Synthesis and Characterization of Biodegradable Low Molecular Weight Aliphatic Polyesters and Their Use in Protein-Delivery Systems

Shaobing Zhou,[1] Xianmo Deng,[1] Xiaohong Li,[1] Wenxiang Jia,[2] Li Liu[2]

[1]*Chengdu Institute of Organic Chemistry, Chinese Academy of Sciences, Chengdu 610041, People's Republic of China*
[2]*Department of Microbiology and Immunology, Hua Xi Medical Center, Sichuan University, Chengdu 610041, People's Republic of China*

Received 10 December 2002; accepted 9 July 2002

ABSTRACT: Poly(lactic acid) (PLA) and poly(lactic-*co*-GA) (PLGA) with low molecular weights were synthesized by a one-step polycondensation of lactic acid (LA) with glycolic acid (GA) molecules using stannous octoate as a catalyst at 160°C. A high yield (>80%) of all the polymers was obtained in the study. The PLA and PLGA copolymers were characterized by ^{1}H-NMR, GPC, and DSC measurements, etc. We elaborated HSA-loaded microspheres based on PLA and PLGA copolymers with different monomer ratios (LA/GA = 85:15, 75:25, 65:35, and 50:50) by the solvent-extraction method based on the formation of double w/o/w emulsion. Microspheres were characterized in terms of the morphology, size, and encapsulation efficiency (E.E.). The highest E.E. (69.3%) of HSA was obtained for HSA-loaded PLGA (65/35) microspheres among all the formulations. *In vitro* matrix degradation and protein release of these microspheres were performed in phosphate-buffer saline (PBS; 154 m*M*, pH 7.4). The degradation profiles were characterized by measuring the loss of the microsphere mass and the decrease of the polymer intrinsic viscosity. The release profiles were investigated from the measurement of the protein presented in the release medium at various intervals. It was shown that the matrix degradation and protein-release profiles were highly LA/GA ratio-dependent. It is suggested that these matrix polymers may be optimized as carriers in protein- and peptide-delivery systems for different purposes. © 2003 Wiley Periodicals, Inc. J Appl Polym Sci 91: 1848–1856, 2004

Key words: biodegradable; polyesters; proteins

INTRODUCTION

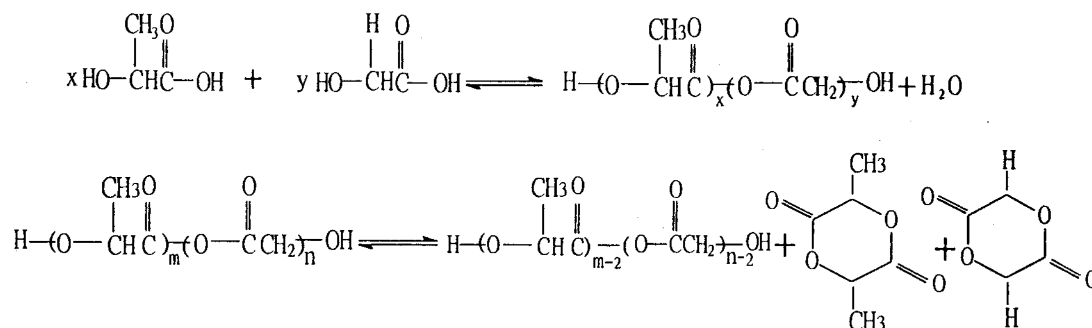
In recent years, biodegradable polymer systems have been widely investigated, especially for the controlled delivery of vaccines, proteins, and peptides.[1–5] Within these systems, biodegradable microspheres and nanoparticles have been extensively investigated as drug-delivery technologies for the sustained and/or controlled release of drugs.[6,7] The microsphere-delivery systems based on poly(lactic acid) (PLA) and poly(lactic-*co*-GA) (PLGA) have received considerable interest because they are biodegradable and biocompatible polymers, which are nonimmunogenic and have a long history of safe use in humans as sutures and as controlled-delivery systems.[8–10] The choice of PLA and PLGA as the matrix for protein formulations is based on their long-term safety in humans, their biodegradability, and the commercial availability of a variety of polymers of different molecular weights and monomer ratios.[5]

PLA homopolymers and PLGA copolymers are usually synthesized by ring-opening copolymerization of lactide with glycolide using stannous octoate ($SnOct_2$) as a catalyst at high temperatures (130–220°C) because $SnOct_2$ is a highly efficient commercial catalyst and a food additive permitted in numerous countries.[11,12] High molecular weight PLA and PLGA polymers could be obtained by this bulk-polymerization method. However, some drawbacks exist in the polymerization process. It is well known that the ring-opening polymerization process of cyclic diester monomers must be divided into two steps: The first step was that the lactic acid or GA molecules were converted into cyclic lactides or glycolides by dehydration. Then, the exhaustive purification of lactide and glycolide need time to recrystallize, which would consume a great deal of organic solvent. Thus, a low yield of lactide and glycolide resulted, which made the PLA and PLGA products more expensive. The second step was the copolymerization of lactide with glycolide in the bulk. It was obvious that the techniques of the polymerization process was very prolix and complex. For easier synthesis of PLA and PLGA, simple one-

Correspondence to: S. Zhou (shaobingzhou@163.net).
Contract grant sponsor: National Science Foundation of China; contract grant number: 20004009; 50303018.
Contract grant sponsor: National 973 Project.

Journal of Applied Polymer Science, Vol. 91, 1848–1856 (2004)
© 2003 Wiley Periodicals, Inc.

step polycondensation of lactic acid (LA) with glycolic acid (GA) molecules should be estabilished. In the polycondensation system of PLA and PLGA, two principal equilibrium reactions exist.[13] One is dehydration equilibrium for esterification equication (**1**) and the other is ring-chain equilibrium involving depolymerization to lactide and/or glycolide equication (**2**):



Although what we obtained was low-molecular weight PLA and PLGA by the direct polycondensation, they were successfully used as drug carriers for controlled delivery systems.

In this article, we synthesized low molecular weight PLA homopolymers and a series of PLGA copolymers with different monomer ratios of D,L-LA and GA in the presence of stannous octoate by direct polycondensation. These biodegradable polymers were characterized by ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectrometry, gel permeation chromatography (GPC), and different scanning calorimetry (DSC), etc. Then, an application of these polymers in a protein-delivery system in the form of microspheres was further investigated. The microspheres containing human serum albumin (HSA) based on these PLA and PLGA copolymers were all prepared by a double-emulsion w/o/w based on solvent-extraction methods. The microspheres were compared by their microsphere size and distribution, surface morphology, and amount of encapsulated (A.E.) HSA in the microspheres and the HSA encapsulation efficiency (E.E.) of the process, and so on. *In vitro* matrix degradation profiles of these microspheres were characterized by measuring the microsphere weight loss and the intrinsic viscosity decrease. *In vitro* HSA release profiles from all sample microspheres were also investigated.

EXPERIMENTAL

Materials

D,L-LA (85%) and GA (99%) were purchased from the Aldrich Chemical Co. Poly(vinyl alcohol) (PVA; 88% hydrolyzed, M_w = 130 kdaltons) was purchased from the Guangzhou Chemical Reagents Department (Guangzhou, China). Stannous octoate ($SnOct_2$) (from the Sigma Corp.) was used as received. HSA was purchased from the Institute of Blood-Transfusion, Chinese Academy of Medical Science (China). The other reagents were used as received.

Methods

^{1}H-NMR was performed on a Bruker AM 300 MHz apparatus using tetramethylsilane (TMS) as an internal standard in $CDCl_3$ at 25°C. The actual ratio of D,L-LA and GA in the PLGA copolymers was calculated from the integral height of hydrogen shown in the ^{1}H-NMR. The average molecular weight and its distribution were determined by gel permeation chromatography (GPC; waters ALC/GPC 244, USA) operating with THF and calibrated with polystyrene standards. The intrinsic viscosity was measured with an Ubbelohde viscometer on a 0.5% (g/dL) solution of the polymer at 25°C in chloroform. The transition temperatures of the polymers were measured with a Perkin–Elmer DSC7. The measurements were carried in the range of 10–120°C under a flow of nitrogen at a scanning rate of 10 °C/min. The midpoint of the second run was used for the glass transition temperature (T_g) calculation.

The microspheres were evaluated for surface morphology by scanning electronic microscopy (SEM; Amray). The microsphere size and distribution were determined with a laser diffraction particle-size analyzer (Malven, Mastersizer 2000, U.K.). The actual A.E. HSA in the microspheres was determined as previously reported.[14] The amount of HSA entrapment was measured by placing 100 mg of the microspheres in 1.5 mL of dichloromethane and extracting the HSA three times with 1.5 mL of double-distilled water. The HSA content of the extraction solution was determined using Bradford's method,[15] compared with a standard curve of data obtained by assaying known concentrations of HSA solutions. The A.E. HSA in the microspheres, given as a percentage, indicates the amount (milligrams) of HSA encapsulated per 100 mg of mi-

crospheres. The E.E. of HSA in the polymer microspheres was determined as

$$\text{E.E.}\ (\%) = (L_A/L_T) \times 100$$

where L_A is the actual loading and L_T is the theoretical loading of HSA (percent weight/weight) in the polymer microspheres.

Preparation

Polymerization

The synthesis of a low molecular weight PLA homopolymer and PLGA copolymer was carried out by polyconsendation as described previously.[16] Thus, a mixture of D,L-LA, GA, and $SnOct_2$ with the desired composition was charged into a glass ampule and then nitrogen gas was bubbled into the mixture at a flow rate of 200 mL min^{-1}. The ampule was immersed in an oil bath maintained at 160°C for 23 h. After cooling at room temperature, the ampule was opened, and the resulting polymers were dissolved in methylene chloride and precipitated in an excess of methanol. The purified product was dried under a vacuum at 40°C for 48 h. The yield was determined by weighing the purified and dried polymers. The ^{1}H-NMR spectra of all samples are given in Figure 1.

Preparation of HSA-loaded microspheres

PLA and PLGA microspheres containing HSA were prepared by solvent extraction based on the formation of a modified double-emulsion $w_1/o/w_2$ reported early.[17] Briefly, the W_1 phase, containing an aqueous solution of HSA protein, was dispersed into the organic phase consisting of the polymer dissolved in dichloromethane (50.0 mg/mL), using a high-speed stirrer for 60 s at room temperature. Then, the obtained primary water-in-oil emulsion was immediately added to the external aqueous phase (100 mL of 2.0% PVA solution) and further emulsified again by a high-speed homogenizer. The organic solvent was extracted by adding 100 mL of 6% isopropanol and the mixture was stirred at a moderate speed at ambient temperature for 3 h. After the complete removal of the organic solvent, the microspheres were collected by centrifugation (Tomy Seiko Co., Japan). The resultant microspheres were rinsed with distilled water and centrifuged three more times, then lyophilized overnight and stored at 4°C.

in vitro degradation of microspheres and HSA release test

The degradation experiment was carried out as described previously.[18] Briefly, preweighed microspheres were placed in individual test tubes containing 5.0 mL of PBS (154 m*M*, pH 7.4). The tubes were kept in a thermostated shaking air bath (Hua Li Da Laboratory Equipment Co., China) that was maintained at 37°C and 100 cycles/min. At predetermined intervals, the degradation medium was removed from the vessel containing microspheres by centrifugation. Then, the microspheres were rinsed with distilled water to remove residual buffer salts and dried to a constant weight in a vacuum desiccator. The *in vitro* HSA release from the polymer microspheres was determined as follows: Polymer microspheres containing HSA were incubated into a test tube containing 10.0 mL of PBS (154 m*M*, pH 7.4). These tubes were stored in the same air bath as mentioned in the degradation test. At appropriate intervals, 1.0 mL of the release medium was collected by centrifugation and 1.0 mL of fresh PBS was added back to the test tube. The amount of HSA was measured by the Bradford's protein assay as described above.

Estimation of degradation

The degree of degradation was estimated from the decrease of mass loss and intrinsic viscosity of polymer microspheres. Mass loss was determined gravimetrically by comparing the dry weight remaining at a specific time with the initial weight. Samples of fresh microspheres and microspheres from the degradation experiments were dissolved in chloroform ($CHCl_3$) and filtered to eliminate unsolved proteins. The intrinsic viscosity was measured with an Ubbelohde viscometer on a polymer solution in $CHCl_3$ at 25°C.

RESULTS AND DISCUSSION

Characterization of polymer

The synthesis of PLA and PLGA was performed by direct polycondensation in the presence of $SnOct_2$ as a catalyst at 160°C to obtain polymers with low molecular weights. Figure 1 shows the ^{1}H-NMR spectra obtained for samples of the PLA homopolymer and of PLGA copolymers with different monomer ratios (mol ratios of LA/GA = 85:15, 75:25, 65:35, and 50:50). It is clearly seen from Figure 1(a) that only two peaks at 1.52 and 5.20 ppm appear, which are assigned to the methyl (CH_3) and methine (CH) signals in the PLA homopolymer, respectively. However, besides the two peaks of methyl (CH_3) and methine (CH) signals in PLA units, the other peak at 4.85 ppm exists, which is attributed to the methylene (CH_2) protons of the PGA units, as seen clearly in Figure 1(b–d,e). These results are consistent with those obtained from PLGA copolymers prepared by the ring-opening polymerization of LA with GA in the presence of $SnOct_2$.[19] The LA/GA ratios in the PLGA copolymers were all calculated by comparing the ratios of absorbances at 5.20

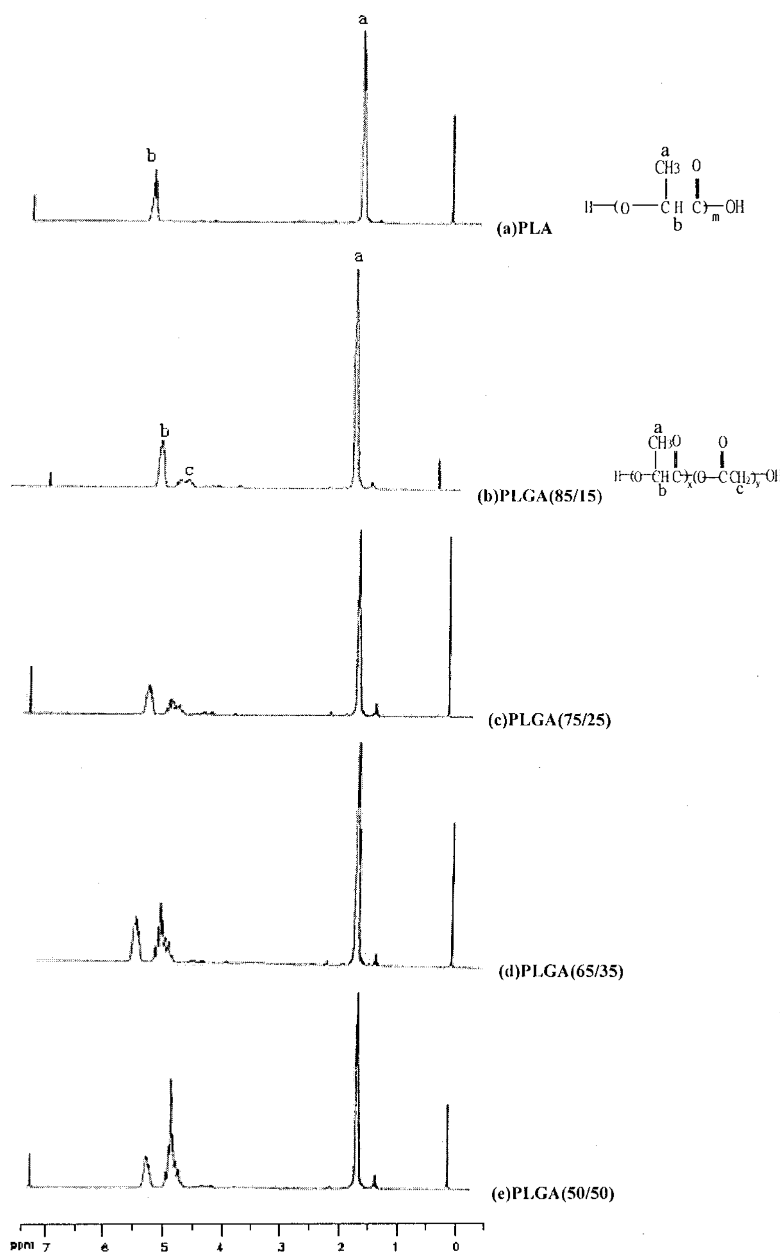


Figure 1 ^{1}H-NMR spectra of (a) PLA, (b) PLGA (85/15), (c) PLGA (75/25), (d) PLGA (65/35), and (e) PLGA (50/50) copolymers.

(CH) and 4.85 ppm (CH_2) as reported previously.[19] The feed monomer ratios (LA/GA) and the copolymer composition calculated from the ^{1}H-NMR spectrum are compared in Table I. The LA contents in the monomers of 85, 75, 65, and 50 mol % was found to be 82, 72, 60, and 45 mol % in the copolymer, respectively. A slight difference in the molar composition between the initial monomer and the final product was observed, but it seems reasonable to conclude that the LA monomer is quantitatively reacted with GA by direct copolycondensation in the presence of $SnOct_2$ at 160°C. The result is consistent with those reported previously.[16] We obtained a high yield (>80%) of all the polymers by direct polycondensation (Table I). The number-average molecular weight (M_n) and polydispersity (M_w/M_n) measured by GPC are also listed in Table I. It can be seen from Table I that the GA content has a great effect on the molecular weight of the resulting PLGA copolymers. The M_w of the copolymer increases and the M_w/M_n decreases with an increased GA content. The PLA homopolymer without GA has the lowest M_n and the largest polydispersity.

The DSC curves of PLA and PLGA as a function of monomer composition are shown in Figure 2. As can be seen, every polymer exhibits only one glass transition temperature (T_g) in the course of the thermo-

TABLE I
Direct Copolycondensation of D,L-LA with GA in the Presence of SnOct and Characteristics of the Copolymers

Sample No.	Feed (mol %) LA/GA	Yield (%)	Polymer composition LA/GA[a]	M_n ($\times 10^3$)[b]	M_w/M_n
1	100/0	80.0	100/0	8.21	2.24
2	85/15	87.0	82/18	8.63	1.89
3	75/25	83.8	72/28	9.84	1.42
4	65/35	81.0	60/40	12.36	1.34
5	50/50	86.1	45/55	13.21	1.29

Copolycondensation conditions: catalyst SnOct, 160°C, N_2, 23 h.
[a] Estimated from the integral height of hydrogen shown in ^{1}H-NMR spectrum.
[b] Number-average molecular weight measured by GPC (calibrated with polystyrene standards).

grams covering from 10 to 120°C, which indicate that all these polymers with low M_w produced in this study are amorphous. Gilding and Reed also showed that the degree of crystallinity in cast polymer films was controllable by the copolymerization of glycolide with lactide at different compositions, with those of 22–66 wt % glycolide being fully amorphous.[19] The T_g of the PLA homopolymer is 42.5°C, which is lower than that of PLA produced by bulk polymerization. It may be due to that the M_w has a great effect on the T_g. The 85:15, 75:25, 65:35, and 50:50 LA/GA copolymers show glass transitions (T_g's) of 38.8, 34.5, 28.3, and 26.1°C, respectively. The decrease of T_g may result from the increase of the GA component in the PLGA copolymers.

Characterization of microspheres

The PLGA copolymers and PLA homopolymer microspheres containing HSA were prepared by a double-emulsion $w_1/o/w_2$ based on the solvent-evaporation method in the same condition. The surface morphology of all microspheres was determined by SEM. The scanning electronic micrographs of all kinds of PLGA microspheres containing HSA are almost identical. The micrographs of the PLA and PLGA (65/35) microspheres containing HSA are shown in Figure 3. The A.E., E.E., the mean diameter, and their size distribution of PLA and all kinds of PLGA microspheres containing HSA are summarized in Table II. As seen from Figure 3, there was no significant difference between the two micrographs for HSA-loaded PLA microspheres [Fig. 3(a)] and HSA-loaded PLGA microspheres [Fig. 3(b)]. All these microspheres had a smooth spherical surface structure and there was no evidence of collapse. As seen from Table II, the mean diameter of PLA and all kinds of PLGA microspheres is less than 5 μm, which could be targeted to the immunization-related tissues.[20] The actual A.E. HSA and its E.E. in various microsphere formulations are also shown in Table II. We achieved more than 48% of E.E. of HSA for all kinds of microspheres. Especially for HSA-loaded PLGA (65/35) microspheres, the highest E.E. (69.3%) was observed among all the for-

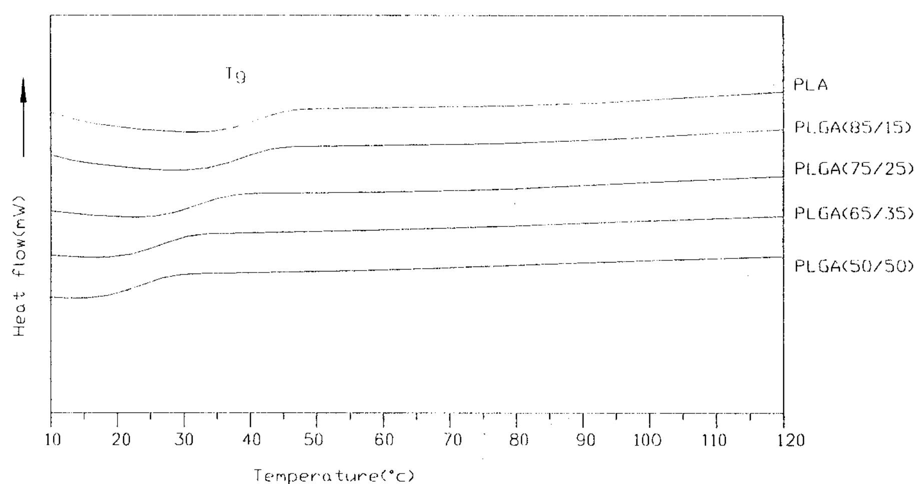


Figure 2 DSC curves of PLA homopolymer and PLGA with 85:15, 75:25, 65:35, and 50:50 LA/GA copolymers.

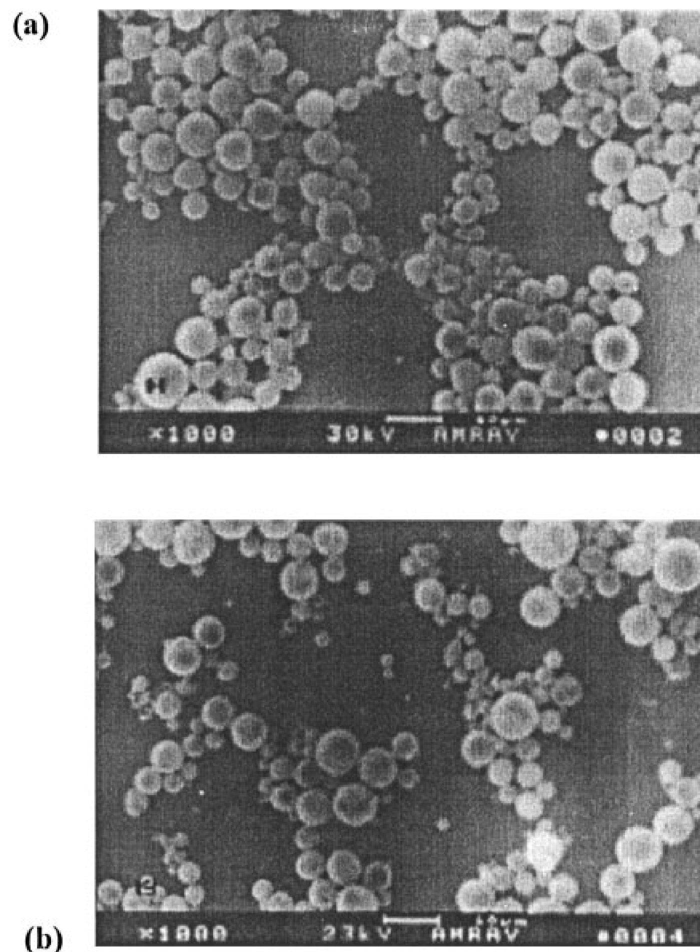


Figure 3 Dispersion pattern and morphology determined by SEM of (a) HSA-loaded PLA and (b) HSA-loaded PLGA (65/35) microspheres.

mulations. The reason may be that all the polymers with a low M_w produced in this study have good hydrophilicity, compared with high M_w polyesters synthesized by bulk polymerization. The result is consistent with the conclusion reported in our previous study that the hydrophilic domains of the polymer could promote the stability of proteins and increase the water-soluble drug and protein E.E.[21] Another likely reason is that the introduction of a suitable ratio of glycolide into PLA chains is beneficial to improve the E.E. of HSA.

In vitro degradation of microspheres

Figure 4 shows that the erosion of HSA-loaded PLA and PLGA microspheres leads to weight-loss profiles that are in agreement with the kinetics of bulk erosion. The weight-loss profiles have the same shape for all the investigated polymers and consist of two parts: During the first period, there is no significant loss of weight as seen from Figure 4. The period of time is very short, which is about 4 days. During the second period, the weight loss sets in spontaneously. The rate

TABLE II
Characteristics of PLA and PLGA Microspheres

Sample No.	Mean diameter (μm)	Size range (μm)	A.E. (%)	E.E. (%)
PLA	3.85 ± 0.15	0.5–10	0.57 ± 0.03	58.6 ± 0.5
PLGA (85/15)	3.50 ± 0.12	0.5–10	0.52 ± 0.03	51.4 ± 0.5
PLGA (75/25)	3.59 ± 0.15	0.5–10	0.55 ± 0.02	55.6 ± 0.5
PLGA (65/35)	3.95 ± 0.16	0.5–10	0.71 ± 0.04	69.3 ± 0.5
PLGA (50/50)	4.05 ± 0.17	0.5–10	0.47 ± 0.02	48.3 ± 0.3

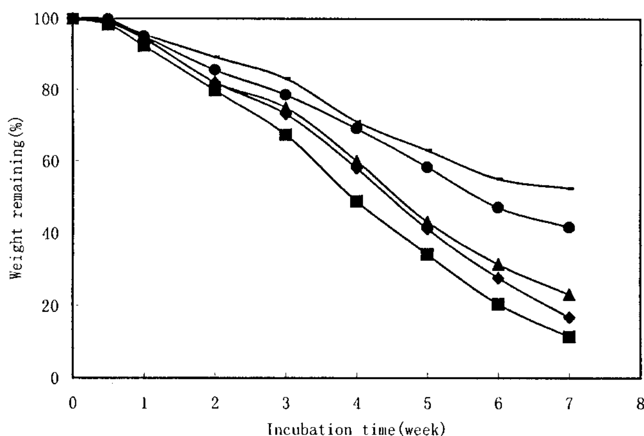


Figure 4 Weight remaining percentage of (-) PLA, (●) PLGA (85/15), (▲) PLGA (75/25), (●) PLGA (65/35), and (■) PLGA (50/50) microspheres containing HSA incubated in PBS at 37°C. Each point represents the mean of three individual samples of microspheres.

of weight loss is increased with increase of the GA mol ratio in the copolymer. The rate of weight loss is the lowest among the investigated polymers for the PLA homopolymer. During the predetermined time, HSA-loaded PLA and 85/15, 75/25, 65/35, and 50/50 PLGA microspheres show percentages of a decrease in mass loss of 47.4, 58.3, 76.8, 83.1, and 89.5, respectively.

The intrinsic viscosity of all samples of microspheres decreased continuously after being exposed to PBS at 37°C. Figure 5 shows the decrease in $\ln(\eta/\eta_0)$ with the incubation time. In all cases, the intrinsic viscosity decrease almost follows a linear profile. The rate of the decrease in $\ln(\eta/\eta_0)$ also depends on the GA mol ratio in the copolymer, which corresponds to the results of Figure 4.

Most studies have dealt with the degradation patterns in form of biodegradable microspheres based on PLA and PLGA polymers with more than 20 kdaltons of M_w under a physiological (pH 7.4) condition, but few investigations have been conducted to evaluate the degradation pattern of microspheres based on PLA and PLGA with low M_w (<15 kdaltons) and different monomer compositions. *In vitro* investigation provides a convenient method to study the degradable characteristics of PLA and PLGA polymers. The degradation process was very complicated. Degradation of the polymer was influenced by the polymer composition, polymer properties such as M_w, crystallinity, and the glass transition temperature,[22,23] and the pH of the polymer solution.[24]

Although a variety of definitions exists in the contemporary literature, it is well accepted that polyester degradation is defined as the process of polymer chain cleavage. Degradation is triggered by water which hydrolyzes the functional groups by which the monomers are usually connected.[25] In the study, the first period of no significant mass loss was much shorter than that for high M_w polymer matrices. For example, PLGA matrices with an initial M_w of 81.5 kdaltons maintained a constant mass for weeks.[26] The reason is that the initial M_w of the polymer has a great effect on its mass loss in the first period. The investigated polymers with low M_w have a good hydrophilicity compared with high M_w PLA and PLGA polymers. The mass of the polymer did not decrease significantly because degraded polymer chains may not have been able to leach out of the polymer microspheres. As seen from Figures 3 and 4, the intrinsic viscosity decrease sets in right from the beginning of the experiment, while the weight loss shows a marked lag period. The reason for the weight-loss profile not coinciding with the degradation profile is that the polymer must undergo sufficiently extensive degradation to produce water-soluble monomers and oligomers; thus, no reduction in mass is observed until degradation is well advanced.[27] When the sample microspheres were exposed to PBS, water permeated into the microsphere matrix, resulting in random hydrolysis of ester bonds and decrease of the intrinsic viscosity. It is well known that poly(glycolic acid) (PGA) is a highly crystalline hydrophilic polymer. So, the reason that the rate of weight loss and the intrinsic viscosity decrease depends on the GA mol ratio in the copolymer is an increased hydrophilicity by introducing the GA into PLA chains. Therefore, we can control the degradation rate by adjusting the monomer ratio in the polymer.

in vitro HSA release from microspheres

Figure 6 shows the percent release of protein from all samples of the microspheres against the incubation

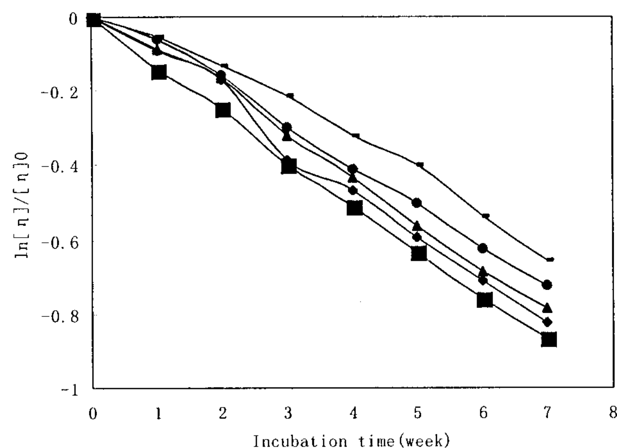


Figure 5 Semilogarithmic relationship between the intrinsic viscosity of (-) PLA, (●) PLGA (85/15), (▲) PLGA (75/25), (◆) PLGA (65/35), and (■) PLGA (50/50) microspheres containing HSA incubated in PBS at 37°C. Each point represents the mean of three individual samples of microspheres.

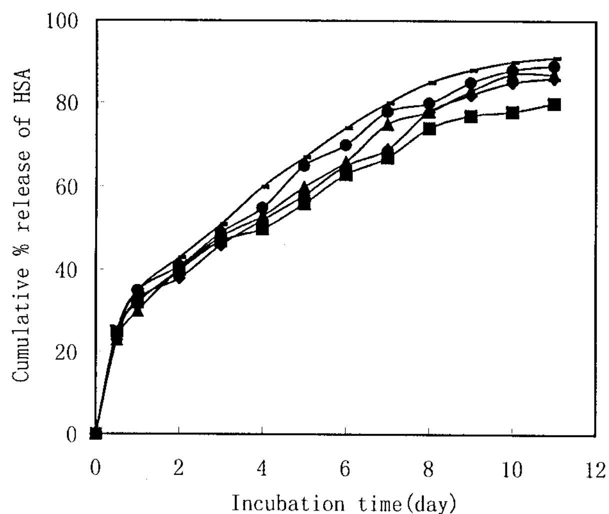


Figure 6 *In vitro* HSA release from (▪) PLA, (●) PLGA (85/15), (▲) PLGA (75/25), (◆) PLGA (65/35), and (■) PLGA (50/50) microspheres incubated in PBS at 37°C. Each point represents the mean of three individual samples of microspheres.

time. The HAS-release profiles of all samples consist of a burst release followed by a gradual release phase over the 11-day study period. The extent of the HSA burst release of all samples of the microspheres at the initial phase is about 23% within 12 h. The gradual release rate of HSA from the microspheres is also dependent on the GA mol ratio in the copolymer, which is in agreement with results reported previously.[28] The cumulative HSA release from these microsphere formulations at the end of 11 days was 80–91% of the initial protein loading. It was concluded that the HSA burst release could be controlled and the sustained, gradual release profiles could be obtained by the biodegradable microsphere system.

The release involved two different mechanisms, that is, diffusion of protein molecules and degradation of the polymer matrix. The burst release of the protein is associated with those protein molecules dispersing close to the microsphere surface, which diffuse out in the initial incubation time. As seen from Figure 6, the gradual release rate of HSA is approximately linear with the incubation time for all samples, indicating that HSA is entrapped within the polymer matrix. So, the faster the degradation, the faster is the HSA release from the microspheres. The proteins gradually release from the microsphere matrix, showing some similarities to the diffusion of macromolecules through a hydrogel-like structure after immersion in water.[29] It is indicated that the gradual release of HSA is due to the swollen inner structure formed by contacting with the aqueous release medium and protein diffusion through the swollen phase.

CONCLUSIONS

PLA and PLGA with a low M_w and different LA/GA ratios can be synthesized by direct polycondensation using $SnOct_2$ as a catalyst. Furthermore, the LA monomer could be quantitatively reacted with GA in our experiment. HSA had been set as a model protein to examine the loading efficiency. HSA-loaded microspheres based on PLA and a series of PLGA copolymers can be successfully prepared by a double-emulsion w/o/w based on solvent-extraction methods. All polymers could form smooth and spherical microspheres with an ideal mean diameter (<5 μm), which could be targeted to the immunization-related tissues. More than 48% of the E.E. of HSA for all kinds of microspheres can be achieved. Especially for HSA-loaded PLGA (65/35) microspheres, the highest E.E. (69.3%) was obtained among all the formulations. Microspheres based on different LA/GA ratio polymers were used to investigate the *in vitro* degradation and protein-release profiles, which were highly LA/GA ratio-dependent. The degradation kinetics of low molecular weight aliphatic polyesters is also consistent with bulk erosion. A gradual release of HSA over an 11-day period can be achieved. The HSA release rate shows some relations with the porous and water-swollen inner structure of the microsphere matrix. Hence, we can draw a conclusion that we can prepare protein-loaded microspheres with a high-protein E.E. and a smooth surface and control the degradation rate and drug release from microspheres by adjusting the LA/GA ratio in the polymer. However, it is clear that more detailed investigations are necessary to clarify the effect of the matrix polymer on the protein-releasing procedure, the *in vitro* degradation mechanism, and the influence factors and the drug-release profiles.

This work was supported by the National Science Foundation of China (Grant Number 20004009) and the National 973 Project.

References

1. Cohen, S.; Yoshioka, T.; Lucarelli, M.; Hwang, L. H.; Langer, R. Pharm Res 1991, 8, 713.
2. Hora, M. S.; Rana, R. K; Nunberg, J. H.; Tice, T. R.; Gilley, R. M.; Hudson, M. E. Pharm Res 1990, 7, 1190.
3. Bittner, B.; Witt, C.; Mader, K.; Kissel, T. J Control Rel 1999, 60, 297.
4. Benoit, M. A.; Baras, B.; Gillard, J. Int J Pharm 1999, 184, 73.
5. Singh, M.; Li, X. M.; Wang, H. Y.; McGee, J. P.; Zamb, T.; Koff, W.; Wang, C. Y.; O'Hagan, D. T. Infect Immun 1997, 65,1716.
6. Couvreur, P.; Puisieux, F. Adv Drug Deliv Rev 1993, 10, 141.
7. Okada, H.; Toguchi, H. Crit Rev Therap Drug Carrier Syst 1995, 12, 1.
8. Yamaguchi, K.; Anderson, J. M. J Control Rel 1993, 24, 81.
9. Langer, R. Science 1990, 249, 1527–1533.
10. Wise, D. L.; Fellman, T. D.; Sanderson, J. E.; Wentworth, R. L. In Drug Carriers in Medicine; Gregoriadis, G., Ed.; Academic: London, UK, 1979; p 237.
11. Kricheldorf, H. R.; Boettcher, C.; Tonnes, K. U. Polymer 1992, 33, 2817.

12. Kowalski, A.; Duda, A.; Penczer, S. Macromolecules 2000, 33, 689.
13. Takahashi, K.; Taniguchi, I.; Miyamoto, M.; Kimura, Y. Polymer 2000, 41, 8725.
14. Maa, Y. F.; Hsu, C. C. J Microencapsul 1997, 14, 225.
15. Braford, M. Anal Biochem 1976, 72, 248.
16. Fukuzaki, H.; Yoshida, M.; Asano, M.; Kumakura, M. Polymer 1990, 31, 2006.
17. Ogawa, Y.; Yamamoto, M.; Okada, H.; Yashiki, T.; Shimamoto, T. Chem Pharm Bull 1988, 36, 1095.
18. Deng, X. M.; Zhou, S. B.; Li, X. H.; Zhao, J.; Yuan, M. L. J Control Rel 2001, 71, 165.
19. Gilding, D. K.; Reed, A. M. Polymer 1979, 20, 1459.
20. Tomlinson. E. Int J Pharm Tech Prod Manuf 1983, 4, 49.
21. Deng, X. M.; Xiong, C. D.; Cheng, L. M. J Polym Lett 1990, 28, 411.
22. Von Recum, H. A.; Cleek, R. L.; Eskin, S. G.; Mikos, A. G. Biomaterials 1995, 16, 441.
23. Pistner, H.; Bendix, D. R.; Muehling, J.; Reuther, J. F. Biomaterials 1993, 14, 291.
24. Vert. M. In Biodegradable Materials; Barenberg, S. A.; Brash, J. L.; Narayan, R.; Redpath, A. E, Eds.; CRC: Boca Raton, FL, 1990; p 11.
25. Göpferich, A. In Sixth World Biomaterials Congress, 2000.
26. Holy, C. E.; Dang, S. M.; Davies, J. E.; Schoichet, M. S. Biomaterials 1999, 20, 1177.
27. Dunne, M.; Corrigan, O. I.; Ramtoola, Z. Biomaterials 2000, 21, 1659.
28. Singh, U. V.; Bisht, K. S.; Rao, S.; Devi, P. U.; Udupa, N. India J Pharm 1997, 29, 166.
29. Li, X. H.; Deng, X. M.; Yuan, M. L.; Xiong, C. D.; Huang, Z. T.; Zhang, Y. H.; Jia, W. X. J Appl Polym Sci 2000, 78, 140.