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Biomaterials 22 (2001) 231–241

Biomaterials

Morphology, drug distribution, and in vitro release profiles of biodegradable polymeric microspheres containing protein fabricated by double-emulsion solvent extraction/evaporation method

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Received 10 December 1999; accepted 28 April 2000

Abstract

The surface and internal morphology, drug distribution and release kinetics at 22°C of polyesters such as PCL (polycaprolactone) and PLGA (poly(DL-lactic-*co*-glycolic acid)) 65:35 microspheres containing BSA (bovine serum albumin) have been investigated in order to understand the relationship amongst morphology, drug distribution and in vitro release profiles and to develop controlled release devices for marine fishes in tropical area. CLSM (confocal laser scanning microscope) micrographs reveal that the polyvinylalcohol (PVA as an emulsifier) concentration in the external water phase strongly influences drug distribution within microspheres and release profiles. The presence of PVA in the internal water phase enhances the stabilization of inner water droplets against coalescence. This results in a more uniform drug distribution and a slower BSA release. Different oil-phase volumes and polymer concentrations yield different solvent exchange and precipitation mechanisms, which lead to different morphologies. A low oil-phase volume yields microspheres with a porous matrix and defective skin surface, which gives a high initial BSA burst as well as a fast release profile. Microspheres fabricated from a low polymer concentration have less defective skin surface, but with a less tortuous inner matrix which results in a more rapid BSA release. A higher BSA loading yields a larger concentration gradient between the emulsion droplet and the continuous water phase as well as between the microspheres and the in vitro medium. The former results in a lower encapsulation efficiency, whereas the latter yields a faster initial burst and a more rapid release profile. High stirring speed can reduce microsphere size, but decreases the yield of microspheres.

Keywords: Microsphere morphology; Drug distribution; In vitro release profiles; Polycaprolactone; Poly(DL-lactic-co-glycolic acid) 65:35

1. Introduction

The marine fish culture industry faces significant problems in synchronized spawning and reproduction of groupers. Groupers are one of the favorite food fishes in the Southeast Asian region. Female groupers outnumber males because males are produced only when adult females reverse sex [1,2]. In order to meet the increasing demand for functional male brood stock in the sustained growth of the grouper culture industry, various efforts were made to induce sex reversal and spawning of groupers by multiple injections of hormone [3–5]. LHRH-controlled release microsphere system may provide a better approach because it may induce sex reversal and synchronized spawning of fish as planned. Double-emulsion solvent extraction/evaporation technique is the most commonly used method to encapsulate hydrophilic drugs, especially protein and peptide drugs, into polymeric microspheres [6–10]. Size and release properties of microspheres are the key considerations to design microsphere delivery systems. Since the release kinetics of protein dominantly depends on polymer nature, morphology and drug distribution within microspheres, fundamental understanding of the relationship among these key characteristics and release mechanisms is essential to yield useful products.

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0142-9612/01/$ - see front matter
PII: S0142-9612(00)00178-2

Biodegradable polyesters such as PLA (poly (lactic acid)), PLGA (poly(lactic-*co*-glycolic acid)) and PCL (polycaprolactone) have been widely used as carriers in controlled-release delivery systems due to their biocompatibility. The degradation of these polyesters involves a bulk erosion process [11,12]. Since the bulk erosion can accelerate the diffusion and release of drug, the drug release mechanism based on these polyesters is quite complicated. An unpredictable release profile brought about by the bulk erosion may lead to a lack of control in drug release. Polyorthoesters and polyanhydrides provide alternative approaches to achieving desired release profiles and zero-order release kinetics due to their surface erosion mechanism [13,14]. Numerous efforts have also focused on optimization of fabrication variables, blending of polymers and synthesis of copolymers with different compositions [15–17] to yield better products. Giunchedi et al. [18] utilized blends of PCL and cellulose acetate butyrate to modulate the release rate of ketoprofen from microspheres. Embleton et al. [19] reported that poly-hydroxybutyrate–hydroxyvalerate microcapsule porosity was controlled using PCL blending. Lemmouchi et al. [20] synthesized copolymers of PCL with lactides and trimethylene carbonate and suggested that the degradation rates of the copolymers could be varied using different chemical compositions. Since polyesters with high molecular weights such as PLA, PLGA and PCL have a very slow degradation rate especially at low temperatures [21,22], it is a challenge to design a microsphere-controlled-release matrix based on these polyesters for marine fish applications. To make a breakthrough, one has to fully understand the relationship among morphology, drug distribution and release kinetics. This piece of information is essential for scientists to design biodegradable polymeric microspheres with desirable protein release profiles for marine fish.

In this work, we use biodegradable polyesters (PCL and PLGA) to encapsulate a model protein, BSA (bovine serum albumin) within microspheres. The aim of this study is to further investigate the effects of fabrication variables on surface and internal morphology, drug distribution and release kinetics profiles at 22°C which is close to marine fish body in tropical area, and to study the relationship among these factors. PCL is chosen because of its ideal physical properties, biocompatibility and biodegradability. Compared to other synthetic polymers such as PLA and PLGA, PCL has a much lower cost, which makes it more practical for applications in aquaculture.

2. Experimental

2.1. Materials

PCL polymers with M_n averages of 10 000, 42 500 and 80 000 were supplied by Aldrich Chemical Company, while PLGA 65:35 polymer with a M_w range of 40 000–75 000 (Lot 77H1208) were obtained from Sigma Chemical Company. PVA hydrolyzed 88% (Polyvinylalcohol) with a M_w range of 31 000–50 000 which was purchased from Fluka Company. BSA (fraction V, 58 kDa) was supplied by Sigma Chemical Company. Methylene chloride of liquid chromatography grade was obtained from Merck. All other reagents were of reagent grade and used as received.

2.2. Preparation of microspheres

A modified water-in-oil-in-water (W/O/W) double-emulsion solvent extraction/evaporation method was employed to fabricate BSA-containing microspheres. A BSA/PBS (phosphate buffer saline) solution (referred to as the internal water phase) with a certain percentage of PVA was emulsified for 20 s with a polymer/methylene chloride solution (oil phase) using a sonicator with a 3 mm probe (Sonics & Materials Inc., Connecticut, USA) at an output power of 50 W. The resulting first emulsion (W/O) was injected using a glass syringe with a 21.5 G needle into a 250 ml PBS (pH 7.4) solution (referred to as the external water phase) with varying concentrations of PVA to produce a double W/O/W emulsion. This solution was stirred for 30 min using a mixer (Cole-Parmer Instrument Co., Illinois, USA) at constant room temperature (22°C). In order to remove methylene chloride to the external phase, 640 ml PBS buffer solution containing the same concentration of PVA as the internal water phase was continuously added to the solution at a rate of 3 ml/min. The resulting BSA-containing microspheres were filtered out and washed three times with de-ionized water. The microspheres were then vacuum-dried overnight and stored at 4°C.

2.3. In vitro BSA release study

In vitro release tests were carried out in triplicate at constant room temperature (22°C) which is close to the body temperature of marine fish in tropical area. A 20 mg aliquot of dried microspheres was placed in a 2 ml microfuge tube and incubated in 1 ml of PBS buffer. Sample tubes were placed on a Stuart Scientific 3D Rocking Platform. The supernatant from each tube was periodically removed and replaced with fresh buffer after being centrifuged for 5 min at 14 000 rev/min using an Eppendorf Centrifuge (Model 5415C). The BSA content of the supernatant was analyzed using HPLC (HP1050, Hewlett Packard). A Zorbax GF-250 column (4.6 mm × 25 cm, Dupont Company) packed with spherical silica was used as the analytical column. The mobile phase was PBS (pH 7.0) and the flow rate was 1.0 ml/min. Sample injection volume was 20 μl and the UV detection was at 210 nm. According to our experimental results, BSA absorbance is more sensitive at 210 nm than 220 nm

which was used by Sah et al. (17). The total analysis time was 8 min.

2.4. Determination of BSA content in the microspheres

The amounts of BSA encapsulated per unit weight of microspheres were determined by an extraction method. 10 mg of dried microspheres was dissolved in 1 ml of methylene chloride for 20 min and 1.0 ml of PBS (pH 7.4) was then added. The mixture was vigorously shaken for 2 min in order to extract BSA into PBS from the organic solution. After centrifuging, the aqueous solution was withdrawn and the BSA content of the solution was analyzed using a bichinchoninic acid (BCA) protein micro-assay. The encapsulation efficiency was expressed as the ratio of actual-to-theoretical BSA content.

2.5. Particle size analysis

The size of microspheres was estimated using a Nikon polarizing microscope (Optiphot2-pol, Japan). Dried microspheres were first re-dispersed in distilled water and placed onto a glass slide. The images were captured using a personal computer running on ImagePro and analyzed using built-in software to calculate individual microsphere size and their mean value. The average microsphere size employed in this paper was referred as the number average diameter.

2.6. Scanning electronic microscope (SEM)

The surface morphology of microspheres was observed by SEM (Model JSM-5310, JEOL, Tokyo, Japan). Microspheres were mounted onto metal stubs using double-sided adhesive tape. After being vacuum-coated with a thin layer (100–150 Å) of gold, the microspheres were examined by SEM at 15 or 10 kv.

2.7. Drug distribution

A Bio-Rad confocal laser scanning microscope (CLSM, MRC 1024, England) equipped with filters for 488 nm (Blue), 568 nm (Yellow), 647 nm (Red) and 488 + 568 nm excitation wavelengths were employed to observe BSA distribution within microspheres because BSA fluoresces (see the appendix picture) [23]. The microspheres were re-dispersed in distilled water and placed onto a glass slide, and the image was taken. In this work, an excitation wavelength (laser power: 100%) of 488 nm and a 522 DF 32 emission filter were used, and a Photo Miltiplier Tube 2 (Iris: 6, Gain: 1300, low signal) was selected. Filter blocks were T2A (560 DRLP) and B1 (Beamsplitter). All the Z section images were obtained under the same resolution.

2.8. Determination of polymer molecular weights

Molecular weights of polymer raw materials and microspheres used for in vitro release were determined by gel permeation chromatography (GPC) (Waters 2690, MA, USA). A differential refractometer detector (Waters 410, MA, USA) was employed. Two columns (Styragel@HR 5E, Lot No. T81081, 7.8 mm × 300 mm and Styragel@HR 4E, Lot No. 780751, 7.8 mm × 300 mm) with the effective molecular weight ranges of 2000–4 000 000 and 50–100 000, respectively, (MA, USA) were connected in series. The mobile phase was tetrahydrofuran (THF) with a flow rate of 1 ml/min. The dried microspheres were dissolved in THF and filtered. The polymer solution injection volume was 100 μl. The data collection and analysis were done using Waters Millennium^{32} software. Weight and number average molecular weights were calculated from a calibration curve using a series of polystyrene standards (Polymer Laboratories Inc., MA, USA) with molecular weight ranging from 1350 to 151 700. The total analysis time of every sample was around 50 min.

3. Results and discussion

3.1. Stirring speed

Size of microspheres is determined by the stirring speed, viscosity of polymer solution and internal water phase, volume ratio of oil phase to external water phase and PVA concentration in external water phase [24–27]. Stirring speed is the dominating factor because it provides the energy to disperse the oil phase in water. Our experimental results demonstrate that a high stirring speed yields smaller microspheres because the second emulsion is broken up into smaller droplets at a higher input power, as illustrated in Fig. 1. However, yield is lower because microspheres are broken down more easily at a higher input power. For example, the yield is decreased from 90.0 to 85.1% when the stirring speed is increased from 500 to 700 rpm. Thus, the stirring speed needs to be optimized in order to obtain a sufficiently high yield of microspheres with a desired size distribution. In this work, all of the double emulsions were produced at 500 rpm.

3.2. PVA concentration in the internal water phase

In this study, the addition of PVA to the internal water phase is to enhance the primary emulsion stability and protein encapsulation. It has been reported [28–31] that BSA, Pluronic F68, Poloxamer 188 and 311 have characteristics of a stabilizer for the water-in-oil emulsion in PLA/PLGA–CH_2Cl_2 systems because of their

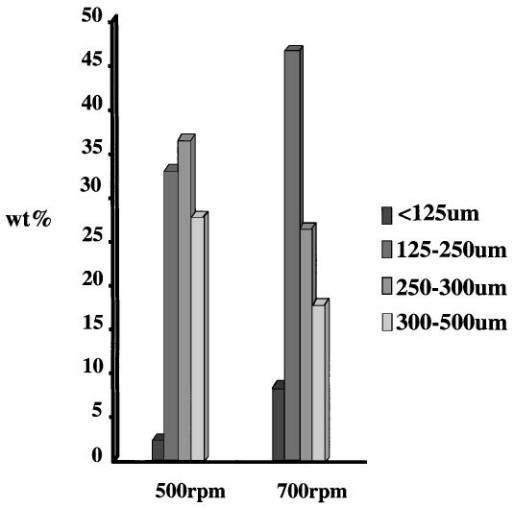


Fig. 1. Size distribution of microspheres fabricated at different stirring speeds. PCL M_n 10 000.

interactions with PLA or PLGA. Since PCL is more hydrophobic than PLA or PLGA, the interactions between BSA and PCL might not be strong. Therefore, PVA may help to stabilize the water-in-oil emulsion in the PCL–CH_2Cl_2 system. Fig. 2 shows the CLSM images of microspheres fabricated with different PVA concentrations in the internal water phase. Since PCL is not fluorescent, the bright color area qualitatively represents BSA. It is clear that a low PVA concentration (e.g. 0.025%) yields microspheres with much bigger pores in the inner structure. However, the drug distribution within the microspheres fabricated at a high PVA concentration (e.g. 0.5%) is more uniform. This may be due to the fact that the presence of PVA stabilizes inner water droplets against coalescence. In other words, it is easy for inner water droplets containing a low concentration of PVA to coalesce with each other and form interconnecting water channels that can increase release rate of BSA. Fig. 3 confirms our hypothesis that a high PVA concentration yields microspheres with a low BSA release rate. It is also observed that decreasing PVA concentration of the internal water phase results in a decrease in encapsulation efficiency (Table 1). This may arise from a possible interaction between PVA and BSA, which can protect BSA from solvent and prevent BSA diffusion towards the external water phase. Consequently, a higher encapsulation efficiency and a lower initial burst are obtained at a high PVA concentration. In addition, we also noticed that a high PVA concentration (0.1%) in the internal water phase led to an increase in the viscosity of the primary emulsion [25]. Hence, it was more difficult to break up the solution into smaller droplets at the same power of mixing, resulting in slightly bigger microspheres (Table 1).

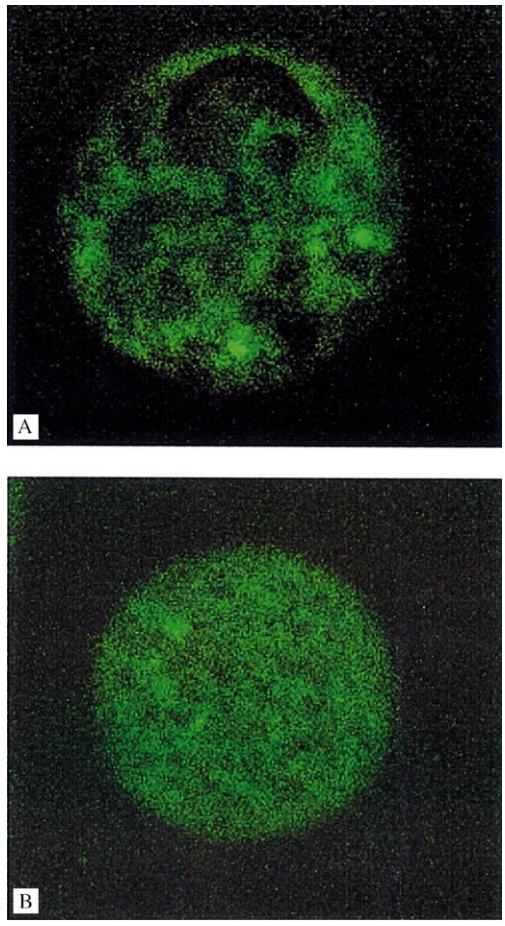


Fig. 2. CLSM images of microspheres fabricated at different PVA concentrations in the internal water phase. A: 0.025%, B: 0.1%. PCL M_n 10 000.

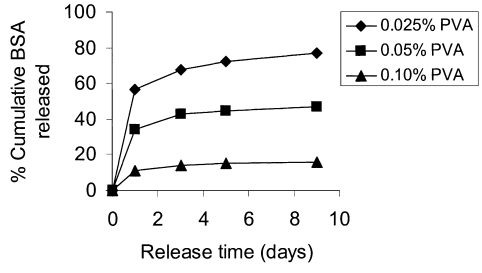


Fig. 3. Effect of PVA concentration in the internal water phase on release profiles. PCL M_n 10 000.

3.3. PVA concentration in the external water phase

PVA concentration in the external water phase is known to be a key factor to influence the size of microspheres [25,32–34]. In the present work, the microspheres are fabricated at 0.05, 0.1 and 0.5% PVA in order to examine the effect of PVA concentration in the external water phase. Fig. 4 shows the surface morphology of corresponding microspheres. All the microspheres

Table 1
Effect of PVA concentration in the internal water phase on the properties of BSA microspheres[a]

PVA conc. (g/l) (%)	Size (μm)	Encapsulation efficiency (%)	Initial BSA burst[b] (%)
0.025	98.2	42.8	56.9
0.05	103.8	46.8	34.3
0.1	121.5	70.0	11.1

[a] Preparation conditions: PCL (M_w 10 000), inner water/solvent: 1/40, polymer concentration: 33.3 g/l, DP/CP: 1/21, theoretical BSA loading: 9.09%, 0.1%PVA in the external water phase.
[b] BSA released during the first 24 h.

have spherical shapes with porous outer skins. The sizes of microspheres fabricated at 0.05, 0.1 and 0.5% PVA are 142.6, 125.8, and 103.8 μm, respectively. A significant decrease in microsphere size can be achieved by increasing PVA concentration in the external water phase. PVA concentration has an offsetting effect on size of microspheres. Since PVA is a polymer with a high molecular weight, the presence of PVA in the external water phase may increase the viscosity of the double emulsion, resulting in an increased difficulty in breaking up the emulsion into smaller droplets. Thus, this yields bigger microspheres. On the other hand, the presence of PVA in the external water phase stabilizes emulsion droplets against coalescence, resulting in smaller emulsion droplets. In the present work, it can be concluded that the stabilization effect is dominant at higher PVA concentrations and leads to the decrease in the size of microspheres. Moreover, we observed an interesting phenomenon (Fig. 5) that PVA concentration in the external water phase has a significant effect on release profiles of the prepared microspheres. Roy et al. [27] reported that drug release was inversely proportional to the size of the microspheres due to decreased diffusional path length and increased effective surface area of the microspheres. In contrast, our experimental results show that the larger microspheres fabricated with a low PVA concentration in the external water phase have a more rapid BSA release. This phenomenon may arise from two factors: (1) a higher PVA concentration increases the viscosity of the external water phase and results in an increased difficulty for the BSA aqueous solution to diffuse out, (2) a higher PVA concentration yields a more stable emulsion which hinders the mass transfer of BSA with surroundings. Thus, drug is distributed more evenly within the interior of the microspheres, as shown in Fig. 6.

However, at a low PVA concentration, a less stable emulsion coupled with possible convective mass transfer at skins during the mixing facilitate BSA-containing droplets to diffuse outward. Thus, the drug is distributed more within the exterior of microspheres than within the interior. From Fig. 5, we can also see that the

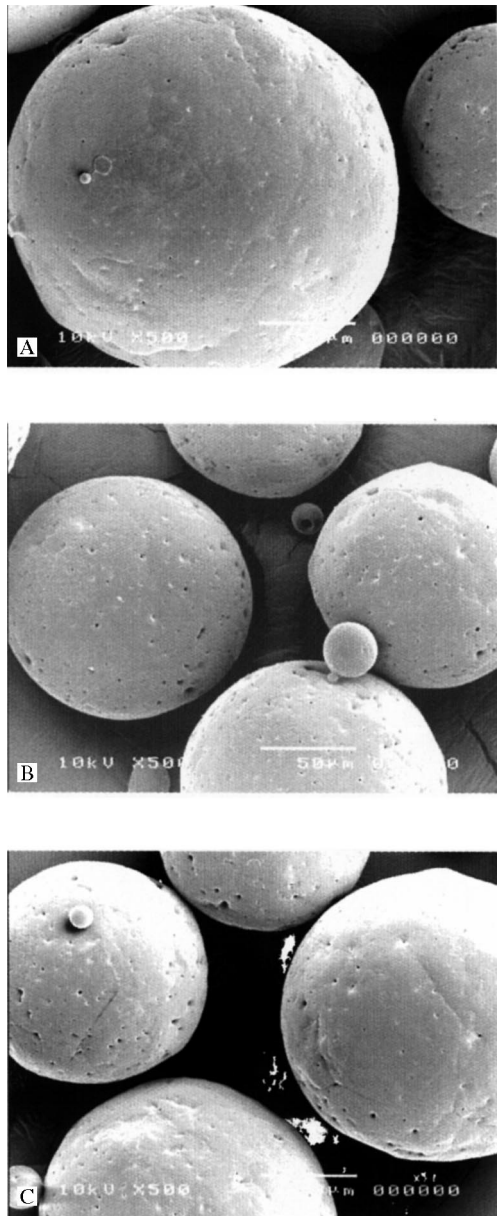


Fig. 4. SEM pictures of microsphere surface fabricated at different PVA concentrations in the external water phase. A, B, C represent 0.05, 0.1, 0.5%, respectively, at 500×, the size of the bar is 50 μm.

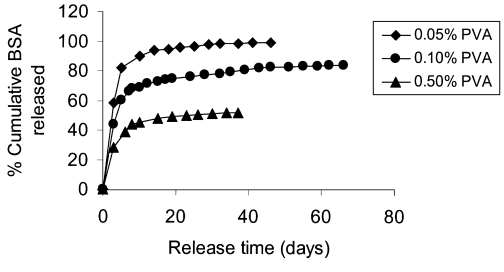


Fig. 5. Effect of PVA concentration in the external water phase on release profiles. PCL M_n 80 000.

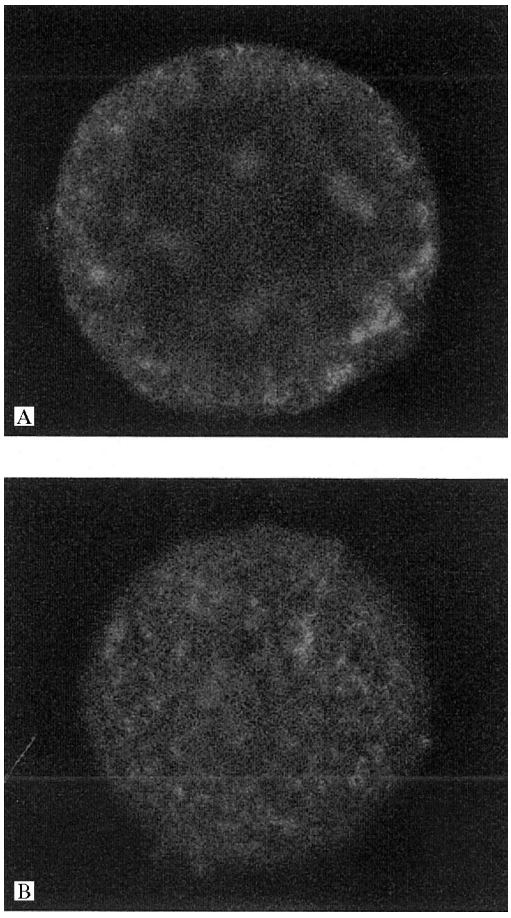


Fig. 6. CLSM images of microspheres fabricated at different PVA concentrations in the external water phase. A: 0.05%, B: 0.5%. PCL M_n 80 000.

microspheres are characterized by a more significant initial release, followed by a slower release. The initial release is attributed to the pore-dependent BSA diffusion. After the accessible protein molecules have diffused, there is a significant reduction in release rate. This is due to the lack of degradation in the PCL microspheres. The encapsulated protein may be loosely bounded to the surface, embedded in the surface layer, or trapped within the microsphere matrix. The protein loosely bounded to the surface and embedded in the surface layer should give rise to the initial release (within a few days). However, the release of protein trapped within the microsphere matrix could depend on the degradation of the microsphere matrix. Based on the GPC results of molecular weight before and after in vitro releases, the degradation of the PCL microspheres almost does not exist even after 60 days of release. As a result, the predominant mechanism of BSA release in these microspheres is diffusion. Therefore, designing morphology and drug distribution is critical to obtain polymeric microspheres with a desired release profile for cases where the polymer has a low degradation rate.

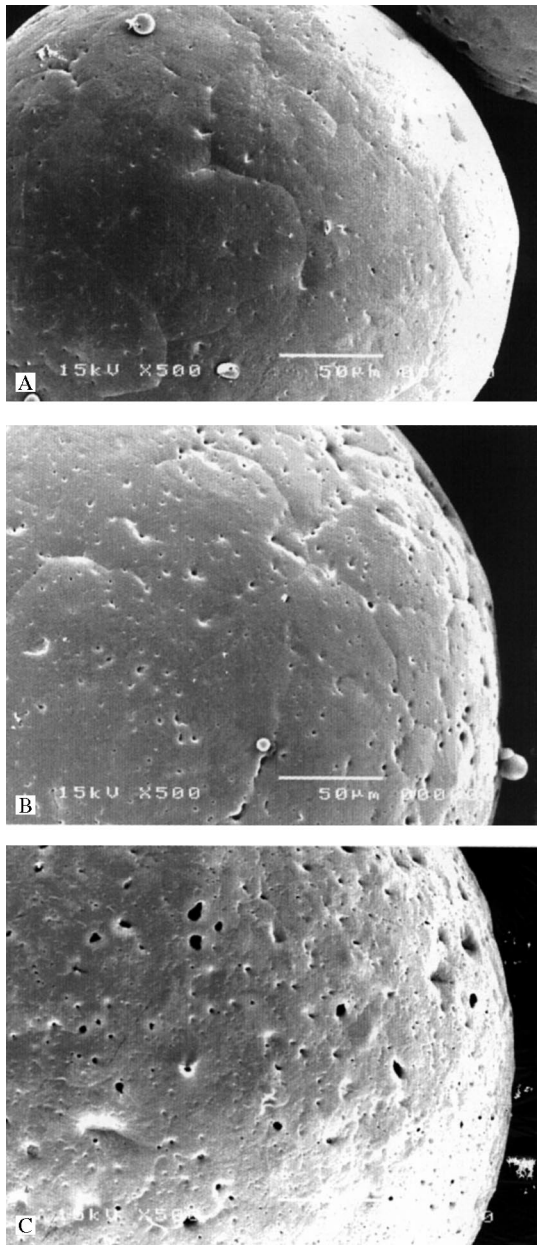


Fig. 7. SEM pictures of microsphere surface fabricated at different oil volumes (the internal water volume is 1.0 ml for all cases). A: 15.0 ml, B: 12.0 ml, C: 9.0 ml at 500×. The size of the bar is 50 μm. PCL M_n 80 000.

3.4. Effect of oil-phase volume

The oil-phase volume also plays a critical role to determine morphology and release profiles of microspheres. Fig. 7 illustrates the surface morphology of microspheres produced with different oil-phase volumes (15.0, 12.0 and 9.0 ml) and a constant internal water-phase volume (1.0 ml). The size of the corresponding microspheres is 96.7, 161.2, and 181.8 μm, respectively. The oil-phase volume has a significant effect on size of microspheres. A low oil volume yields a viscous and concentrated

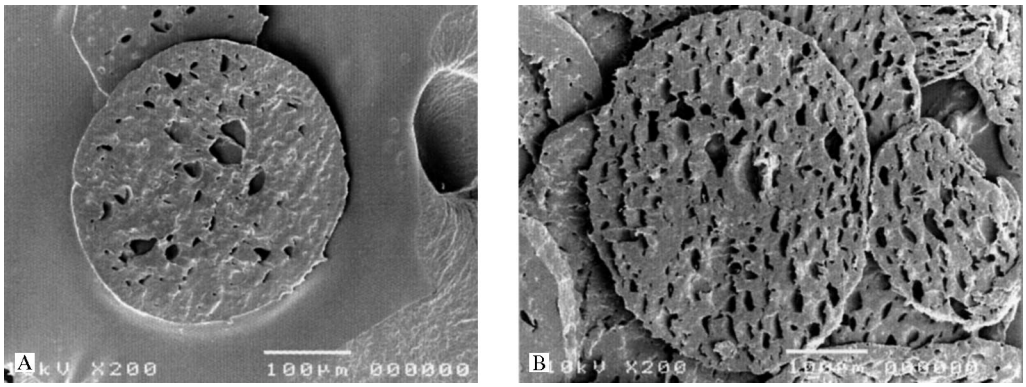


Fig. 8. SEM pictures of microsphere crosssection fabricated at different oil volumes (the internal water volume is 1.0 ml for all cases). A and B represent 15.0 and 9.0 ml, respectively, at 200×, the size of the bar is 100 μm.

polymer solution, so that it is more difficult for the polymer solution to be broken up into smaller oil droplets during the second emulsion. Moreover, oil-phase volume also has a significant impact both on the surface and the inner morphology of the microspheres (Figs. 7 and 8). A low oil volume yields a matrix characterized by more numerous and bigger pores. Since a low methylene chloride (oil) content results in a more viscous solution, it is more difficult to break up the internal water into smaller droplets during the first emulsion induced by sonication. The water droplets trapped at the interior of microspheres evaporate and leave empty spaces after drying. In addition, since water has a very high surface energy (72.8 mJ/m^2), it is very likely that the internal water droplets may coalesce with one another when the oil volume is low and yields a porous internal matrix with big holes, confirmed by CLSM (Fig. 9).

The size of the microspheres fabricated at 9 ml of oil volume is much bigger (181.8 μm) than those fabricated at 15 ml of oil volume (96.7 μm). The intensity of fluorescence within the big microsphere (Fig. 9B) is weaker near its center than the skin since the emitted fluorescence of BSA travels a longer distance from the interior than from the exterior. Since the tortuosity of diffusion paths for BSA within a porous microsphere is significantly reduced, a low oil volume yields microspheres with a fast BSA release, as illustrated in Fig. 10.

3.5. Polymer concentration

Polymer concentration is also a key factor influencing the characteristics and release profiles of microspheres. The size of the microspheres fabricated at 16.7 and 33.3 mg/ml of polymer solutions (volume ratio of internal water to oil: 1 : 24) is 97.9 and 125.8 μm, respectively. The increase in size of the microspheres with increasing polymer concentration arises from the more viscous (concentrated) polymer solution. Even though the microspheres fabricated at these two polymer concentrations have no sharp differences in surface porosity, the CLSM images show that the microspheres fabricated from the lower polymer concentration have a more porous matrix compared with the microspheres fabricated from the higher concentration (Fig. 11). This may be due to two factors: (1) the internal water droplets in the low polymer concentration solution tend to coalesce together more easily, leading to bigger pores and a less tortuous network, (2) the high polymer concentration solution coagulates faster during the second emulsion and yields a tighter structure because of chain entanglement. Consequently, a low polymer concentration yields microspheres with a more rapid release profile (Fig. 12). Moreover, the smaller microspheres resulting from a low polymer concentration may also contribute to the increase in the release rate.

3.6. Molecular weight

PCL molecular weight also plays an important role in microspheres preparation. At a constant solvent volume, the PCL with a M_n of 80 000 kg/mol yields a more viscous solution than the PCL with a lower M_n (42 500 kg/mol). Therefore, the former yields larger microspheres (125.8 μm) than the latter (95.0 μm). In addition, the high Mn PCL leads to microspheres with a more defective (porous) surface (*pictures not shown*) possibly due to a less stable primary emulsion and a much more rapid polymer coagulation. Fig. 13 illustrates the release profiles for these two types of PCL microspheres and indicates a slightly higher initial release rate for microspheres made of the low Mn PCL. Diffusion is the dominant release process for PCL microsphere systems. The slightly higher initial release rate is probably due to the fact that the low Mn PCL produces smaller sized microspheres and thus have more surface area for diffusion.

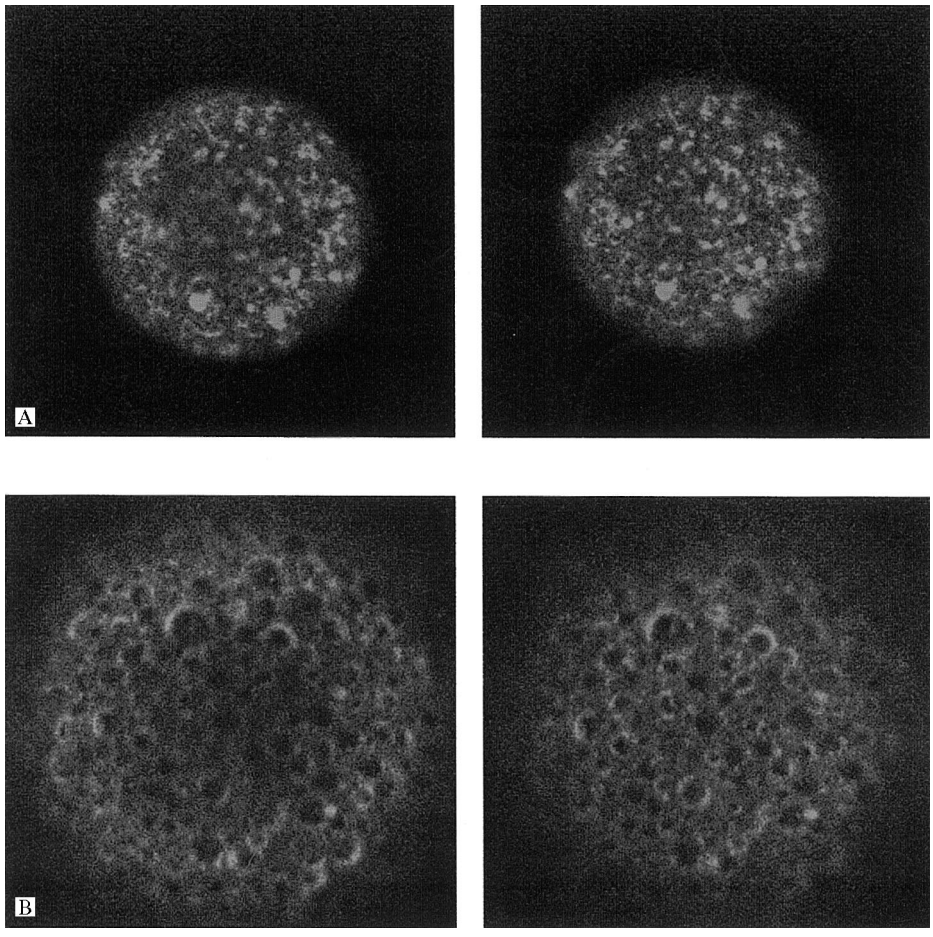


Fig. 9. Z section CLSM images of microspheres fabricated at different oil volumes. A: 15.0 ml, B: 9.0 ml. PCL M_n 80 000.

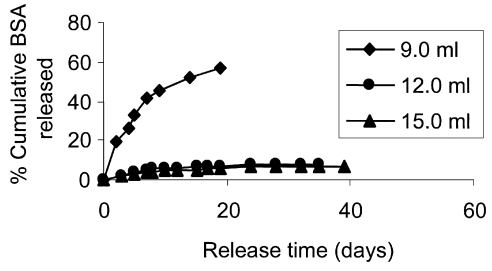


Fig. 10. Effect of oil volume on release profiles. PCL M_n 80 000.

3.7. BSA loading

The effect of BSA loading on the properties of microspheres is listed in Table 2. The results shown are consistent with the previous work [16,35,36]. Basically, BSA loading has no significant effect on the size of microspheres. However, an increase in BSA loading reduces the encapsulation efficiency of BSA. A higher loading provides a higher BSA concentration in the emulsion droplets. This increase in the concentration gradient of BSA between the emulsion droplets and the continuous water phase increases the amount of BSA dissolving into the continuous water phase.

The release profiles and the initial bursts are also related to the degree of actual BSA loading (Fig. 14). The lower the actual BSA loading, the lower the initial bursts. The release profile of microspheres with an actual BSA loading of 2.64% is found to be quite different from those with 0.66 and 0.45% of actual BSA loading. At 0.66 or 0.45% loading, BSA follows a very slow and pseudo-linear release after an initial burst. However, at 2.60% loading, BSA release rate varies, decreasing as a function of time.

The difference in release rates is due to the fact that there is a large BSA concentration gradient between the microspheres and the outer water phase when the BSA loading is high. Since the gradient is the driving force for BSA diffusion, 2.64% BSA loading leads to a higher initial burst and a more rapid release rate. Moreover, at a high actual loading level, there may be more BSA distributed near the surface area of microspheres. This leads to the greater initial release. As the BSA releases, it leaves more pores and interconnecting channels for the release of the remaining BSA. However, BSA now has to

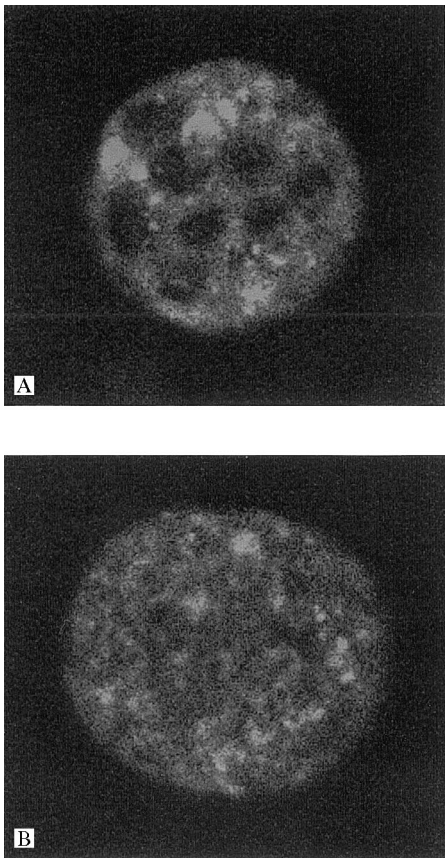


Fig. 11. CLSM images of microspheres fabricated at different polymer concentrations. A: 16.7 mg/ml, B: 33.3 mg/ml. PCL M_n 80 000.

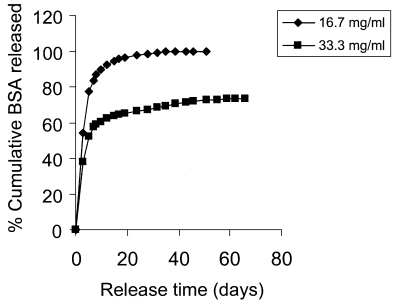


Fig. 12. Effect of polymer concentration on release profiles. PCL M_n 80 000.

travel a longer path under a lower concentration gradient. Therefore the release rate of BSA from the microspheres at the later stage decreases as shown in Fig. 14. When the BSA loading is very low, there is no significant change in the concentration gradient of BSA during the release. Therefore, the BSA release with either 0.66 or 0.45% loading is fairly constant after initial bursts, although much slower. These results suggest that protein loading should be taken into consideration when trying to achieve a desired release profile.

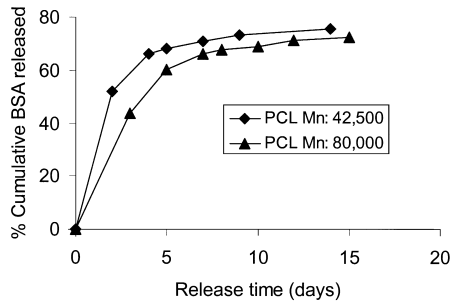


Fig. 13. Effect of polymer molecular weight on release profiles.

Table 2
Effect of BSA loading on the properties of BSA microspheres[a]

Loading of BSA (%), theoretical	Size (μm)	Encapsulation efficiency (%)	Initial BSA burst[b] (%)
4.80	63.1	55.0	41.5[c]
1.10	69.1	59.8	7.57
0.57	61.1	79.1	4.20

[a] Preparation conditions: PLGA 65:35, innter water/oil: 1/24, polymer concentration: 33.3 g/l, DP/CP: 1/21, 0.5% and 0.05% PVA in the internal and external water phase, respectively.
[b] BSA released during the first 24 h.
[c] BSA released during the first 36 h.

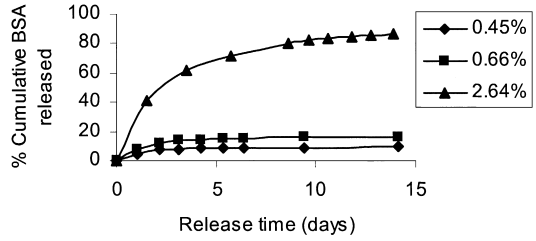


Fig. 14. Effect of BSA loading on release profiles. PLGA 65:35.

4. Summary and conclusions

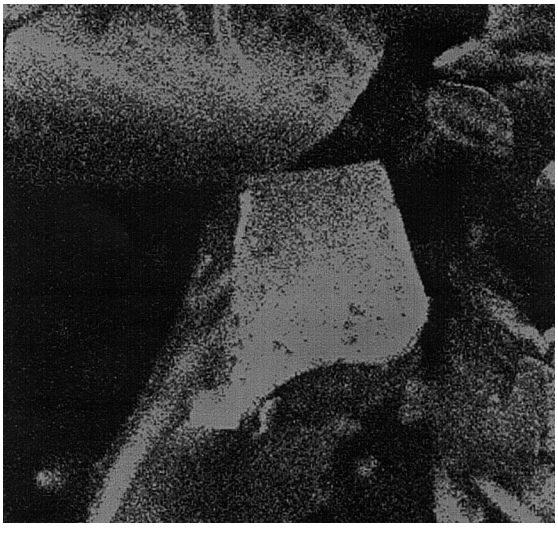
Diffusion through interconnecting pores and channels is a predominant release mechanism for PCL and PLGA microspheres containing BSA at room temperature. In this case, morphology and drug distribution are crucial factors that influence the release profile of the microspheres. CLSM provides a good approach to exploring the internal structure of the microspheres and drug distribution. In this study, we have investigated the effect of fabrication variables of polymeric microspheres on morphology, drug distribution and release kinetics. The presence of PVA in the internal water phase enhances the stability of the primary emulsion for the PCL–BSA–H_2O system. PVA concentration in the external water phase

has a great impact on the size of resultant microspheres and affects the drug distribution within microspheres. We have shown that the viscosity of the polymer solution significantly influences the stability of the primary emulsion and polymer precipitation. Furthermore, since BSA diffusion is driven by concentration gradient, a high BSA loading leads to lower encapsulation efficiency values and a more rapid BSA release.

Acknowledgements

The authors thank Dr. J. Heller at Advanced Polymer Systems Inc., USA, and Dr. W. K. Chan at NUS for their valuable suggestions in this work. They also appreciate the technical assistance from Dr. X. Q. Chen, Mr. K. W. Lee, Ms. L. Wang, Ms. X. L. Bai, Ms. Y. S. Chang at IMA and Mr. Q. W. Lin at IMA.

Appendix A. CLSM image of BSA powder



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