Development of Polylactide Microspheres for Protein Encapsulation and Delivery

Thomas Chandy,[1] Gladwin S. Das,[1] Robert F. Wilson,[1] Gundu H. R. Rao[2]

[1] *Department of Cardiology, University of Minnesota, Mayo Mail Code 508, 420 Delaware Street SE, Minneapolis, Minnesota 55455*
[2] *Department of Lab Medicine Pathology, University of Minnesota, Mayo Mail Code 508, 420 Delaware Street SE, Minneapolis, Minnesota 55455*

Received 22 March 2001; accepted 14 February 2002

ABSTRACT: The development of injectable microparticles for protein delivery is a major challenge. We demonstrated the possibility of entrapping human serum albumin (HSA) and thrombin (Thr) in poly(ethylene glycol) (PEG)-coated, monodisperse, biodegradable microspheres with a mean diameter of about 10 μm. In our earlier studies, diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) analysis was used to characterize the surface of PEG-coated, taxol-loaded poly(lactic acid) (PLA) microspheres. An analysis by DRIFTS revealed that PEG was incorporated well on the PLA microsphere surface. An emulsion of protein (in water) and PLA dissolved in an acetone–dichloromethane (or acetone–chloroform) mixture were poured into an aqueous solution of PEG [or poly(vinyl alcohol) (PVA)] with stirring with a high-speed homogenizer for the formation of microparticles. HSA recovery in microspheres ranged from 13 to 40%, depending on the solvent and emulsification systems used for the preparation. PLA dissolved in a dichloromethane/acetone system and albumin loaded via a PEG emulsification solution (PLA–PEG–HSA) showed maximum drug recovery (39.5%) and drug content (9.9%). Scanning electron microscopy revealed that PEG-coated microspheres had less surface micropores than PVA-based preparations. The drug-release behavior of microspheres suspended in phosphate-buffered saline exhibited a biphasic pattern. An initial burst release (30%) followed by a constant slow release for 20 days was observed for HSA and Thr from PLA–PEG microspheres. PEG-coated PLA microspheres show great potential for protein-based drug delivery. © 2002 Wiley Periodicals, Inc. J Appl Polym Sci 86: 1285–1295, 2002

INTRODUCTION

Poly(lactic acid) (PLA) and copolymers of lactic and glycolic acids are well-known biodegradable and histocompatible aliphatic polyesters. They are commonly used as biodegradable sutures,[1,2] and they have more recently contributed to the reconstruction of deficient or injured organs and to improved galenic formulations.[3,4] Protein delivery from biodegradable polymer systems has been a challenging area of research because of the necessity of improving the delivery of newly developed macromolecular drugs and antigens.

During the last few years, several techniques for drug encapsulation have been developed that currently use aliphatic polyesters. A large variety of organic molecules have accordingly been encapsulated, ranging from low molecular weight synthetic drugs to biological proteins.[5,6] Proteins are efficiently encapsulated by a modified solvent-evaporation method based on double emulsions[7–9] and by a phase-separation or coacervation process.

Biodegradable poly(ethylene glycol) (PEG)-coated nanospheres and microcapsules[8,9] have important potential therapeutic applications as injectable blood-persistent systems for the controlled release of drugs, site-specific drug delivery,[10] and medical imaging.[11] Bazile et al.[12] found that hydrophilic coatings with PEG could increase the blood half-life of PLA nanospheres in rats up to several hours. The main advantage of PEG-coated nanospheres over other long-circulating systems is their shelf stability and ability to control the release of an encapsulated compound.[9,10] Recent studies of Quellec et al.[11] have shown that nanosphere preparations of amphiphilic diblock PEG–PLA copolymers constitute an efficient protein-delivery system.

Our goal was to use PLA as a matrix to develop stable microspheres for protein delivery. A PEG coating was employed to increase the capsule shelf stability and biocompatibility. Human serum albumin (HSA) and thrombin (Thr) were chosen as model proteins for the encapsulation studies in this modified PLA–PEG system. Drug-loaded PLA–PEG microspheres were found to have great potential for protein-based drug delivery.

Correspondence to: T. Chandy (chand025@tc.umn.edu).

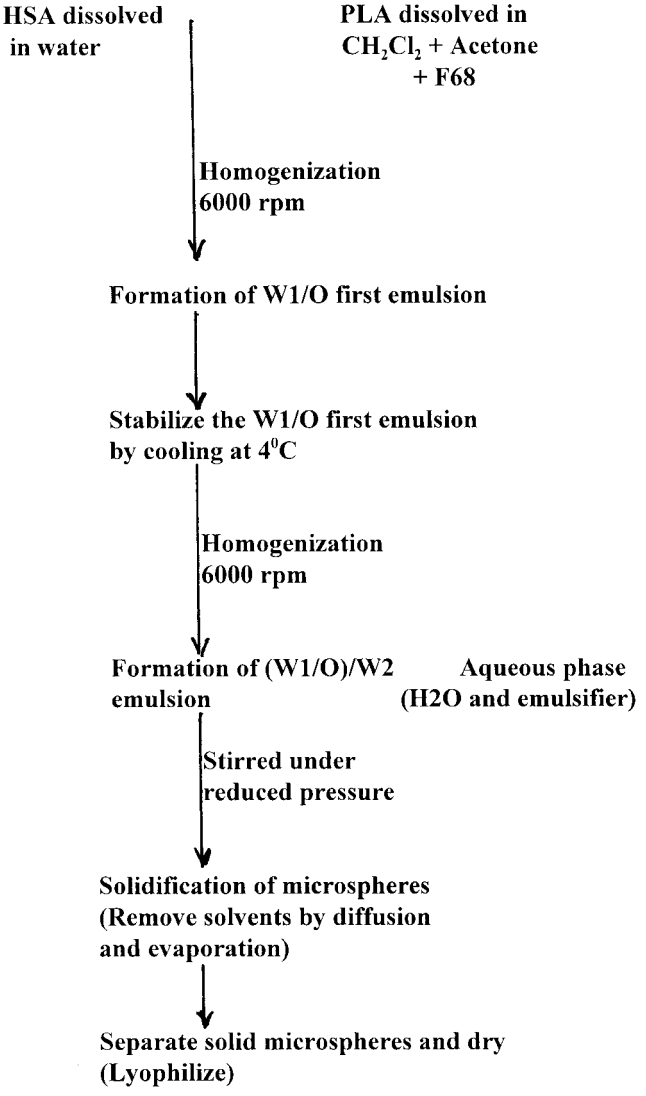
Journal of Applied Polymer Science, Vol. 86, 1285–1295 (2002)

MATERIALS AND METHODS

PLA (molecular weight = 25,000), poly(DL-lactic-*co*-glycolic acid) (PLGA; 70:30, molecular weight = 10,000), and poly(vinyl alcohol) (PVA; 98% mole-hydrolyzed, molecular weight = 25,000) were obtained from Polysciences, Inc. (Warrington, PA). PEG (molecular weight = 20,000), albumin (human, fraction V, 96–98% pure), Thr, pluronic F68, and a protein assay kit (the Lowry method) were acquired from Sigma Chemical Co. (St. Louis, MO). All organic solvents were either HPLC-grade or American Chemical Society analytical-grade reagents.

Microsphere preparation process

The concept of the preparation method was based on the double-emulsion/solvent diffusion technique.[6] Typically, 100 mg of HSA was dissolved in 0.5 mL of distilled water, and 300 mg of PLA was dissolved in dichloromethane/acetone (8:2) or chloroform/acetone containing 0.05% pluronic F68. The aqueous HSA solution was added to the PLA organic solution, and a water-in-oil (W/O) emulsion was formed through stirring at 6000 rpm with a homogenizer (Polytron, Kinematica GmbH, Littau, Switzerland). This W/O emulsion was added to a 2.5% PEG or PVA aqueous solution with stirring to achieve the water-in-oil-in-water (W/O/W) double-emulsion system. Then, the emulsified system was stirred with a magnetic stirrer under reduced pressure. During the evaporation of the water-immiscible organic solvent (dichloromethane or chloroform) from the droplets of the mixed organic solution (for 3–4 h), the dispersed microdroplets solidified in the aqueous solution. The whole dispersed system was filtered with a membrane filter (pore size = 0.8 μm; Nalgene IL, USA) for the separation of microspheres. They were further washed three times with distilled water for the removal of PEG or PVA residues and any free drugs. The microspheres were dried in vacuo or were lyophilized. The schematic procedure for preparation is shown in Scheme 1. Microspheres of HSA-loaded PLGA were also prepared by the same procedure.



Scheme 1 Flow chart of the water-in-oil double-emulsion (W1/O)/W2 method for the preparation of HSA-loaded PLA–PEG microspheres. W1 refers to the inner aqueous phase consisting of HSA dissolved in water. W2 is the outer aqueous phase consisting of PEG in water. The oil phase is the PLA layer to form the solid microspheres.

Process characterization

The effect of the processing parameters on the preparation process was assessed by the determination of the total microsphere yield, size, surface morphology, protein-loading efficiency, and protein delivery. The total microsphere yield was calculated gravimetrically on the basis of polymer/drug recovery.

Particle size distribution and surface morphology

The microsphere size distribution was obtained with a Coulter Z_2 particle counter (Coulter Electronics, Ltd., Lalton Beds, England) on a suspension of microspheres in an isotonic-buffered saline solution. The shapes and surfaces of the microparticles were observed with a Model S-800 scanning electron microscope (Hitachi, Tokyo, Japan). Dry microcapsules were sputter-coated with 50 Å of gold and examined with a scanning electron microscope.

Drug content in the microspheres and encapsulation efficiency

HSA or Thr-loaded PLA microspheres were dissolved in chloroform, and the protein was extracted by the shaking of the organic solution with a 2× volume of water at 37°C for at least 30 min. The extraction was

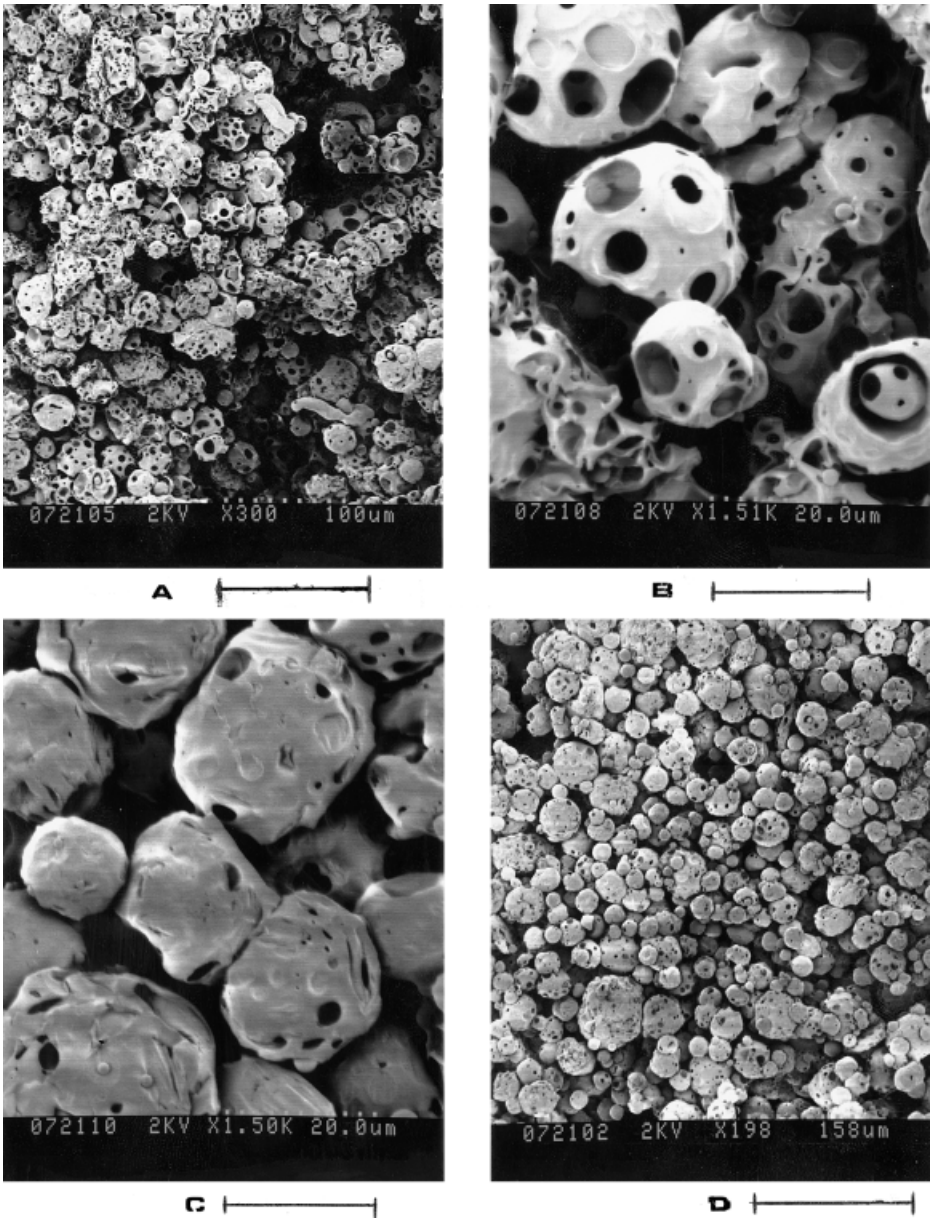


Figure 1 SEM micrographs of HSA-loaded PLA/PLGA microspheres prepared from the dissolution of the polymer in the $CHCl_3$/acetone system: (A) dry PLA–PVA microspheres (scale bar = 100 um, magnification = 300×), (B) the surface morphology (scale bar = 20 μm, magnification = 1510×), (C) PLGA–PVA microspheres (scale bar = 20 μm, magnification = 15000×), (D) PLA–PEG microspheres (scale bar = 158 μm, magnification = 108×), and (E) the surface morphology (scale bar = 20 μm, magnification = 1500×).

repeated three times. The protein concentration in water extracts was analyzed with Lowry's method (Sigma procedure 690) for protein estimation.[13] The absorbance was measured at 725 nm on a Beckman ultraviolet–visible spectrophotometer. The drug (HSA or Thr) recovery and content in the microspheres are represented by eqs. (1) and (2), respectively:

$$\text{Drug Recovery (\%)} = \frac{\text{Amount of Drug in Microspheres}}{\text{Amount of Drug Fed in the System}} \quad (1)$$

$$\text{Drug Content (\%)} = \frac{\text{Amount of Drug in Microspheres}}{\text{Amount of Microspheres Recovered}} \quad (2)$$

In vitro hsa/thr release

In vitro protein release from the microparticles was performed in 0.1*M* phosphate-buffered saline (PBS, pH 7.4) at 37°C in a closed container. At appropriate intervals, a small amount of the sample was withdrawn, centrifuged, and assayed for protein concen-

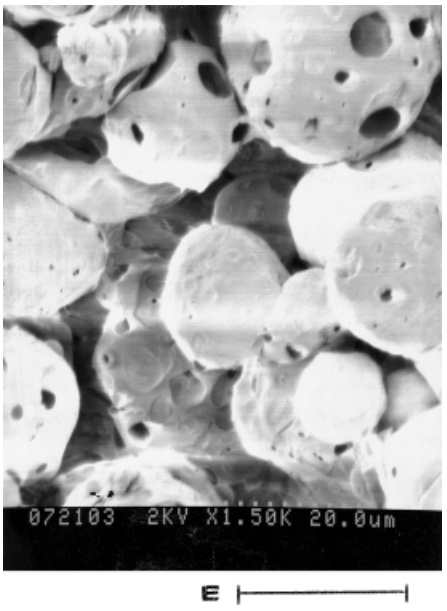


Figure 1 (*Continued from the previous page*)

tration with Lowry's method.[13] An equal volume of PBS was added to the dissolution medium so a constant volume could be maintained. Each determination was carried out in triplicate, and the release results were plotted as the cumulative content and percentage of the content in the dissolution medium versus time.

Evaluation of the thr time

The Thr time was determined with released Thr to certain specific time intervals with a coagulometer (Diagnostica Stago, American Bioproducts Co., New Jersey). The test system consisted of a known amount of a released Thr solution (0.45–0.6 NIH units), incubating 0.1 mL of citrated human plasma (1 mL of 3.8% sodium citrate for 9 mL of blood) and 0.1 mL of $CaCl_2$ (0.025*M*) at pH 7.4 and 37°C. The time required to form a firm clot was registered automatically. A known amount of soluble Thr (0.1 NIH units) was also used as standard for comparing the biological activity of released Thr. The test was repeated at least five times, and the Thr time is expressed in seconds with the standard deviation.

Statistical analysis

All data are presented as the mean plus or minus the standard deviation. PEG-coated and uncoated PLA microspheres were analyzed with an unpaired *t* test. Only *p* values less than 0.05 were considered significant.

RESULTS

Scanning electron micrographs of HSA-loaded PLA and PLGA microspheres and their surface morphology are shown in Figure 1. The microbeads were prepared by the dissolution of the polymer in the chloroform/acetone (8:2) solvent system and were emulsified in an aqueous PVA or PEG dispersing medium. The microspheres were about 15–25 μm in size and spherical in shape, and they had microporous structures (Fig. 1). The surface morphology of microspheres prepared from PLA [Fig. 1(B)] and PLGA [Fig. 1(C)] emulsified with a PVA solution was highly porous with open channels. However, when PEG was used as the emulsion dispersing medium, the surface structures of the microspheres were modified with fewer micropores [Fig. 1(E)]. The open, porous structures on PLA microspheres were filled with PEG.

Figure 2 shows scanning electron microscopy (SEM) photomicrographs of HSA- or Thr-loaded PLA–PVA or PLA–PEG microspheres and their surface morphology. These microparticles were derived by the dissolution of PLA in the dichloromethane/acetone (8:2) system and were emulsified in a PVA or PEG dispersing medium. The microspheres were spherical in shape and 8–12 μm in diameter and had a smooth surface structure [Fig. 2(B,E)]. The surface morphology was modified by the PEG coating on PLA microspheres, as is evident from Figure 2(E,F). It seems that hydrophilic PEG made the PLA microspheres smoother and nonporous.

Table I summarizes the particle size, microsphere yield, drug recovery, and drug content of PLA microspheres prepared in the chloroform/acetone system. The drug recovery and drug content in the PLA microspheres were higher in PEG-coated cases than in PVA emulsions. The diameter of the PLA microspheres varied from 15 to 25 μm for all preparations from the chloroform/acetone system, as presented in Figure 3.

The properties of drug PLA/PLGA microspheres prepared from the dichloromethane/acetone system are presented in Table II. The microsphere yield was higher (52–81%) with the dichloromethane/acetone system with chloroform/acetone (51–57%), as evident from Tables I and II. The drug recovery and content were also greater in the microspheres prepared from the dichloromethane/acetone solvent system. Furthermore, the PEG-coated PLA microspheres had greater drug recovery and content than PVA-coated microcapsules. The mean diameter of HSA-loaded microspheres measured with the Coulter particle counter was around 8–15 μm, and the standard deviations were rather low (monodispersity), as shown in Figures 4 and 5. These values agreed with the observations by SEM (Fig. 2). The particle size measurements (Figs. 3–5) indicated a sharp size cutoff below 10 μm.

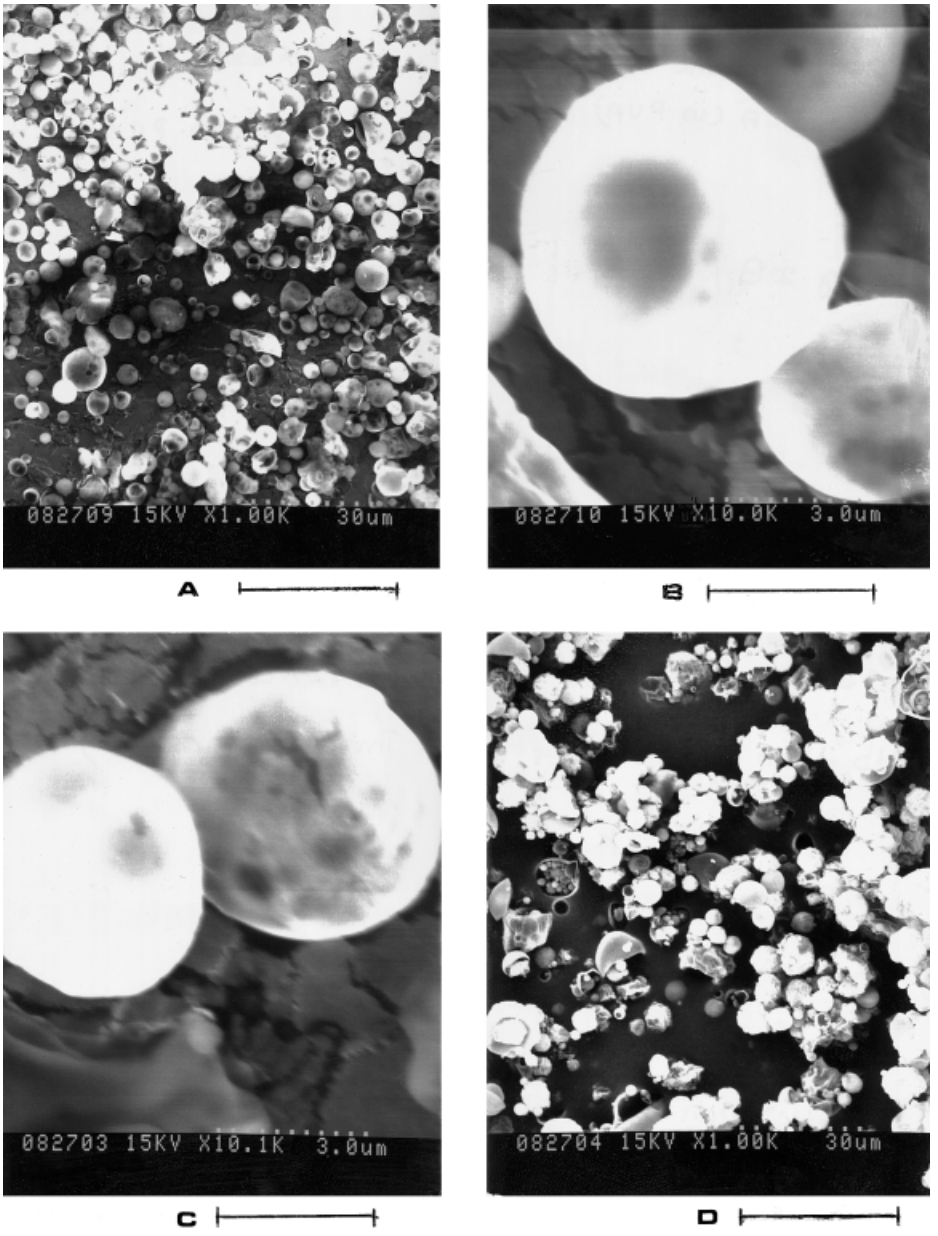


Figure 2 SEM micrographs of PLA microspheres prepared from the dissolution of the polymer in the CH_2Cl_2/acetone system: (A) HSA-loaded PLA–PVA microspheres (scale bar = 30 μm, magnification = 1000×), (B) the surface morphology (scale bar = 3 μm, magnification = 10,000×), (C) Thr-loaded PLA–PVA microspheres (scale bar = 3 μm, magnification = 10,000×), (D) HSA-loaded PLA–PEG microspheres (scale bar = 30 μm, magnification = 1000×), (E) the surface morphology (scale bar = 3 μm, magnification = 9900×), and (F) Thr-loaded PLA–PEG microspheres (scale = 1.5 μm, magnification = 20,000×).

However, a detailed size distribution analysis with a Gaussian distribution may provide a better picture.

Figures 6 and 7 show the dissolution rates of HSA from PLA/PLGA microspheres, as a function of time, in 0.1*M* PBS (pH 7.4). Here, an initial burst release followed by a constant release of HSA from microspheres was observed over 20 days. The amount and percentage of protein delivery was higher with PEG-coated PLA and PLGA microspheres than with PVA-coated PLA microcapsules. For example, at 20 days, the microspheres prepared with PLA–PVA released 40% of the entrapped HSA and PLA–PEG released 49% of the entrapped HSA, whereas the microcapsules prepared with PLGA–PVA released 83% of the entrapped HSA. In other words, PEG coatings on PLA modified the release profile of HSA from the microcapsules.

The amount and percentage of Thr from PLA microspheres as a function of time in PBS (pH 7.4) are depicted in Figures 8 and 9, respectively. An initial burst release (23–30%) followed by a constant release of Thr from the PLA microspheres was observed over

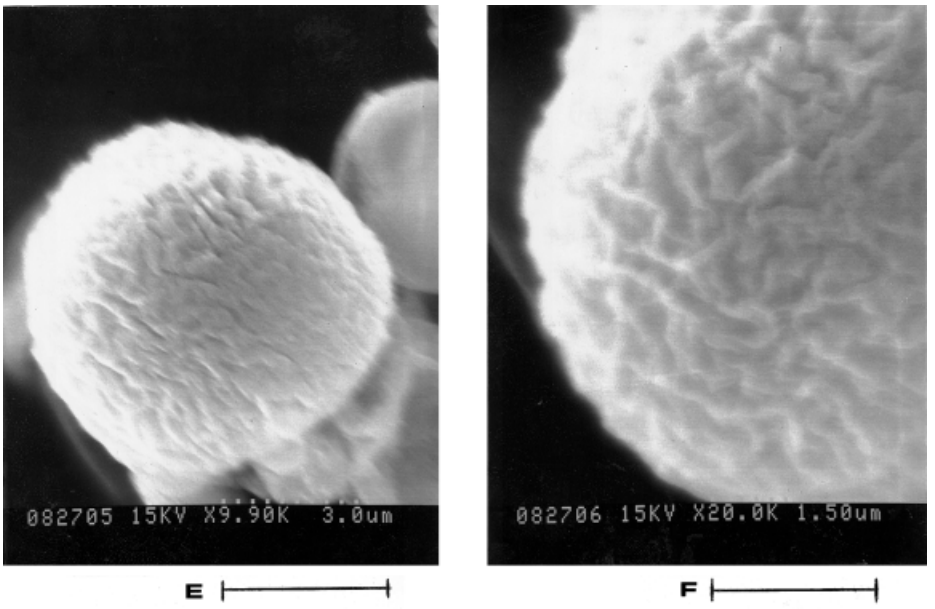


Figure 2 (*Continued from the previous page*)

20 days. The amount of the release was higher (10 μg/mg of beads for 10 days) with PEG-coated PLA microcapsules, although the release percentage was low (42%). However, a reverse pattern of the release was observed with PLA–PVA microspheres (amount of Thr = 8.6 μg/mg of beads, release percentage = 62% for 10 days).

Table III indicates the amount of Thr released at specific time intervals from various PLA microspheres and their Thr times. The plasma Thr time was reduced because of the released Thr from PLA microspheres. Furthermore, the procoagulant properties of the released Thr were higher with the PEG-coated PLA system than with the PVA-based PLA microcapsules (Table III). In other words, the released Thr sustained its procoagulant properties, demonstrating the absence of any adverse effect by this procedure on biological activities. Furthermore, Thr released from PEG-coated microspheres had more bioactivity (39.8 s) than Thr from PVA-based microspheres (47.4 s), as evident from their procoagulant activities.

DISCUSSION

Biodegradable polymers of the lactide/glycolide series have been extensively evaluated for the controlled release of pharmacologically active substances.[3,14,15] Microsphere formulations of these polymers have shown promising results in the delivery of many bioactive peptides and proteins.[8,15,16] However, the development of a surface-modified PLA/PLGA system for protein delivery has hardly been reported. This study demonstrates that biocompatible PEG-coated PLA microspheres provided near zero-order, in vitro release of albumin and Thr.

We have shown in this study the possibility of encapsulating a model protein, HSA, in PEG-coated PLA microspheres with a mean diameter of about 10 μm with loading up to 10% (w/w). An initial burst release (~25%) followed by a constant slow release of proteins from PLA microspheres was observed (Figs. 6 and 7) for 20 days. It is believed that hydrophilic drugs attract water inside the matrix because

TABLE I
Entrapment of Albumin in Various Polymeric Microspheres Prepared in a Chloroform/Acetone (8:2) System

Polymer	Mean diameter ± standard deviation (μm)	Recovery of microspheres (%)	Drug recovery (%)	Drug content (%)
PLA–PVA	21.9 ± 6.3	51.5	13.0	3.24
PLA–PEG	21.3 ± 5.8	57.4	20.7	5.20
PLGA–PVA	20.9 ± 5.0	53.2	18.4	4.60

Polymer = 300 mg; HSA = 100 mg.

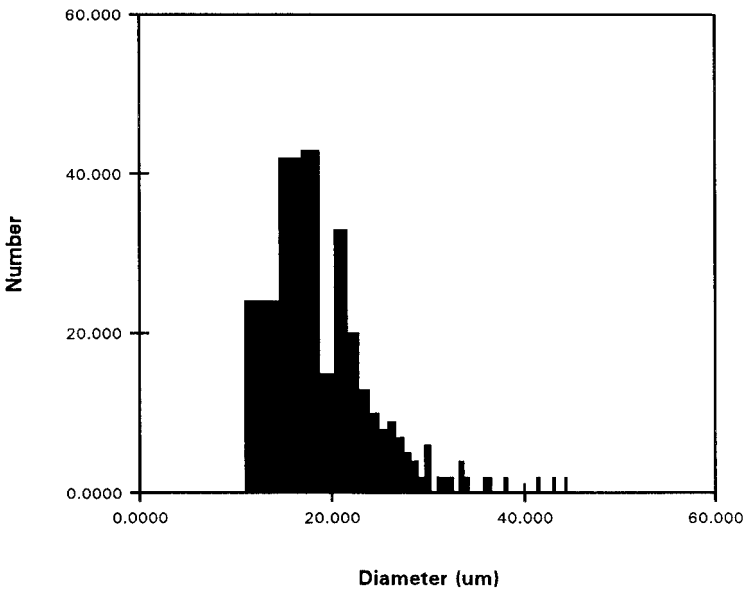


Figure 3 Particle size distribution of HSA-loaded PLA–PVA microspheres prepared from the dissolution of the polymer in the $CHCl_3$/acetone system.

of Donnan equilibrium, leading to swelling of the matrix.[17] The diffusion- and dissolution-based mechanism for the release of hydrophilic drugs from such polymers with interconnected pores filled with a water phase is well established.[18] These SEM studies (Fig. 1) of PLA microspheres (prepared from chloroform/acetone) revealed that their surface had open macroporous and microporous channels. However, as seen in Figure 2(E,F), the open channels of PLA microcapsules (prepared from dichloromethane/acetone) were filled with PEG. The low payload for HSA in the chloroform/acetone system may be due to substantial diffusion of the drug through the open pores. The diffusion of HSA was substantially reduced by PEG coatings on PLA microspheres from the dichloromethane/acetone system to increase the drug content. Furthermore, the observed initial burst release of HSA in all PLA microcapsules may be due to the diffusion of the drugs through micropores rather than the degradation of the polymer and dissolution.

So far, only hydrophobic drugs have been encapsulated in PEG-coated nanospheres or microspheres prepared from amphiphilic diblock PEG–PLA copolymers. Among them, cyclosporin A[19] and lidocaine[20] were entrapped with high loadings with the emulsion/solvent-evaporation technique. In this system, we have fabricated HSA-loaded, PEG-coated PLA microspheres with an emulsification/solvent-evaporation technique. PEG has been used as the aqueous dispersing medium for the formation of PLA microspheres. PEG appears to work as a protective colloid for the emulsion droplets during the preparation. The PEG molecules adsorbed on the surface of the droplets prevented the coalescence of droplets. Therefore, it appears that the PEG coating can increase the payload of drugs and ensure better stabilization.

TABLE II
Entrapment of Albumin and Thr in Various Polymeric Microspheres Prepared in Dichloromethane/Acetone (8:2) System

Polymer/drug		Mean diameter ± standard deviation (μm)	Recovery of microspheres (%)	Drug recovery (%)	Drug content (%)
PLA–PVA	HSA	9.7 ± 1.9	52.5	24.5	6.1
	Thr	10.5 ± 2.9	63.1	56.1	1.4
PLA–PEG	HSA	11.4 ± 3.6	50.6	39.5	9.9
	Thr	10.7 ± 2.8	81.2	93.5	2.3
PLGA–PVA	HSA	10.4 ± 2.9	56.0	37.6	9.4

Polymer = 300 mg; HSA = 100 mg; Thr = 7.8 mg.

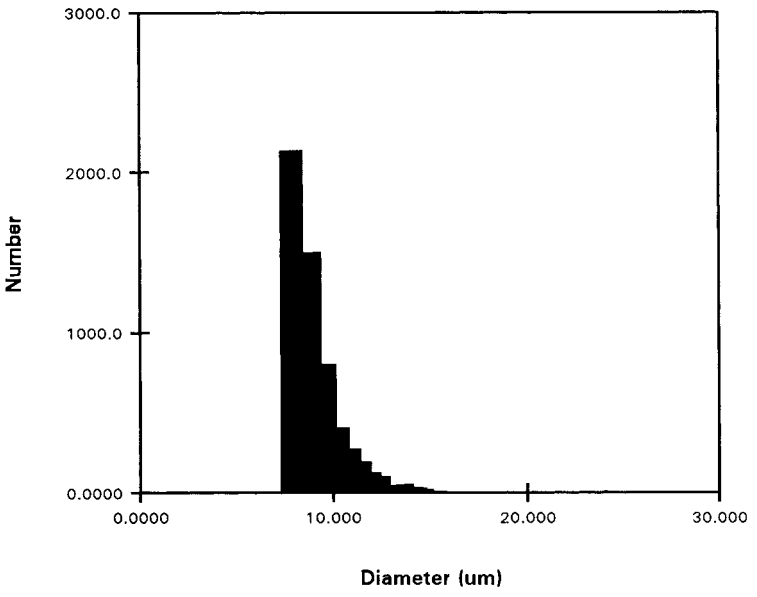


Figure 4 Particle size distribution of HSA-loaded PLA–PVA microspheres prepared from the dissolution of the polymer in the CH_2Cl_2/acetone system.

PEG-coated alginate–chitosan microspheres have shown improved biocompatibility and have been demonstrated to be a better system for the oral delivery of proteins.[6] However, we lack direct evidence that the PEG polymer forms an interpenetrating network near the surface of the PLA microspheres. Previous studies using a diffuse reflectance infrared Fourier transform spectroscopy analysis of PEG-coated PLA microspheres indicated the presence of specific functional groups of both PLA and PEG.[21] These SEM studies of PEG-coated PLA microspheres [Fig. 2(E,F)] revealed the incorporation of PEG within the PLA matrix surface. However, more physicochemical characterization methods are needed to determine the presence of PEG on PLA microspheres.

The use of PEG to reduce protein adsorption and cell adhesion is well documented in the literature.[6,22] The grafting of PEG to polyacrylonitrile surfaces was demonstrated by Miyama et al.[22] to render the surface less

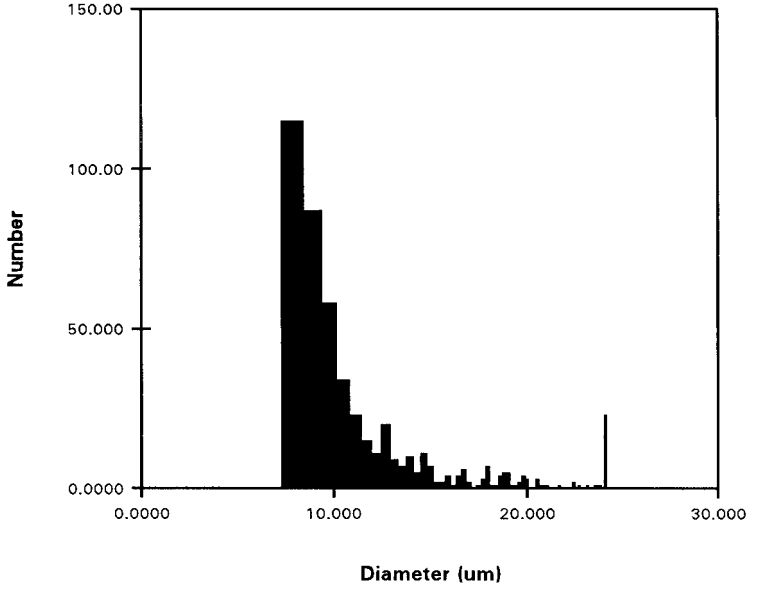


Figure 5 Particle size distribution of HSA-loaded PLA–PEG microspheres prepared from the dissolution of the polymer in the Ch_2Cl_2/acetone system.

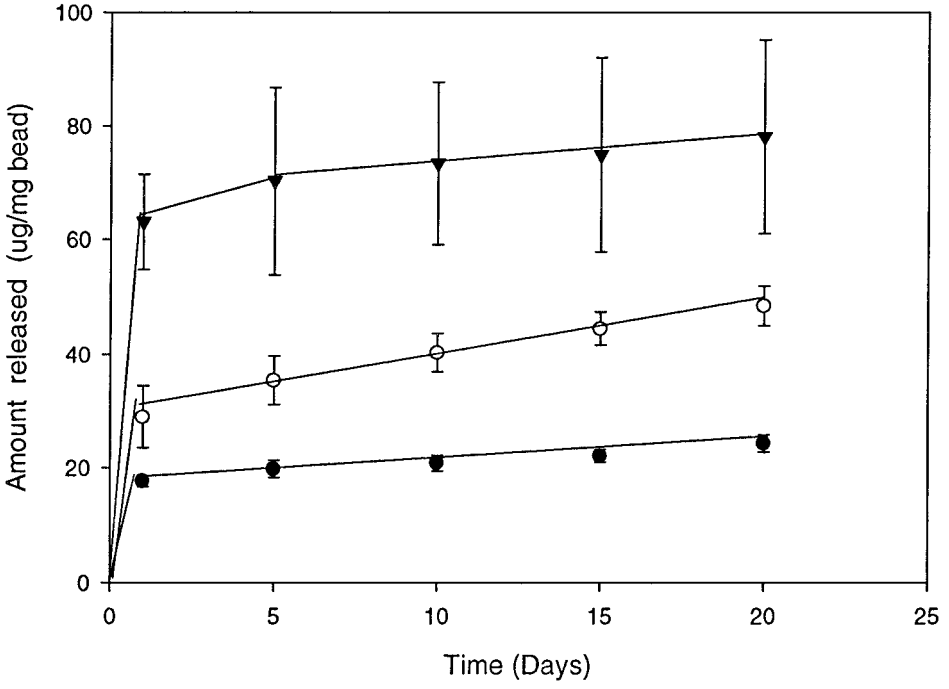


Figure 6 Amount of HSA released from the PLA/PLGA microspheres in 0.1*M* PBS (pH 7.4): (○) PLA–PVA, (●) PLA–PEG, and (▲) PLGA–PVA. The bars indicate the standard deviation.

thrombogenic. Nagaoka et al.[23] synthesized a graft copolymer of methacrylates with PEG and found the resulting polymer to be quite nonthrombogenic. PEG-grafted polymer surfaces have also been shown to reduce protein adsorption[24] and are highly resistant to mammalian and bacterial cell adhesion.[25] Therefore, these new microcapsules fabricated from PLA/PEG may serve to provide controlled protein delivery and immunoprotection, whereas the outer layer of PEG may serve to enhance biocompatibility and reduce biodegradation.

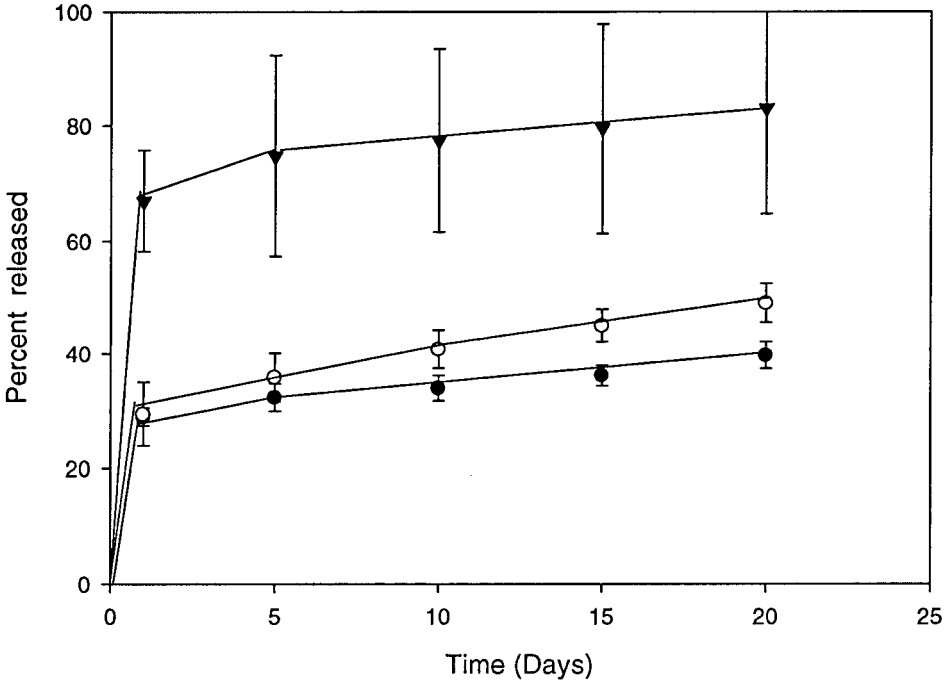


Figure 7 Percentage of HSA released from the PLA–PLGA microspheres in 0.1*M* PBS (pH 7.4): (○) PLA–PVA, (●) PLA–PEG, and (▲) PLGA–PVA. The bars indicate the standard deviation.

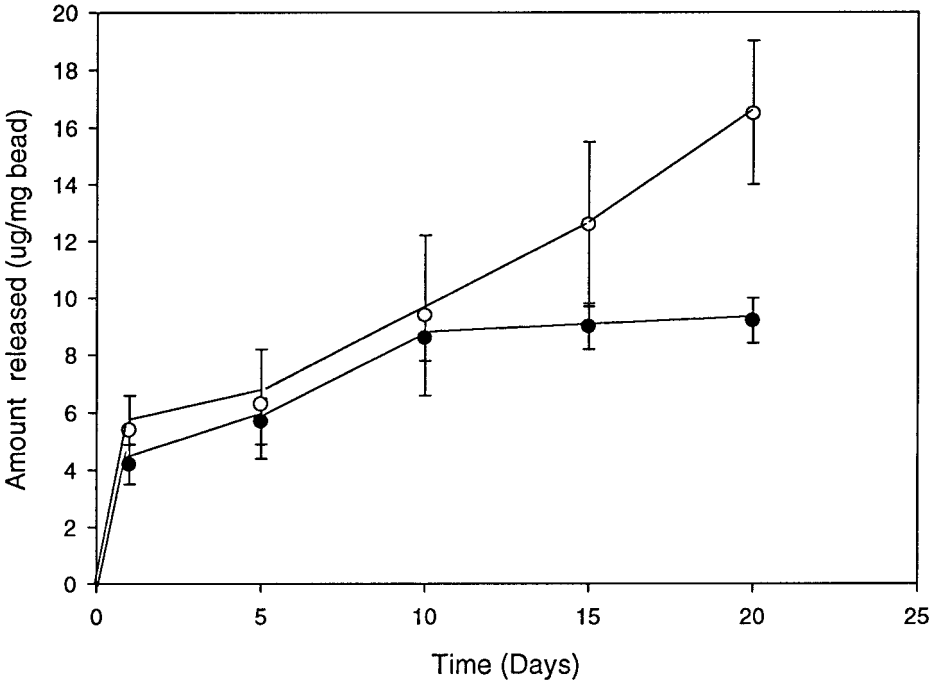


Figure 8 Amount of Thr released from the PLA microspheres in 0.1*M* PBS (pH 7.4): (○) PLA–PVA and (●) PLA–PEG. The bars indicate the standard deviation.

CONCLUSIONS

This work has demonstrated that PLA–PEG microspheres may be used as vehicles for the delayed release of protein drugs. The incorporation of biocompatible PEG may protect the protein from degradation and, subsequently, their bioavailability. The processing parameters can be varied to obtain high protein incorporation in the microspheres. We can, therefore, speculate that the PLA–PEG system is a good candidate for the sustained delivery of bioactive proteins.

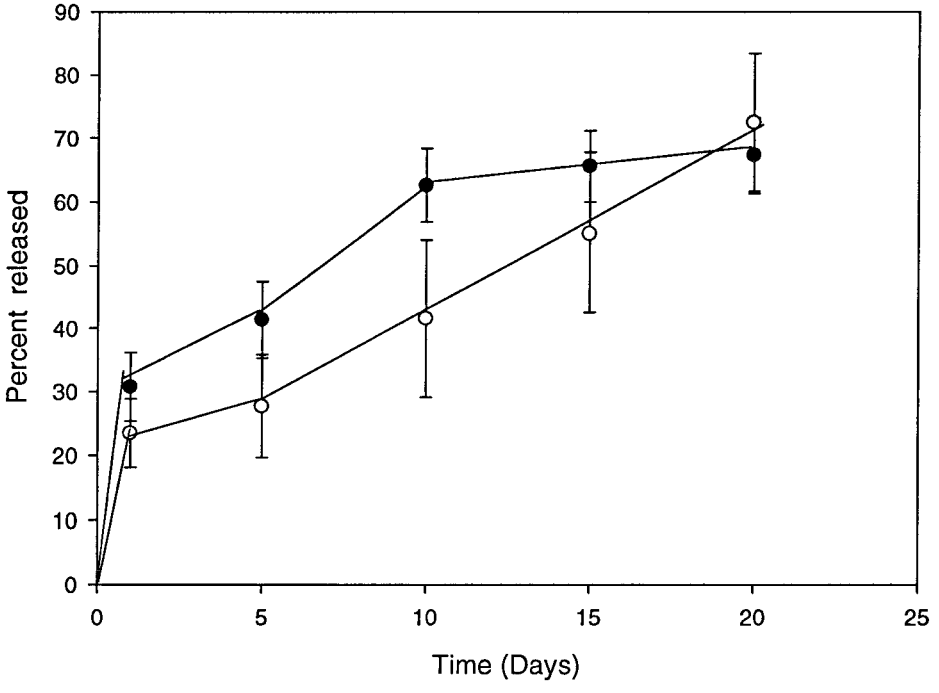


Figure 9 Percentage of Thr released from the PLA microspheres in 0.1*M* PBS (pH 7.4): (○) PLA–PVA and (●) PLA–PEG. The bars indicate the standard deviation.

TABLE III
Thr Time Assay for Released Thr

Amount of Thr (NIH units)	Thrombin time in seconds ± standard deviation	
	PLA–PVA	PLA–PEG
Zero	127.5 ± 8.9	
0.1 (soluble Thr)	34.7 ± 2.7[a]	
0.45–0.53 (released Thr, 4 h)[b]	53.8 ± 3.5[a]	46.4 ± 3.1[a]
0.55–0.64 (released Thr, 6 h)[b]	47.4 ± 4.0[a]	39.8 ± 2.9[a]

[a] $p < 0.001$, where the Thr time values of all cases were compared with the zero Thr levels.
[b] 10 mg of Thr-loaded microspheres were incubated in 1 mL of saline at 37°C. After specific time periods, 0.1 mL was used for the assay.

However, a more detailed physicochemical characterization of microspheres and studies of the bioactivity and biocompatibility of encapsulated proteins are needed to find applications.

References

1. Heller, J. Biomaterials 1980, 1, 51.
2. Niwa, T.; Takeuchi, H.; Hino, T.; Kunou, N.; Kawashima, Y. J Controlled Release 1993, 25, 89.
3. Ruiz, J. M.; Benoit, J. P. J Controlled Release 1991, 16, 177.
4. Nihant, N.; Grandfils, C.; Jerome, R.; Teyssie, P. J Controlled Release 1995, 35, 117.
5. Hari, P. R.; Chandy, T.; Sharma, C. P. J Appl Polym Sci 1996, 59, 1795.
6. Chandy, T.; Mooradian, D. L.; Rao, G. H. R. J Appl Polym Sci 1998, 70, 2143.
7. Jayanthi, R.; Thanoo, B. C.; Mehta, R. C.; Deluca, P. P. J Controlled Release 1996, 38, 235.
8. Song, C. X.; Labhasetwar, V.; Murphy, H.; Qu, X.; Humphrey, W. R.; Shebuski, R. J.; Levy, R. J. J Controlled Release 1997, 43, 197.
9. Couvreur, P.; Puisieux, F. Adv Drug Delivery Res 1993, 10, 141.
10. Gref, R.; Minamitake, Y.; Peracchia, M. T.; Trubetskoy, V.; Torchilin, V.; Langer, R. Science 1994, 263, 1600.
11. Quellec, P.; Gref, R.; Perrin, L.; Dellacherie, E.; Sommer, F.; Verbavatz, J. M.; Alonso, M. J. J Biomed Mater Res 1998, 42, 45.
12. Bazile, D.; Prudhomme, C.; Bassoullet, M. T.; Marlard, M.; Spenlehauer, G.; Veillard, M. J Pharm Sci 1995, 84, 493.
13. Lowry, O. H.; Rosenbrough, N. J.; Ferr, A. L.; Randall, R. J. J Biol Chem 1951, 193, 265.
14. Mehta, R. C.; Jeyanthi, R.; Calis, S.; Thanoo, B. C.; Burton, K. W.; Deluca, P. P. J Controlled Release 1994, 29, 375.
15. Sah, H.; Toddywala, R.; Chien, Y. W. J Controlled Release 1995, 35, 137.
16. Cleland, J. L. Pharm Biotechnol 1997, 10, 1.
17. Carelli, V.; Dicolo, G. J Pharm Sci 1983, 72, 316.
18. Lee, T. K.; Sokoloski, T. D.; Roger, G. P. Science 1981, 213, 233.
19. Quellec, P.; Gref, R.; Calvo, P.; Almso, M. J.; Dellacherie, E. Proc Int Symp Controlled Release Bioact Mater 1996, 3, 815.
20. Peracchia, M. T.; Gref, R.; Minamitake, Y.; Domb, A.; Lotan, N.; Langer, R. J Controlled Release 1997, 46, 223.
21. Das, G. S.; Rao, G. H. R.; Wilson, R. F.; Chandy, T. J Biomed Mater Res 2001, 55, 96.
22. Miyama, H.; Shimada, H.; Fujii, N.; Nosaka, Y. J Appl Polym Sci 1988, 35, 115.
23. Nagaoka, S.; Mori, Y.; Takiuchi, H.; Yokota, K.; Tanzawa, H.; Nishiumi, S. In Polymers as Biomaterials; Shalaby, S. W.; Hoffman, A. S.; Ratner, B. D.; Horbett, T. A., Eds.; Plenum: New York, 1984; p 361.
24. Gombotz, W. R.; Guanghui, W.; Hoffman, A. S. J Appl Polym Sci 1989, 37, 91.
25. Desai, N. P.; Hossainy, S. F. A.; Hubbell, J. A. Biomaterials 1992, 13, 413.