was then added. The mixture was vigorously agitated for 5 min to extract BSA into PBS from the organic solution. After centrifugation, the aqueous phase was withdrawn and the amount of BSA was analyzed by the BCA assay. The BSA encapsulation efficiency was expressed as follows:

Encapsulation efficiency (%) = (Actual BSA:PLGA-mPEG ratio) / (Initial BSA:PLGA-mPEG ratio) × 100

All the experiments were run in triplicate and the data were shown as mean ± standard deviation.

In vitro BSA Release Study

The *in vitro* release experiment of BSA-loaded microspheres was conducted in PBS (pH 7.4, 0.01% sodium azide, 0.02% Tween 80). In triplicate, 30 mg of microspheres was placed in centrifuge tube and suspended in 5 mL PBS. The tube was sealed and placed in a shaking water bath, which was maintained at 37 °C under middle shaking (50 strokes/min). At scheduled time intervals, the tubes were taken out and centrifuged, then, 3 mL of supernatant was withdrawn to determine the amount of BSA released by BCA assay kit and the tube was replenished by the same volume of fresh medium.

RESULTS AND DISCUSSION

Characterization of PLGA-mPEG

The PLGA-mPEG block copolymer was synthesized by the ring opening polymerization of DL-lactide (LA) and glycolide (GA) using monomethoxypoly(ethylene glycol) (mPEG) (M_n =5000 g/mol) as a macroinitiator, in the presence of a catalytic amount of $Sn(Oct)_2$. The GPC analysis showed that the weight average molecular weight of the product was 36650, with a narrow molecular weight distribution (P.I = 1.6), shown in Fig. 1.

The chemical structure of the PLGA-mPEG diblock copolymer was investigated *via* FTIR (Fig. 2) and ^{1}H-NMR spectroscopy (Fig. 3). The IR absorption peaks at 2850–3000 cm^{-1} were assigned to C—H stretching. The peaks at 1750 and 1085 cm^{-1} were assigned to the carbonyl (C=O) stretching mode of the PLGA, and the ether (C—O) bending mode of the mPEG and PLGA ester, respectively. It was characteristic that the broad absorption band at 3500 cm^{-1} in the spectrum of mPEG, assigned to O—H stretching, was practically eliminated from the spectrum of PLGA-mPEG, indicating that the free hydroxyl groups of mPEG had reacted with the carbonyl groups of lactide/glycolide[20].

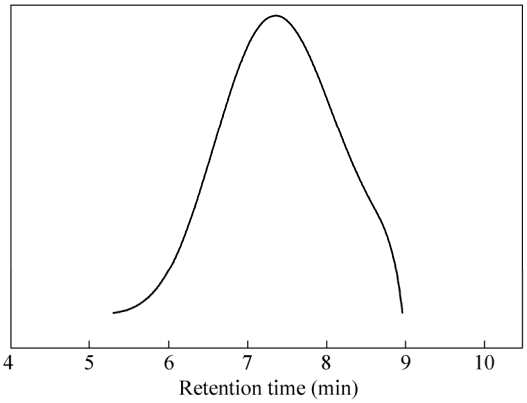

Fig. 1 GPC trace of PLGA-mPEG diblock copolymer

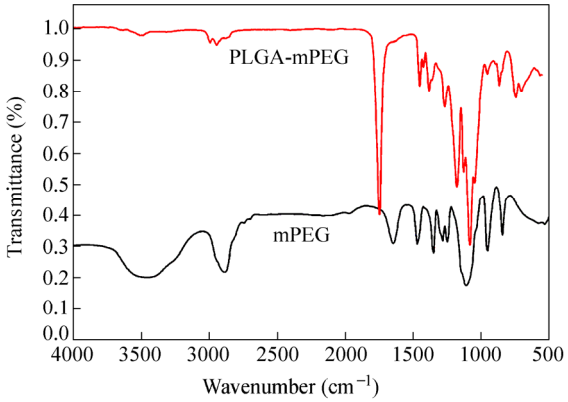

Fig. 2 IR spectra of mPEG and PLGA-mPEG, over the range of 4000–500 cm^{-1}

The ^{1}H-NMR spectrum of the PLGA-mPEG block copolymer showed the terminal methoxy proton signal of mPEG at $\delta = 3.36$ and the methylene proton signal at $\delta = 3.65$ as reported[26, 27]. The methoxy proton signal of LA unit in the PLGA segments was shown at $\delta = 1.55$ and the methyne proton signal at $\delta = 5.18$. The methylene proton signal of GA unit in the PLGA segments was shown at $\delta = 4.82$. In comparison with the ^{1}H-NMR spectrum of mPEG, new proton signals which belonged to LA and GA appeared, indicating the successful synthesis of PLGA-mPEG block copolymer.

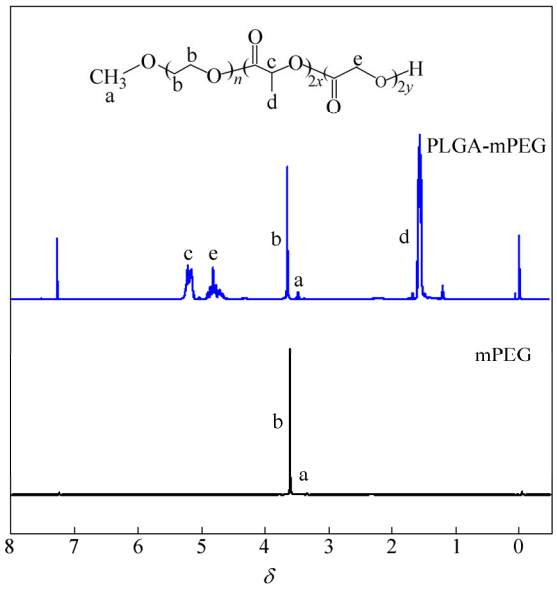

Fig. 3 ^{1}H-NMR spectra of mPEG and copolymer PLGA-mPEG in $CDCl_3$

Preparation of Microspheres

Figure 4 exhibited the optical micrographs of the primary emulsion (W_1/O) and the double emulsion (W_1/O/W_2). A normal double emulsion consisted of small-sized water droplets (W_1) contained within larger oil droplets (O) that are dispersed within an aqueous continuous phase (W_2). We divided the double emulsion into three typical droplet structures: with no entrapped inner water (marked "1" in Fig. 4); with little entrapped inner water, occupying appropriate volume within the oil droplet (marked "2" in Fig. 4); with much inner water, occupying large volume within the oil droplet (marked "3" in Fig. 4).

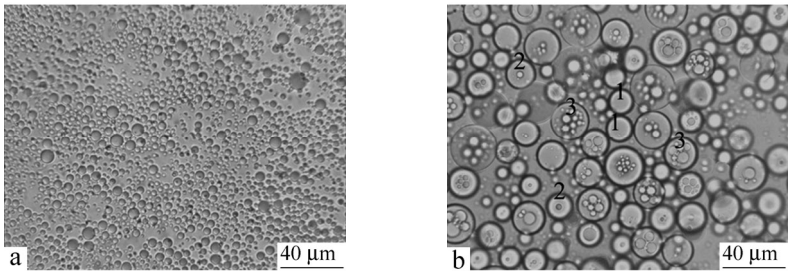

Fig. 4 (a) Optical micrograph of the primary (W_1/O) emulsion; (b) Optical micrograph of the double ($W_1/O/W_2$) emulsion

In order to observe in detail the transformation of the double emulsion, one drop of the double emulsion was poured on a microscope slide and sealed with cover glass. In this case, the solvent evaporation is restricted only through the thin air-emulsion interface located at the edges of the cover glass, thus, the process was slowed down allowing a proper observation of the microsphere formation. The solvent evaporation at the edges of the cover glass initiated the solvent elimination from the oil droplets. Once the solvent elimination initiated, the transforming process was accelerated (only 30 s from A1 to A3 and B1 to B3 shown in Fig. 5) by the polymer precipitation and the remaining solvent was practically expulsed from the oil droplets, generating the final microspheres. To those oil droplets entrapping little inner aqueous phase (occupying appropriate volume within the oil droplet) or none, polymer content in oil droplet was sufficient to form a resistant layer to wrap around the inner aqueous phase, thus, closed microspheres were generated (marked "b", "a" in Fig. 5 respectively), and after freeze drying, the microsphere appearing as perfect round dense ball on the SEM (Fig. 5. A4). For the oil droplets that entrapped too much inner water (occupying large volume within the oil droplet), polymer content in oil droplet was insufficient to form a resistant layer to wrap around the incompressible inner water, thus, the polymer layer collapsed during the solvent elimination, forming holes through which the encapsulated BSA was partly expulsed (marked "c" in Fig. 5), and after freeze drying, the microsphere appeared like deflated ball (Fig. 5. B4).

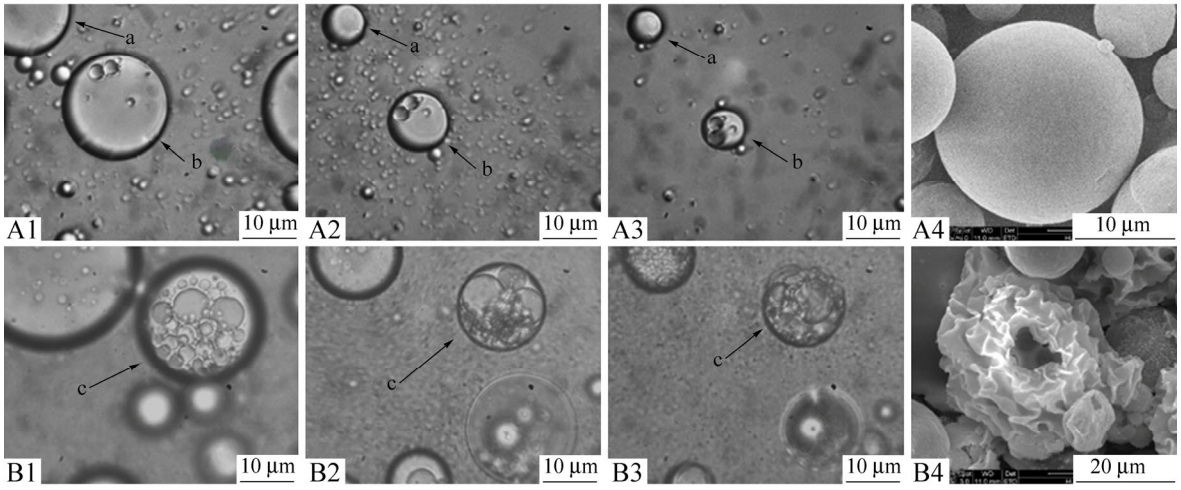

Fig. 5 Picture sequence of the double emulsion ($W_1/O/W_2$) transformation: (A1, A2, A3) oil droplets with little inner droplets, (B1, B2, B3) oil droplets with much inner droplets, Scanning electron micrographs of (A4) the closed microsphere and (B4) the broken microsphere

Through the optical microscope observation results, we assumed that the proportion of inner aqueous phase was one of the most critical factors that affected the morphology and encapsulation efficiency of microspheres, so, we prepared three types of microspheres variable in proportion of inner aqueous phase, and the conditions are shown in Table 1. The double emulsion droplets in each group were observed immediately after the second

emulsification, and the droplets microphotographs were taken around the central area of cover glass for comparing their inner water volume within oil droplets. Figure 6 shows that the inner water volume proportion within oil droplet increased obviously with the inner aqueous phase increased from 0.1 mL to 0.4 mL. The absolute value of inner water volume within the double emulsion droplet was difficult to calculate, instead, we counted the inner droplet number as the emulsification conditions were all the same. In each group, one hundred emulsion droplets were randomly chosen in the microphotographs, the inner water droplet number and number distribution were counted, shown in Fig. 6. As the inner aqueous phase increased from 0.1 mL to 0.4 mL, the inner droplet proportion tended to increase, from less than 5 inner droplets in most oil droplets (Fig. 6A) to more than 10 inner droplets in most oil droplets (Fig. 6C), which means that more oil droplets like that marked "c" in Fig. 5 were formed and microspheres broken percentage would increase. Though the inner droplets and oil droplets got a size distribution, but the preparation and measurement in each group were conducted under the same conditions and the oil droplets were chosen at random, so the inner droplet number comparison could relatively reflected the inner droplet volume.

Table 1. Characteristics of microspheres with different proportion of inner aqueous phase

Sample	PLGA-mPEG (g)	CH_2Cl_2 (mL)	Inner aqueous phase (mL)	Drug-encapsulation (%)	Microsphere broken percentage (%)
A	0.25	4	0.1	67.74 ± 1.87	4.33
B	0.25	4	0.2	32.66 ± 3.21	46.67
C	0.25	4	0.4	25.43 ± 2.04	60.33

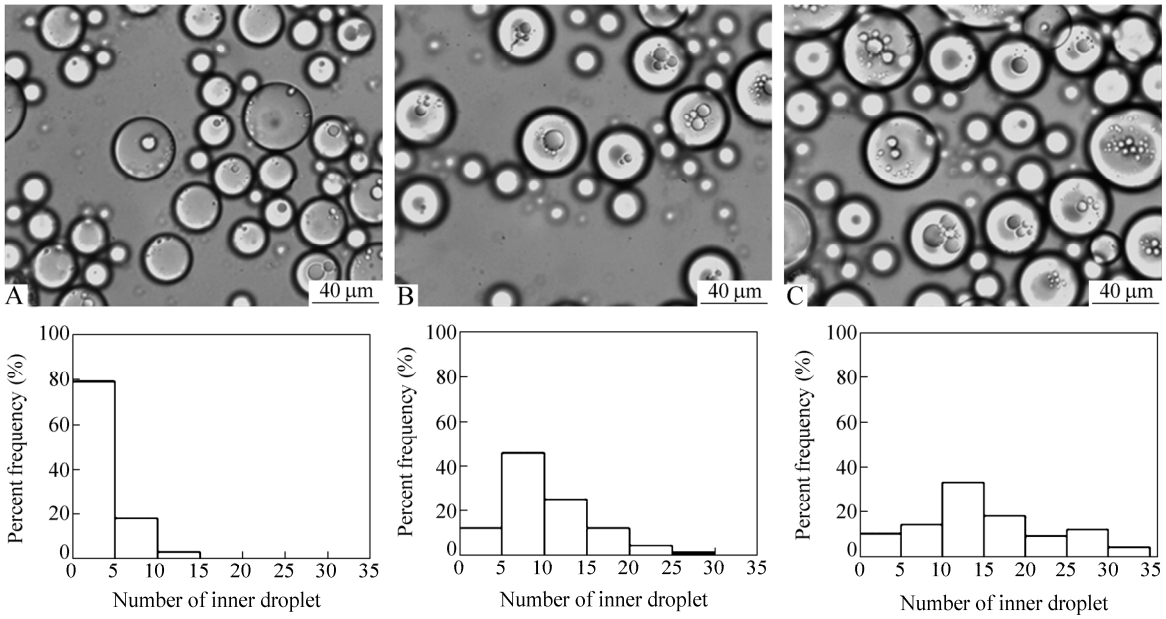

Fig. 6 Optical micrographs of the double emulsions variable in proportion of inner aqueous phase and corresponding number distribution of inner droplets: (A) 0.1 mL inner aqueous phase, (B) 0.2 mL inner aqueous phase and (C) 0.4 mL inner aqueous phase

Microsphere Characterization

SEM micrographs in Fig. 7 reveal that most of the microspheres fabricated with 0.1 mL inner water phase get a spherical shape, smooth surface, and low broken percentage. With the inner aqueous volume increased, a higher broken percentage of microspheres appeared. We chose three hundred microspheres in each group at random, recorded their morphology and calculated the broken percentage, shown in Table 1. The results showed an obvious rise in broken percentage, from 4.33% to 60.33% along with the inner aqueous phase volume increase from 0.1 mL to 0.4 mL. The results from the SEM analysis (Fig. 7) cooperated with the optical microscopic

observation (Fig. 6) indicated that the inner aqueous volume proportion of oil droplet was a critical factor that would affect the microsphere morphology. Higher broken percentage of microspheres means a more severe drug loss, as the encapsulated BSA would be continuously expulsed through the holes during the solvent evaporation process. As the inner aqueous phase increased from 0.1 mL to 0.4 mL, the BSA encapsulation efficiency decreased from 67.74 ± 1.87% to 25.43 ± 2.04%. Figure 7 also revealed a difference in size distribution among the three groups. Fude[12] reported that the average particle size increased slightly with increasing the inner aqueous phase volume, but in this study, the particle size analysis didn't show an obvious distinction between group A and B (11.71 μm and 11.55 μm), which may due to the higher microspheres broken percentage of group B, causing a decrease in size. But with the inner aqueous phase increased to 0.4 mL, the microsphere size showed a slight increase, which may be ascribed to that the effect of increased inner aqueous phase on microspheres size was larger than the effect of microsphere rupture.

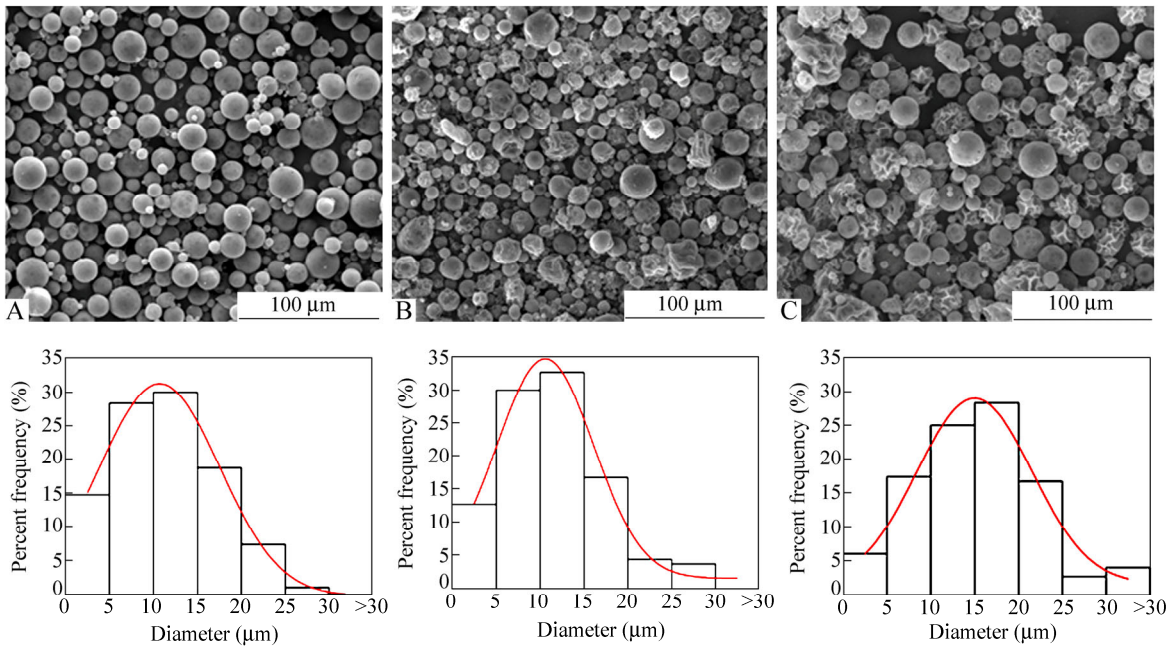

Fig. 7 Scanning electron micrographs of microspheres fabricated with different proportion of inner aqueous phase and the corresponding microspheres size distribution: (A) microspheres fabricated with 0.1 mL inner aqueous phase, (B) microspheres fabricated with 0.2 mL inner aqueous phase and (C) microspheres fabricated with 0.4 mL inner aqueous phase

***In vitro* BSA Release Study**

Figure 8 shows the release behaviors of the three types of microspheres, which revealed a different initial burst release but similar time for drug released in full. A burst release of 20.23% was obtained in group A, which was largely due to the BSA placed on the most superficial part of the microspheres as reported[12]. Microspheres in groups B and C showed more sever initial burst releases (46.47% and 50.39% respectively), which should due to the portion of broken microspheres, so the encapsulated BSA could diffuse quickly through holes and gaps, aggravating the initial burst release[28]. As microspheres in group C were prepared with maximum inner aqueous phase, it revealed the worst morphology and most sever burst release. Comparing to the microspheres in groups B and C, group A showed an obvious lag time, with little drug released during the following 6 days after the initial burst release. The lag time in BSA release profile depended on the degradation rate of polymer. To the groups B and C, part of the microspheres were broken and porous, which caused not only more sever burst release, but faster release rate of BSA, resulting in a disappearance of lag time. The time for BSA release in full was similar in the three types of microspheres, as even in group C, there were also closed microspheres existed,

which would contribute to the later period of release. Besides, in the BSA release analysis, almost 100% of the encapsulated BSA was detected, which was higher than that reported in PLGA and PCL microspheres[1, 17], indicating that PLGA-mPEG could maintain the intrinsic properties of proteins.

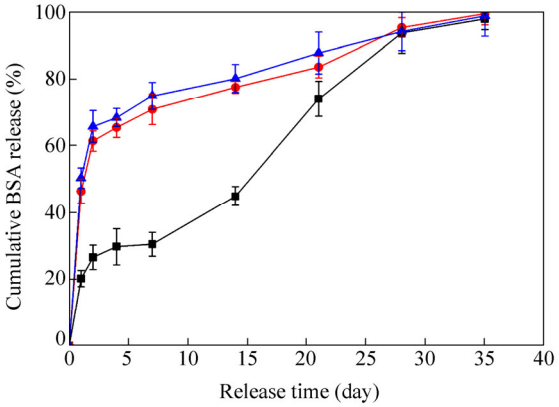

Fig. 8 Effect of inner phase amount on the release behavior of BSA from microspheres in PBS: (■) microspheres fabricated with 0.1 mL inner aqueous phase, (●) microspheres fabricated with 0.2 mL inner aqueous phase and (▲) microspheres fabricated with 0.4 mL inner aqueous phase

CONCLUSIONS

In this study, PLGA-mPEG was successfully synthesized by bulk ring-opening polymerization method and was used as matrix to encapsulate BSA. BSA-loaded microspheres were prepared by double emulsion solvent evaporation method under microscope observation, through which, we found that the proportion of inner aqueous phase greatly affected the morphology of microspheres. As the inner aqueous phase increased, inner droplet (W_1) volume proportion within most oil droplets (O) increased, the polymer matrix was insufficient to wrap around the incompressible inner droplets, causing broken microspheres and thus, severe drug loss and initial burst release, and also, a faster release rate. Microspheres containing protein or peptide as controlled release devices had been widely used for the treatment of human diseases and this result could optimize the fabrication conditions to obtain microspheres with well morphology and long-term release profile.

ACKNOWLEDGEMENTS Thanks to the testing center of HUST for useful characterization. The assistance in the measurement of IR and ^{1}H-NMR by Li-hua Zhao, is highly appreciated.

REFERENCES

1 Wang, M., Feng, Q., Niu, X., Tan, R. and She, Z., Polym. Degrad. Stab., 2010, 95(1): 6
2 Ho, M.L., Fu, Y.C., Wang, G.J., Chen, H.T., Chang, J.K., Tsai, T.H. and Wang, C.K., J. Control. Release, 2008, 128(2): 142
3 Kakizawa, Y., Nishio, R., Hirano, T., Koshi, Y., Nukiwa, M., Koiwa, M., Michizoe, J. and Ida, N., J. Control. Release, 2010, 142(1): 8
4 Bae, S.E., Son, J.S., Park, K. and Han, D.K., J. Control. Release, 2009, 133(1): 37
5 Yeh, M. and Chiang, C., J. Microencapsulation, 2004, 21(1): 91
6 Gasparini, G., Holdich, R.G. and Kosvintsev, S.R., Colloids Surf., B, 2010, 75(2): 557
7 Anderson, J.M. and Shive, M.S., Adv. Drug Delivery Rev., 1997, 28(1): 5
8 De la Ossa, D.H.P., Ligresti, A., Gil-Alegre, M.E., Aberturas, M.R., Molpeceres, J., Di Marzo, V. and Suarez, A.I.T., J. Control. Release, 2012, 161(3): 927

9 Yoo, N.Y., Youn, Y.S., Oh, N.M., Oh, K.T., Lee, D.K., Cha, K.H., Oh, Y.T. and Lee, E.S., Colloids Surf., B, 2011, 88(1): 419
10 Lecaroz, C., Gamazo, C., Renedo, M.J. and Blanco-Prieto, M.J., J. Microencapsulation, 2006, 23(7): 782
11 Karal-Yilmaz, O., Serhatli, M., Baysal, K. and Baysal, B.M., J. Microencapsulation, 2011, 28(1): 46
12 Fude, C., Dongmei, C., Anjin, T., Mingshi, Y., Kai, S., Min, Z. and Ying, G., J. Control. Release, 2005, 107(2): 310
13 Zhou, S.B., Liao, X.Y., Li, X.H., Deng, X.M. and Li, H., J. Control. Release, 2003, 86(2–3): 195
14 Li, J., Jiang, G. and Ding, F., J. Appl. Polym. Sci., 2008, 108(4): 2458
15 Lee, S.Y., Hyun, H., Youn, J.Y., Kim, B.S., Song, I.B., Kim, M.S., Lee, B., Khang, G. and Lee, H.B., Colloids Surf., A, 2008, 313–314: 126
16 Thomasin, C., Ho, N.T., Merkle, H.P. and Gander, B., J. Pharm. Sci., 1998, 87(3): 259
17 Yang, Y.Y., Chung, T.S. and Ng, N.P., Biomaterials, 2001, 22(3): 231
18 Tao Y., Zhang, H.L., Hu, Y.M., Wan, S. and Su, Z.Q., Int. J. Mol. Sci., 2013, 14(2): 4174
19 Yang, X.Z., Dou, S., Sun, T.M., Mao, C.Q., Wang, H.X. and Wang, J., J. Control. Release, 2011, 156(2): 203
20 Beletsi, A., Leontiadis, L., Klepetsanis, P., Ithakissios, D.S. and Avgoustakis, K., Int. J. Pharm., 1999, 182(2): 187
21 Yang, Y.Y., Chung, T.S., Bai, X.L. and Chan, W.K., Chem. Eng. Sci., 2000, 55(12): 2223
22 Petros, R.A. and DeSimone, J.M., Nat. Rev. Drug Discov., 2010, 9(8): 615
23 Jiang, H.H., Kim, T.H., Lee, S., Chen, X., Youn, Y.S. and Lee, K.C., Biomaterials, 2011, 32(33): 8529
24 Bajgai, M.P., Aryal, S., Parajuli, D.C., Khil, M.S., Lee, D.R. and Kim, H.Y., J. Appl. Polym. Sci., 2009, 111(3): 1540
25 Meng, F.T., Ma, G.H., Liu, Y.D., Qiu, W. and Su, Z.G., Colloids Surf., B, 2004, 33(3–4): 177
26 Zou, P., Suo, J.P., Nie, L. and Feng, S.B., J. Mater. Chem., 2012, 22(13): 6316
27 Zou, P., Suo, J. P., Nie, L. and Feng, S.B., Polymer, 2012, 53(6): 1245
28 Rosca, I.D., Watari, F. and Uo, M., J. Control. Release, 2004, 99(2): 271