Effects of the Process Parameters on the Initial Burst Release of Poly(lactide-*co*-glycolide) Microspheres Containing Bovine Serum Albumin by the Double-Emulsion Solvent Evaporation/Extraction Method

Zheng Wang

Department of Chemical and Biomolecular Engineering, National University of Singapore, 4 Engineering Drive 4, Singapore 117576

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ABSTRACT: The effects of fabrication parameters on the morphology, drug loading, and initial burst release of poly (lactide-*co*-glycolide) microspheres loaded with bovine serum albumin were investigated to establish an optimal process and system for the *in vivo* delivery of therapeutic proteins. Through the addition of salts or sugars to induce an osmotic pressure in the external water phase, large microspheres were seen to have their morphology, drug loading, and initial burst release significantly affected. However, the effect was not observed for compact microspheres less than 10 μm in diameter. The presence of poly(vinyl alcohol), Pluronic F127, and Tween 80 in the internal water phase had detrimental effects on the drug loading because of the depressed stability of the primary emulsion and competitive interactions of surface-active substances with the polymer. However, the simultaneous addition of salts to the external water phase resulted in enhanced drug loading and decreased initial burst. The polymer concentration and volume of the internal water phase were important factors influencing the characteristics of the microspheres. These parameters were optimized for achieving the maximal drug loading and a low initial burst. The solvent extraction method yielded microspheres with a higher drug loading and a lower initial burst in comparison with the solvent evaporation method. Different ranges of protein encapsulation efficiencies were obtained with blends of poly(lactide-*co*-glycolide) and poly (ethylene glycol), depending on the molecular weight and content of poly(ethylene glycol). © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 115: 2599–2608, 2010

Key words: biodegradable; bioengineering; biomaterials; drug delivery systems

INTRODUCTION

Controlled drug release using biodegradable polymers such as poly(lactide-*co*-glycolide) (PLGA) has been shown to hold great promise for implant therapy. Although inorganic drugs have been released in a well-controlled manner,[1–3] the increasing use of proteins as drugs compels the use of the same polymer systems for the controlled delivery of proteins.[4] The advantages of such systems would be (1) controllable protein release kinetics over periods of days to months,[5] (2) complete biodegradability and good biocompatibility,[6] and (3) easy administration using standard syringes.

The double-emulsion (water-in-oil-in-water) method has been successfully used for the preparation of protein-loaded PLGA microspheres.[7–9] Typically, these microspheres will provide a very large burst of drug release once they are placed in the release medium. This initial burst release (i.e., the percentage of drug released after 24 h) depends on the immediate diffusion of proteins from the polymer matrix[10] and is complicated by its correlation with the effective drug loading.[11] Too high a burst will reduce the effective lifetime of the drug delivery device, reducing its effectiveness both therapeutically and economically.[11] Worse, excessive initial release rates can result in drug levels close to or exceeding toxic threshold levels.

Burst phenomena in drug-loaded microspheres have been observed by many groups,[12–20] and several studies have been carried out on technologically preventing the burst effect.[16–20] One promising method for limiting burst release is to use double-walled microspheres with an inner drug-loaded polymer core covered by an outer polymer layer without drugs.[16] However, the release profile of these microspheres typically contains three phases; the characteristic lag phase following the low initial burst can be over 20 days, depending on both the type of drug and the core and shell materials. A second method is to modify the double-emulsion process[19] by, for example, promoting fast polymer precipitation in the second emulsion process to

Correspondence to: Z. Wang (wangzheng@ntu.edu.sg).

reduce porosity inside the microspheres.[11] Another method is to create osmotic pressure in the microencapsulation process[20] by the addition of salts or sugars to the external water phase during the second emulsion process so that an osmotic pressure gradient is generated, resulting in smaller inner cavities within the microspheres and thus a lower initial burst. However, this method is limited as other parameters such as the particle size[21] may also have dominant influences on the burst effect simultaneously.

In this work, a model protein, bovine serum albumin (BSA), was encapsulated in biodegradable PLGA microspheres with the double-emulsion solvent evaporation/extraction method. The aim of this work was to investigate the effects of fabrication variables on the surface and internal morphology, the protein-loading properties, and the initial burst release from the microspheres. The results could form a basis for the further selection of process parameters in the development of injectable microsphere systems for the *in vivo* delivery of therapeutic proteins.

EXPERIMENTAL

Materials

A 75/25 PLGA polymer with an inherent viscosity of 0.93 dL/g was supplied by Turat Co. Poly(vinyl alcohol) (PVA) with a hydrolysis degree of 87–89% and a weight-average molecular weight (M_w) of 13,000–23,000 was obtained from Aldrich Co. Poly (ethylene glycol) (PEG) with M_w = 10,000 (PEG 10,000) and PEG with M_w = 35,000 (PEG 35,000) were supplied by Fluka. BSA (fraction V, 98% minimum) was purchased from Sigma Co. Tween 80 (polyoxyethylene sorbitan monooleate) was obtained from Tokyo Kasei Kogyo Co., Ltd. Pluronic F127 was purchased from Sigma. Sucrose (American Chemical Society reagent) was obtained from Sigma–Aldrich Co. Dichloromethane (liquid-chromatography grade) was purchased from Tedia. All other reagents were reagent-grade and were used as received.

Preparation of the microspheres

The water-in-oil-in-water double-emulsion solvent extraction/evaporation method was employed to fabricate BSA-loaded microspheres. A polymer/dichloromethane solution (7 mL; 57.1 mg/mL unless specified otherwise) was emulsified with 2 mL of an aqueous BSA solution (25 mg/mL unless otherwise specified; called the internal water phase or W1) with a homogenizer (Ultra Turrax T8, IKA-WERKE, Germany). The resultant primary emulsion (water-in-oil) was poured into a 100-mL aqueous solution (called the external water phase or W2) with various concentrations of PVA to produce the second emulsion. This solution was homogenized at 1600 rpm for 30 min with a homogenizer (L4R, Silverson, United States) at room temperature (22°C). The solution was then either laid *in vacuo* for 3 h to allow solvent evaporation or mixed with a 100-mL aqueous isopropyl alcohol solution (2% v/v) for solvent extraction. The microspheres were collected by centrifugation and washed three times with deionized water. The BSA-loaded microspheres were lyophilized and then stored at 4°C.

Determination of the BSA loading of the microspheres

Lyophilized microspheres (20 mg) were added to 2.0 mL of a 0.1*M* NaOH solution containing 5% (w/v) sodium dodecyl sulfate and incubated for 24 h at 37°C with occasional shaking. The mixture was then centrifuged, and the supernatant was drawn to determine the BSA loading with a bicinchoninic acid kit. Each sample was assayed in triplicate. The encapsulation efficiency was expressed as the ratio of the actual BSA content to the theoretical BSA content. The drug loading was expressed as follows:

$$\text{Drug loading (\%)} = \frac{\text{Amount of loaded drug}}{\text{Amount of polymer} + \text{Amount of loaded drug}} \times 100 \quad (1)$$

Determination of the demixing rate of the primary emulsion

The primary emulsion with various components in W1 was prepared according to the method in the previous section and then was stored in an assay vial that was sealed later with parafilm. The time required for an initial macroscopic phase separation to occur was measured at room temperature.

In vitro release of BSA from the microspheres

Dried microspheres (20 mg) were placed in a centrifuge tube and dispersed in 2 mL of a phosphate-buffered saline buffer (pH 7.0). The tube was placed in a 37°C incubator for 24 h with occasional shaking. The supernatant from the tube was then analyzed for the BSA content with a bicinchoninic acid kit. Each sample was assayed in triplicate. The initial burst release was expressed as the percentage of protein released after 24 h with respect to the total amount of loaded protein.

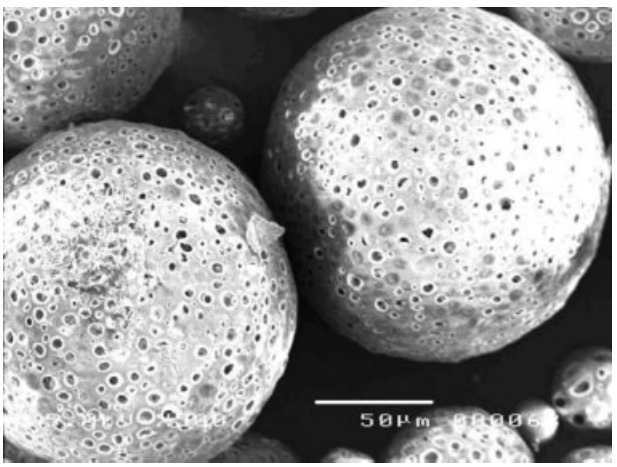

Figure 1 SEM picture of the surface of microspheres fabricated by mechanical stirring (1000 rpm) in the second emulsion process (bar = 50 µm).

Particle size analysis

The size of the microspheres was examined with an Axiotron high-performance microscope (Image & Microscope Technology, Korea). Dried microspheres were dispersed in deionized water and then placed on a glass slide. The images were analyzed with built-in software (i-Solution) to calculate the individual particle size. The mean particle size was called the number-average diameter.

Far-UV circular dichroism (CD) spectroscopy

Far-UV CD scans for native BSA samples and BSA released from microspheres on day 1 were performed on a Chirascan (Applied Photophysics, United Kingdom). Data were collected in millidegrees from 180 to 280 nm at 0.5-nm intervals with a 0.1-cm-path-length quartz cuvette. Buffer subtraction was performed for each BSA sample with a phosphate-buffered saline buffer (pH 7.0).

Scanning electron microscopy (SEM)

Lyophilized microspheres were mounted onto metal stubs with double-sided adhesive tape and then vacuum-coated with gold. The surface morphology and internal structure of the microspheres were examined by SEM (model JSM 6360A, JEOL, Japan) at 5, 10, or 15 kV.

RESULTS AND DISCUSSION

Salts/sugars in W2

In this study, salts or sugars were added to W2 before the second emulsion process to create an osmotic pressure gradient between the internal and external aqueous phases. Because the original particle size and morphology could be important factors balancing the ultimate effects of salt/sugar addition, homogenization or mechanical stirring was involved in the second emulsification to produce microspheres with different particle sizes and morphologies.

Figures 1 and 2 show the surface morphology of microspheres produced with mechanical stirring (1000 rpm) and homogenization (1600 rpm) in the second emulsion process, respectively. Microspheres fabricated with mechanical stirring (called G1) were spherical and highly porous in comparison with those smooth and compact microspheres produced with homogenization (called G2) without any additives. The mean particle diameter of the former group was 86.5 ± 11.8 µm, whereas the value of the latter was 9.1 ± 0.9 µm.

With respect to the G1 group, the protein encapsulation efficiency and initial burst release of the microspheres changed significantly with the addition of NaCl in W2. As shown in Table I, the protein encapsulation efficiency of the microspheres consistently decreased with the increase in the NaCl concentration up to 10% (w/v), and this was consistent with previous findings.[19] It is known that when the primary emulsion is transferred to the bulk continuous phase, microspheres form as the solvent is evaporated through the emulsion–air interface.[22] Because dichloromethane has about 2% solubility in water,[23] the addition of NaCl to W2 increased the polarity of the bulk aqueous phase, resulting in slower solvent removal from the water phase and thus allowing the microsphere to remain soft for a longer period of time. As a result, the encapsulation efficiency decreased because of delayed solidification of the dispersed phase and the concomitant water influx from the continuous phase into the polymer phase.[11,24] The particle size increased simultaneously

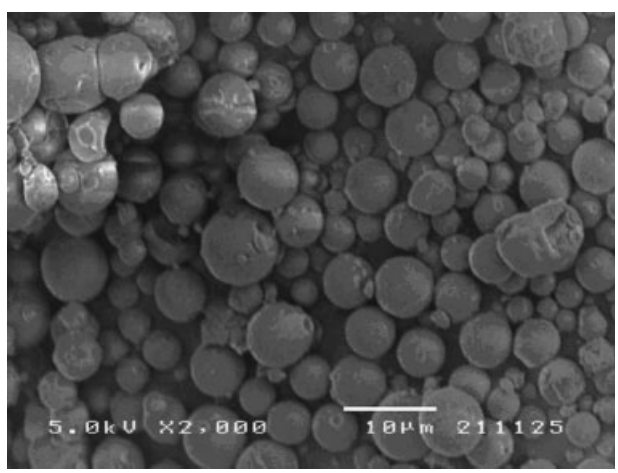

Figure 2 SEM picture of the surface of microspheres fabricated by homogenization (1600 rpm) in the second emulsion process (bar = 10 µm).

TABLE I
Characteristics of Microspheres (G1) Produced with NaCl and Sucrose as Additives in W2

Additive in W2	Concentration (w/v %)	Protein encapsulation efficiency (%)	Drug loading (%)	Mean particle diameter (μm)	Burst release (%)
NaCl (G1)	0	56.6 ± 2.1	6.6 ± 0.2	86.5 ± 11.8	97.2 ± 2.2
	2	55.1 ± 1.0	6.4 ± 0.2	87.6 ± 12.8	20.3 ± 2.6
	5	48.8 ± 1.9	5.7 ± 0.4	106.4 ± 17.8	8.4 ± 2.7
	10	41.7 ± 1.2	5.0 ± 0.1	106.6 ± 27.9	8.6 ± 1.2
Sucrose	0	56.6 ± 2.1	6.6 ± 0.2	86.5 ± 11.8	97.2 ± 2.2
	2	62.7 ± 0.2	7.3 ± 0.1	93.4 ± 19.3	97.2 ± 0.8
	5	61.1 ± 1.7	7.1 ± 0.2	93.3 ± 7.1	94.0 ± 4.1
	10	61.5 ± 0.8	7.1 ± 0.1	87.2 ± 12.6	93.2 ± 2.6
NaCl (G2)	0	56.4 ± 1.6	6.6 ± 0.2	—	62.9 ± 3.2
	2	56.4 ± 0.4	6.6 ± 0.1	—	64.8 ± 3.6
	5	56.2 ± 1.6	6.6 ± 0.2	—	63.1 ± 3.2
	10	52.1 ± 1.0	6.1 ± 0.2	—	65.2 ± 5.3

with the addition of NaCl to W2, as illustrated in Figure 3 and Table I, and this was also due to the slower shrinkage of the solid phase during the solvent expulsion process induced by the slower solidification of the microspheres. As shown in Table I, the initial burst release of the microspheres dramatically decreased from 97.2 ± 2.2% in the absence of NaCl to 8.6 ± 1.2% in the presence of 10% (w/v) NaCl in W2. This can be explained by the largely changed morphology of the microspheres with the addition of NaCl to W2 (Fig. 4) in comparison with the situation without the addition of NaCl (Fig. 1). It is clear that the surface of the microspheres with the addition of 5% (w/v) NaCl became smooth with largely reduced pores in comparison with those microspheres without additives (from a comparison of Figs. 1 and 4). This is also confirmed by a comparison of the internal morphology of the microspheres fabricated in the absence and presence of NaCl in W2 (Fig. 5). In this case, the NaCl concentration of 5% (w/v) was chosen to be in accordance with the previous condition (Fig. 4), as at this concentration of NaCl, the surface morphology differed significantly for the microspheres fabricated in the absence (Fig. 1) and presence of salts (Fig. 4). When NaCl was added to W2 during the second emulsion process, an osmotic pressure gradient was generated between W2 and W1 because of the inability of charged ions of NaCl to diffuse through the oil phase. As a result, W1 was drained because of the osmotic pressure, and this resulted in remarkably reduced coalescence of water droplets within the oil phase and thus smaller and fewer internal cavities in the fabricated microspheres, which led to a lower initial burst. Moreover, the slower solvent removal allowed further water diffusion from the internal phase to W2.

However, Table I shows that the initial burst of the microspheres slightly decreased with the increase in the sucrose concentration in W2 in the same concentration range. This may be due to the lack of influence of sucrose on the polarity. Figure 6 proves that the surface of the microspheres prepared with 5% (w/v) sucrose was still porous, although the surface pore size was reduced in comparison with those without additives [a 5% (w/v) concentration was chosen in this case for comparison with the condition with the addition of NaCl]. The

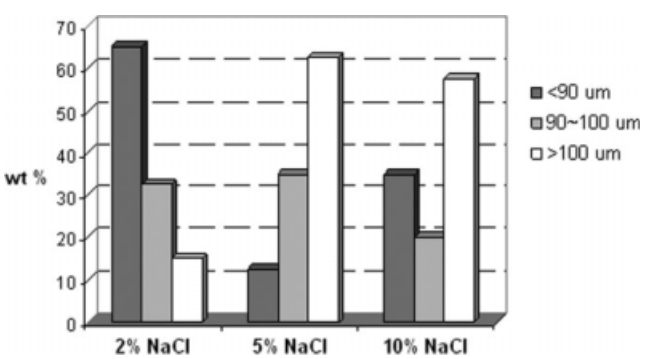

Figure 3 Size distribution of microspheres fabricated with different NaCl concentrations in W2.

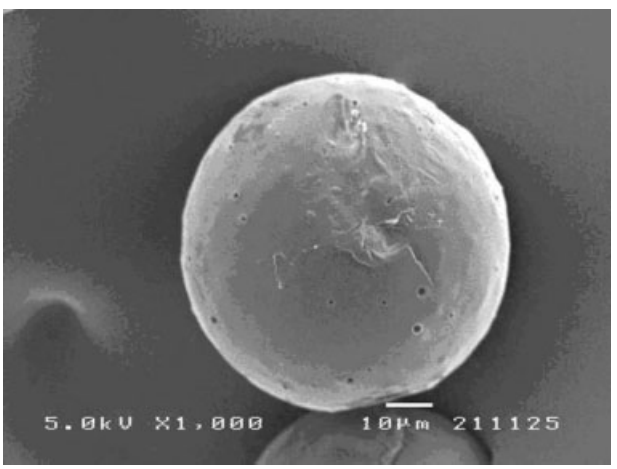

Figure 4 SEM picture of a microsphere surface (G1) with 5% NaCl in W2 during the second emulsion process.

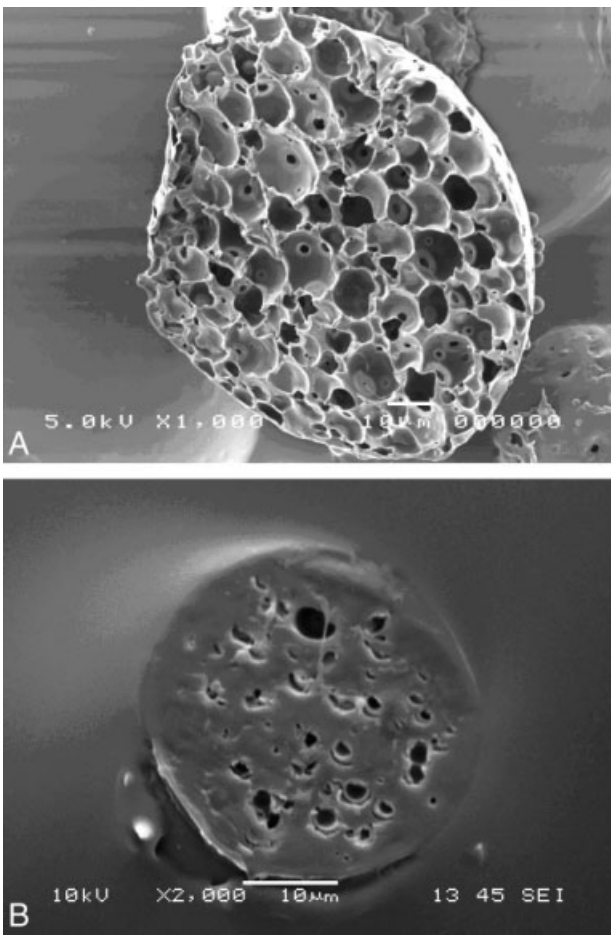

Figure 5 SEM pictures of cross sections of microspheres fabricated (A) in the absence of additives and (B) in the presence of 5% NaCl in W2.

addition of sucrose also showed no remarkable influence on the particle size (Table I). On the other hand, the protein encapsulation efficiency of the microspheres slightly increased with the addition of sucrose (Table I), probably because solidification of the microspheres in the second emulsion process was not retarded in the presence of sucrose in W2, whereas the presence of sucrose could promote the drainage of W1 because of the formation of an osmotic pressure.

In comparison, the G2 microspheres were small and compact. The protein encapsulation efficiency and initial burst of G2 without any additives are listed in Table I. The addition of NaCl in W2 produced no improvement in the initial burst release. The initial burst for the microspheres with 2, 5, and 10% (w/v) NaCl in W2 was 64.8 ± 3.6, 63.1 ± 3.2, and 65.2 ± 5.3%, respectively (Table I). It is known that burst release can be partly due to proteins either associated with the particle surface or situated in pockets close to the surface and easily accessible once the particle is hydrated.[25] The high initial burst release of the small microspheres with NaCl in W2 can be attributed to the decreased diffusional path length and increased effective surface area of the microspheres, which was induced by the small particle size.[26] On the other hand, the protein encapsulation efficiency of G2 with increasing NaCl concentration in W2 slightly decreased (Table I), and this was similar to the previous case of G1. Again, the reason for the decreasing encapsulation efficiency is delayed solidification of the dispersed phase and the concomitant water influx from the continuous phase into the polymer phase due to the addition of salts to W2.[11,26] As the addition of sucrose in W2 had minor effects on reducing the initial burst release for the G1 group, this parameter was not further studied here. Because the microspheres of G2 were small, spherical, and thus injectable with a 27G needle, all microspheres for the later topics were produced with homogenization (1600 rpm) in the second emulsion process. In this case, the much higher speed of 1600 rpm in homogenization versus the maximal speed of 1000 rpm in mechanical stirring definitely led to a smaller particle size with microspheres produced under the former condition[23] and remarkably different particle surface morphologies in the two cases, as discussed earlier. Also, the initial burst release of smaller microspheres produced under the former condition was higher, in accordance with the literature.[26]

Surface-active substances in W1

It is known that the stability of multiple emulsions in the water-in-oil-in-water method is a critical factor determining the encapsulation efficiency of microspheres.[11] An interaction between the drug and polymer is preferred for efficient internalization of the active ingredient.[11] Because the presence of surfactants in W1 is supposed to have important effects on both of these factors, PVA, Pluronic F127, and Tween 80 were used as surface-active additives in

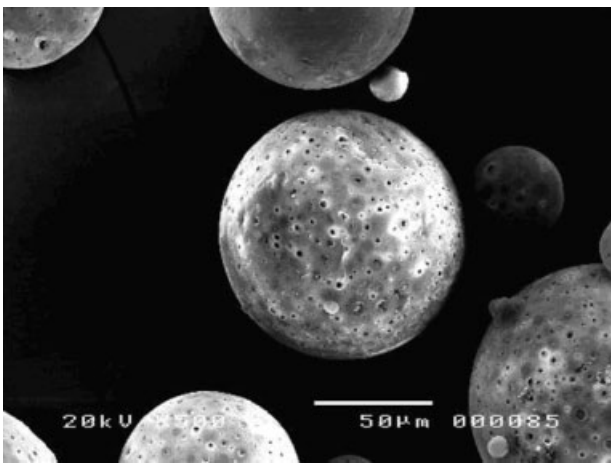

Figure 6 SEM picture of a microsphere surface (G1) with 5% sucrose in W2 during the second emulsion process.

TABLE II
Characteristics of Microspheres (G2) Produced with PVA, Pluronic F127, and Tween 80 as Surface-Active Additives in W1

Additive in W1	Concentration (w/v %)	Protein encapsulation efficiency (%)	Drug loading (%)	Burst release (%)
PVA	0	56.4 ± 1.6	6.6 ± 0.2	62.9 ± 3.2
	0.1	26.1 ± 2.3	3.2 ± 0.2	51.3 ± 4.2
	0.5	14.7 ± 1.8	1.8 ± 0.2	57.3 ± 2.8
	1	14.1 ± 1.7	1.7 ± 0.2	50.4 ± 3.6
Pluronic F127	0	56.4 ± 1.6	6.6 ± 0.2	62.9 ± 3.2
	0.1	16.3 ± 0.8	2.0 ± 0.1	80.8 ± 2.3
	0.5	16.2 ± 1.5	2.0 ± 0.2	74.7 ± 5.6
	1	10.1 ± 2.6	1.2 ± 0.4	72.3 ± 4.8
Tween 80	0	56.4 ± 1.6	6.6 ± 0.2	62.9 ± 3.2
	0.1	20 ± 2.8	2.4 ± 0.4	65.0 ± 3.3
	0.5	17.5 ± 1.4	2.1 ± 0.2	47.1 ± 2.4
	1	16.0 ± 1.6	2.0 ± 0.2	42.5 ± 2.6

W1 in this case. The purpose was to investigate their influence on the characteristics of microspheres.

Table II lists the protein encapsulation efficiency and the initial burst release of the microspheres (G2) produced with and without surface-active substances in W1. The protein encapsulation efficiency dramatically decreased from 56.4 ± 1.6% for microspheres without PVA in W1 to 14.1 ± 1.7% for those with 1% (w/v) PVA. This result is in contrast to the reported case with polycaprolactone microspheres, in which a lower PVA concentration in W1 resulted in decreased protein encapsulation efficiency.[23] Nonionic surfactants of Pluronic F127 and Tween 80 were also selected as additives in W1 for comparison. Similarly, the addition of Pluronic F127 and Tween 80 to W1 up to 1% (w/v) definitely led to decreased protein encapsulation efficiency (Table II).

To investigate the role of stability of the primary emulsion in such a process, the demixing rate of the primary emulsion (measured as the phase-separation time) in the presence of BSA, PVA, or BSA plus PVA in W1 is listed in Table III. The primary emulsion with pure water as W1 was the most unstable, in accordance with the previous finding.[7] When PVA was added to W1, the phase-separation time increased with the increase in the PVA concentration because of the surface activity of PVA. The emulsion stability was dramatically enhanced by the dissolution of a small amount of BSA, which is known to have tensoactive properties.[9] It has been reported that a small concentration of BSA in water promotes a sharp decrease in the water/dichloromethane interfacial tension.[7] On the other hand, the codissolution of PVA in a BSA solution as W1 clearly had a detrimental effect on the stability of the primary emulsion promoted by the presence of only BSA in W1; this was similar to a previous case in which the stability of the emulsion (a BSA solution as W1)

TABLE III
Phase-Separation Time with the Variation of the Components in W1

Component of W1 (2 mL)	Phase-separation time
Deionized water	25 min
0.1% PVA solution	2 h
0.5% PVA solution	4 h
1% PVA solution	5.5 h
BSA solution (25 mg/mL)	24 h
BSA in a 0.1% PVA solution	21 h
BSA in a 0.5% PVA solution	14 h
BSA in a 1% PVA solution	6 h

deteriorated with the dissolution of Poloxamer 188 in the oil phase.[7] This probably occurred because the absorption of surface-active substances to the polymer interface competed with the interaction of tensoactive proteins with the polymer and finally removed proteins from the interface.[27] On the other hand, the binding of surfactants with proteins via hydrophobic interactions or hydrogen bonding also contributes to protein removal.[7] These findings are consistent with the previous protein-loading results because the lower the stability was of the primary emulsion, the lower the drug encapsulation efficiency was. This was due to the tendency of the internal aqueous phase to merge with W2 in an unstable double-emulsion system.

To confirm that the presence of an osmotic pressure could enhance the protein loading even though PVA was present in W1, 5% (w/v) NaCl was added to W2 before the second emulsion process. The protein encapsulation efficiencies increased immediately to 56.8 ± 1.3, 53.3 ± 2.1, and 53.5 ± 0.8% in the presence of 0.1, 0.5, and 1% (w/v) PVA in W1, respectively, with the simultaneous addition of 5% (w/v) NaCl to W2 (Table IV). Figure 7 demonstrates that the internal pores and pore sizes of the microspheres with 0.1% (w/v) PVA in W1 were remarkably reduced by the addition of 5% (w/v) NaCl to W2. On the other hand, the initial burst release of microspheres with 0.1, 0.5, and 1% (w/v) PVA in W1 was 45.1 ± 3.1, 67.7 ± 2.6, and 86.8 ± 1.8%, respectively (Table IV). Such a result proves that the enhanced

TABLE IV
Characteristics of Microspheres (G2) Produced with Various Concentrations of PVA in W1 and 5% NaCl in W2

PVA concentration in W1 (w/v %)	Protein encapsulation efficiency (%)	Drug loading (%)	Burst release (%)
0	56.2 ± 1.6	6.6 ± 0.2	63.1 ± 3.2
0.1	56.8 ± 1.3	6.6 ± 0.2	45.1 ± 3.1
0.5	53.3 ± 2.1	6.2 ± 0.2	67.7 ± 2.6
1	53.5 ± 0.8	6.3 ± 0.1	86.8 ± 1.8

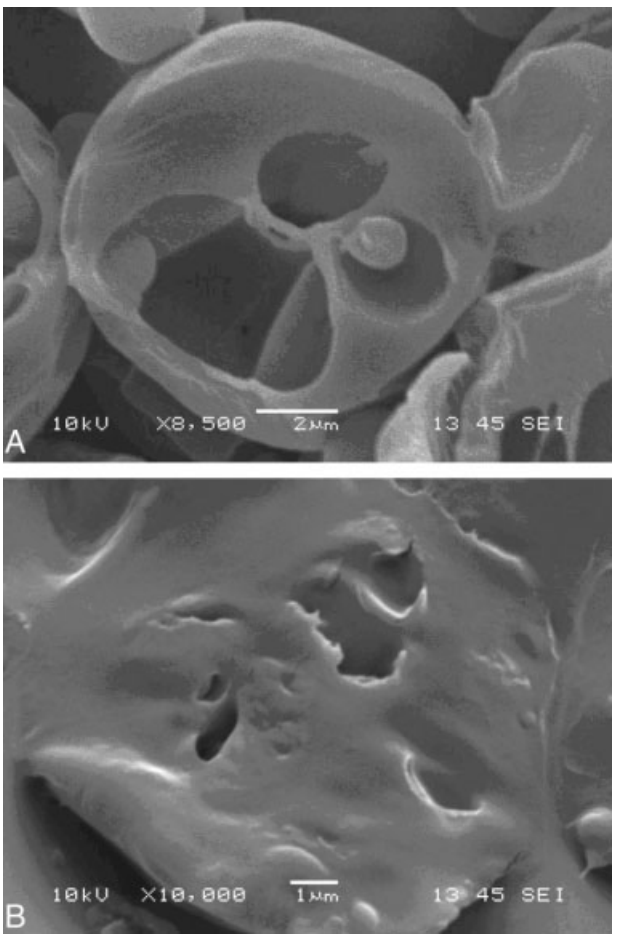

Figure 7 SEM pictures of cross sections of microspheres fabricated (A) with only 0.1% PVA in W1 and (B) with 0.1% PVA in W1 and 5% NaCl in W2.

water outflux from the internal phase to W2 in the presence of an osmotic pressure gradient averted the merging of W1 with W2 in the presence of PVA in W1 and therefore enhanced the protein encapsulation. This also indicates that the formation of insoluble associated species at the water/oil interface, like those found because of BSA–poloxamer interactions,[7] may not be the cause of the decreased protein loading in the presence of PVA. The initial reduction of burst release with the addition of 0.1% (w/v) PVA can be explained by the reduced pore size of the microspheres, as evidenced in Figure 7. However, the addition of a higher concentration of PVA may degrade the stability of the primary emulsion and thus result in an increased initial burst release.

To examine the secondary structure of BSA before and after incorporation into PLGA microspheres, far-UV CD spectroscopy was performed with a native BSA sample and BSA released from microspheres [0.1% (w/v) PVA in W1 and 5% (w/v) NaCl in W2] after 24 h of incubation (Fig. 8). The spectrum of native BSA has two negative bands at 212 and 222 nm as well as a strong positive band at

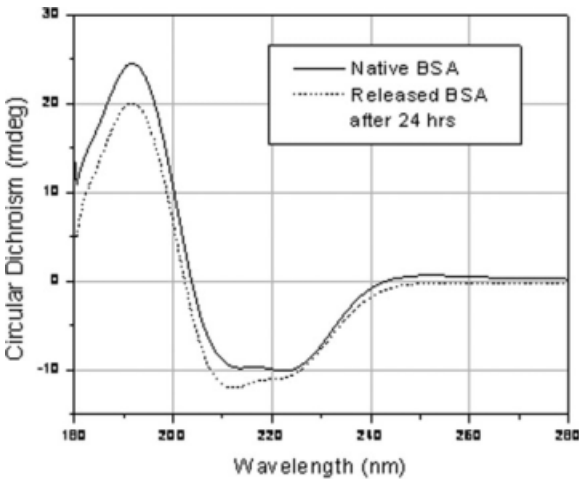

Figure 8 Far-UV CD scans of a native BSA sample and BSA released from microspheres (0.1% PVA in W1 and 5% NaCl in W2) after 24 h of incubation.

192 nm. These characteristic bands are attributed to a high content of the α-helix structure in the proteins.[28] The spectrum of BSA released from microspheres after 24 h shows a small blueshift in the 212-nm band, whereas the band position of 222 nm remains the same. This reflects a slight perturbation of the secondary structure of BSA after its loading into PLGA microspheres. This may be explained by protein denaturation due to the exposure of the proteins to the solvent environment, the hydrophobic surface, and also the air interface in the fabrication process.[29]

Polymer concentration

The polymer concentration is an important factor affecting the properties and release profiles of microspheres. The mean particle diameter of the microspheres fabricated with an increasing concentration of PLGA [5% (w/v) NaCl in W2 and 0.1% (w/v) PVA in W1] remarkably increased (Table V). The homogenization speed of 1600 rpm was used in this case, so the particle size of these microspheres was relatively small. The drug loading of the microspheres decreased with the increase in the polymer

TABLE V
Characteristics of Microspheres (G2) Produced with Various Concentrations of PLGA (5% NaCl in W2 and 0.1% PVA in W1)

PLGA concentration (mg/mL)	Drug loading (%)	Mean particle diameter (μm)	Burst release (%)
57.1	6.6 ± 0.2	15.1 ± 1.9	45.1 ± 3.1
71.4	6.1 ± 0.1	16.4 ± 1.6	43.1 ± 4.2
85.7	3.9 ± 0.2	42.3 ± 8.1	22.0 ± 4.5
100	2.0 ± 0.2	59.1 ± 12.9	20.3 ± 2.6

concentration (Table V). The initial burst release also consistently decreased with the increase in the polymer concentration (Table V). The increase in the size of the microspheres with increased polymer concentration arose from the more viscous polymer concentration. The decrease in the initial burst release of the microspheres can be attributed to the decreased porosity of the microspheres at a higher polymer concentration, as demonstrated earlier.[23]

Volume of W1

To investigate the influence of the volume of W1 on the characteristics of microspheres, 0.5, 1, or 2 mL of W1 was used in the fabrication process in the presence of 5% (w/v) NaCl in W2 (a certain amount of NaCl was added to reduce the initial burst release). The protein encapsulation efficiency increased and the initial burst release decreased with the increase in the internal water volume (Table VI). The particle size also increased at a higher W1 volume while other conditions remained the same (Fig. 9 and Table VI). Here the mean particle size was relatively low because of the use of high-speed homogenization in the secondary emulsification process, as stated earlier. The improvement of the encapsulation efficiency and the increase of the particle size at a higher W1 volume can be explained by the fact that the emulsion viscosity increases with an increase in the internal aqueous phase volume fraction,[30] as a high viscosity of the primary emulsion is preferred for achieving a high encapsulation efficiency.[31,32] On the other hand, internal water droplets are more difficult to coalesce together at a higher viscosity of the primary emulsion, which leads to a lower initial burst.

Comparison of solvent evaporation and solvent extraction

It has previously been shown that the solvent removal rate influences the solidification rate of the dispersed phase.[33] Thus, the solvent evaporation and solvent extraction were comparatively studied for their effects on the characteristics of microspheres. It was observed that the encapsulation efficiency of the latter was 59.1 ± 5.7%, slightly higher than the value of the former. The initial burst release was also remarkably reduced with the use of the solvent extraction method (Table VII). This happened because faster solvent removal by the solvent extraction method in comparison with the solvent evaporation method (several hours *in vacuo*) can accelerate the solidification rate of the polymer phase, thus minimizing the outward diffusion of BSA-containing droplets to the continuous water phase during the solidification process.

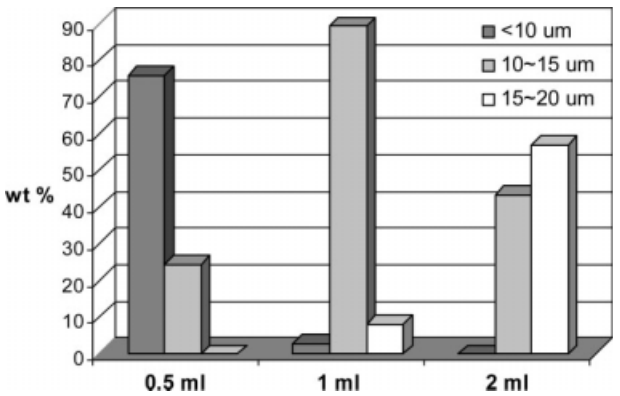

Figure 9 Size distribution of microspheres fabricated with various volumes of W1 in the presence of 5% NaCl in W2 and 0.1% PVA in W1.

Microspheres made from PLGA/PEG blends

Several groups have used PEG to make blends with PLGA in the fabrication of microspheres as a protein stabilizer and a surface modifier of the PLGA network[34,35] or as a water-soluble additive to increase the diffusion of the protein and polymer degradation products.[36] To investigate the influence of PEGs with different molecular weights on the drug loading and the initial burst of PLGA microspheres, PEG 10,000 and PEG 35,000 were selected to make blends with PLGA in this study.

Table VIII shows the drug loading and the initial burst release of microspheres fabricated with different ratios of PEG to PLGA. The protein encapsulation efficiency dramatically decreased when the molecular weight of PEG increased from 10,000 to 35,000 with the same ratio of PEG to PLGA. On the other hand, with the same kind of PEG, the

TABLE VI
Characteristics of Microspheres (G2) with Various Inner Water Volumes (5% NaCl in W2)

Inner water volume (mL)	Protein encapsulation efficiency (%)	Mean particle diameter (μm)	Burst release (%)
0.5	42.9 ± 2.1	9.4 ± 0.9	92.4 ± 3.6
1	50.8 ± 2.4	12.7 ± 1.2	88.2 ± 3.1
2	56.2 ± 1.6	15.1 ± 2.1	63.1 ± 3.2

TABLE VII
Characteristics of Microspheres (G2) Produced with Solvent Evaporation and Solvent Extraction Methods (5% NaCl in W2 and 0.1% PVA in W1)

Fabrication method	Protein encapsulation efficiency (%)	Burst release (%)
Solvent evaporation	56.8 ± 1.3	45.1 ± 3.1
Solvent extraction	59.1 ± 5.7	34.1 ± 6.8

TABLE VIII
Characteristics of Microspheres Fabricated with Different Ratios of PEG to PLGA

PEG	Ratio of PEG to PLGA	Protein encapsulation efficiency (%)	Drug loading (%)	Burst release (%)
PEG 10,000	1 : 4	62.8 ± 5.4	7.3 ± 0.6	77.1 ± 0.6
	1 : 2	19.0 ± 1.8	2.3 ± 0.2	79.1 ± 6.6
PEG 35,000	1 : 4	30.0 ± 2.5	3.6 ± 0.3	80.3 ± 4.6
	1 : 2	16.9 ± 3.3	2.1 ± 0.4	81.2 ± 2.3

The concentration of PLGA was maintained at 57.1 mg/mL in the oil phase.

encapsulation efficiency decreased with the increase in the PEG content in the oil phase. This may be due to the fact that the addition of high-molecular-weight PEG to the polymer phase could create an osmotic pressure in W1 as PEG dissolved in W2 may lead to just a small concentration because of the relatively large volume of the continuous water phase. This osmotic pressure in W1 promoted the water influx from the continuous phase to W1 and thus resulted in the low encapsulation efficiency of BSA. Apparently, the higher the PEG concentration was, the higher the osmotic pressure was. The initial burst release of PEG-containing microspheres was quite high, as reported earlier,[36] and it was higher for microspheres fabricated with PEG 35,000 than for the other group. This result may be related to the porous structure of microspheres fabricated with water-soluble PEG (Fig. 10), which was caused by the dissolution of PEG in water phases during the solidification process.

CONCLUSIONS

The morphology and drug loading are interrelated factors influencing the initial burst release of PLGA microspheres fabricated with the double-emulsion solvent evaporation/extraction method. In this case, different fabrication variables were investigated for their effects on the characteristics of microspheres for optimizing the processing conditions. The presence of salts/sugars in W2 strongly influenced the morphology, drug loading, and initial burst release, but the ultimate effects depended on the particle size. The addition of surface-active substances such as PVA, Pluronic F127, and Tween 80 degraded the protein encapsulation efficiency. However, the simultaneous addition of salts to create an osmotic pressure in W2 helped to significantly increase the encapsulation efficiency. The protein encapsulation efficiency of the microspheres increased with both the polymer concentration and an increase in the internal water volume. The solvent extraction method yielded a higher encapsulation efficiency and a lower initial burst in comparison with solvent evaporation. Finally, for microspheres fabricated from PLGA/PEG blends, the protein encapsulation efficiency dramatically decreased when the molecular weight of PEG increased from 10,000 to 35,000 with the same PEG/PLGA ratio. Conversely, with the molecular weight constant, the encapsulation efficiency decreased with the increase in the PEG/PLGA ratio. This was attributed to the formation of an osmotic pressure in W1, as the water-soluble PEG phase dissolved out during the solidification process.

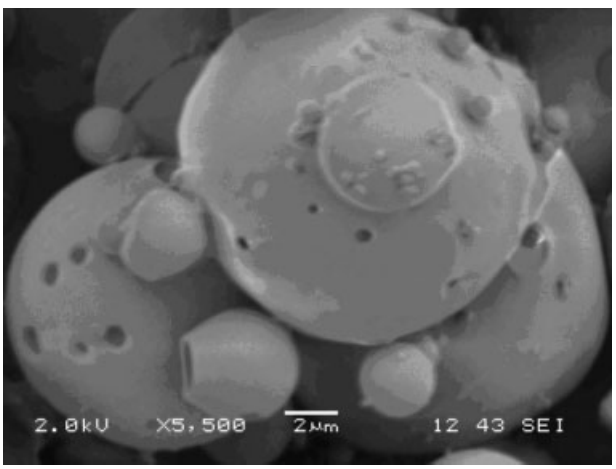

Figure 10 SEM picture of the surface of microspheres fabricated from a PLGA/PEG blend (PEG 10,000; weight ratio of PEG to PLGA = 1 : 2).

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