Preparation and Properties of a Biodegradable Polymer as a Novel Drug Delivery System

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ABSTRACT: Poly (DL-lactic acid-*co*-glycolic acid)-*co*-poly-(ethylene glycol) was synthesized by bulk ring-opening polymerization of DL-lactide/glycolide/poly(ethylene glycol) using stannous chloride as an initiator. The molecular structure of the copolymer was analyzed by IR, ^{1}H NMR, and DSC. The degradation behavior of copolymer was assayed by the reduction of molecular weight, the loss-in-mass, and the changes of pH value for degradation medium. The different contents of PGA and PEG in the molecules of the copolymer could control the degradation rate of polymer. Human Serum Albumin (HSA) was chosen as the model hydrophilic drug and encapsulated in the copolymer. The HA-loaded copolymer microspheres were characterized by the diameter, diameter distribution of the microspheres, and the loading efficiency. © 2003 Wiley Periodicals, Inc. J Appl Polym Sci 90: 3150–3156, 2003

Key words: synthesis; degradation

INTRODUCTION

Many biodegradable polymers have been investigated as potential drug carriers, such as polylactide[1] poly-(lactic-*co*-glycolic acid) (PLGA).[2] The advantages of these biodegradable polymers include biodegradability, biocompatibility, no adverse tissue reaction, and no toxicity. They can be degraded by simple hydrolysis of ester backbone in aqueous environments, such as body fluids. Furthermore, the degradation products of them are ultimately metabolized to carbon dioxide and water or are excreted via the kidneys.[3] Polyglycolide (PGA) is the most simplest linear aliphatic polyester with highly crystalline (45–55%,) which gives rise to a high melting point (220–225°C), a glass transition temperature of 35–40°C, and low solubility in organic solvents. PGA loses about 50% of its strength after 2 weeks and 100% at 4 weeks, and is completely absorbed in 4–6 months. Poly(DL-lactide) (PLA) is an amorphous polymer exhibiting a random distribution of both isomeric forms of lactic acid, and accordingly, is unable to arrange into an organized crystalline structure. This material has lower tensile strength, higher elongation, and a much more rapid degradation time, making it more attractive as a drug delivery system. PLA, however, is more hydrophobic than PGA, owing to the presence of an extra methyl group. PLGA, the copolymer of PGA and PLA, extends the range of homopolymer properties. It degrades faster than either homopolymer, because the adding of DL-lactide disrupts the crystalline structure of PGA. Moreover, there is no linear relationship between the copolymer composition and mechanical and degradation properties of the corresponding materials. PLGA has been investigated extensively as drug carriers.[4] Due to its hydrophilic property, however, the polymer shows some limitations.[5] For example, when the molecules of drug are hydrophilic (such as peptides, genes), the difference in physicochemical properties with hydrophobic polymeric matrix has profound influence on the encapsulation efficiency during the drug-encapsulation procedure.

Poly(ethylene glycol) (PEG) is known to associate with the phospholipid head group of the cell membrane, and assist modified proteins to penetrate into the cell membrane. Thereby, it has been widely used to improve the biocompatibility of the blood contacting materials and hydrophilic property of polymers.[6,7] To improve the hydrophilic property of PLGA, PEG is introduced to form a tri-copolymer poly-DL-lactide-*co*-glycolic acid-*co*-poly (ethylene glycol) (PLGAE). The copolymer is different from the A-B-C model. Actually, the three compositions are randomly bonded covalently. Due to the original order of PEG, the copolymer, PLGAE, is called a tri-block copolymer. The hydrophilic domain of PLGAE improves the affinity between water-soluble drug molecules and the polymeric matrix, the affinity between water and polymer. Thereby, the physicochemical properties of

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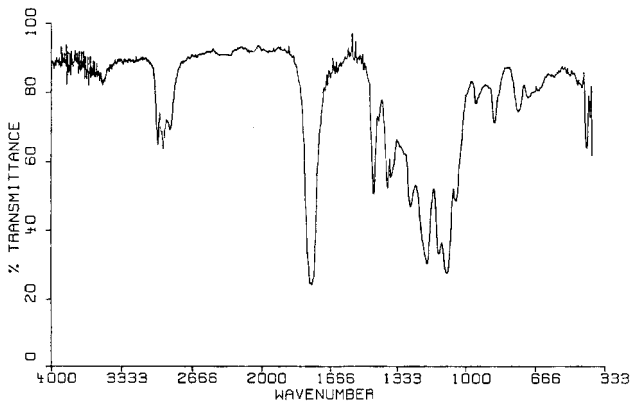


Figure 1 The IR spectrum of PLGAE.

PLGAE are different from either PLA or PLGA. In this article, we report the synthesis of PLGAE and investigate the characteristics of the copolymer.

EXPERIMENTAL

Material

Poly(ethylene glycol) (PEG, molecular weight: 6000 Daltons) was purchased from Guangzhou Chemical Reagents Department, China, and purified by extracting from water and recrystallizing from ether/chloroform, then thoroughly dried at 40°C and a pressure below 10 mmHg for 10 h; DL-lactic acid and glycolic acid were purchased from Aldrich; stannous octoate was from Sigma; ethyl acetate, methylene chloride, and chloroform were commercially available and purified by distillation prior to use. The DL-lactide and glycolide were prepared by dehydration of DL-lactic acid and glycolic acid at 140°C for 8 h, in the presence of 2% zinc oxide, at a pressure of 100 mmHg. The DL-lactide and glycolide were refluxed at about 200°C at a pressure below 10 mmHg. The crude products were recrystallized several times from ethyl acetate. Poly (DL-lactic acid-*co*-glycolic acid) (PLGA) was prepared by bulk ring-opening polymerization of DL-lactide and glycolide using stannous octoate as initiator. All other chemicals and solvents were analytical grade.

Equipment

^{1}H NMR spectra of copolymer were recorded in $CDCl_3$ with a Varian^{UNITY} INOVA-400 MHz apparatus at 25°C. IR spectra were recorded on a NICOLET MX-1 IR apparatus as KBr pellets. The average molecular weight was determined by gel permeation chromatography (GPC, Waters ALC/GPC 244), at room temperature, a differential refractometer as a detector, and calibrated with polystyrene standards. Thermal transition data were collected with a Perkin-Elmer DSC-7. The sample size was 8 mg. Each sample was recorded from 25–250°C with a rate of 20°C/min. Laser Diffractometer (Mastersizer 2000, Malvern, UK) was used to determined the size and size distribution of polymer microspheres.

Synthesis of poly-DL-lactide-*co*-glycolic acid-*co*-poly (ethylene glycol) (PLGAE)

The copolymer was synthesized by bulk ring-opening polymerization of lactide/glycolide/PEG using stannous octoate as an initiator. A prescribed amount of DL-lactide, glycolide, PEG, and stannous octoate were placed in a dried and clean polymerization flask. The

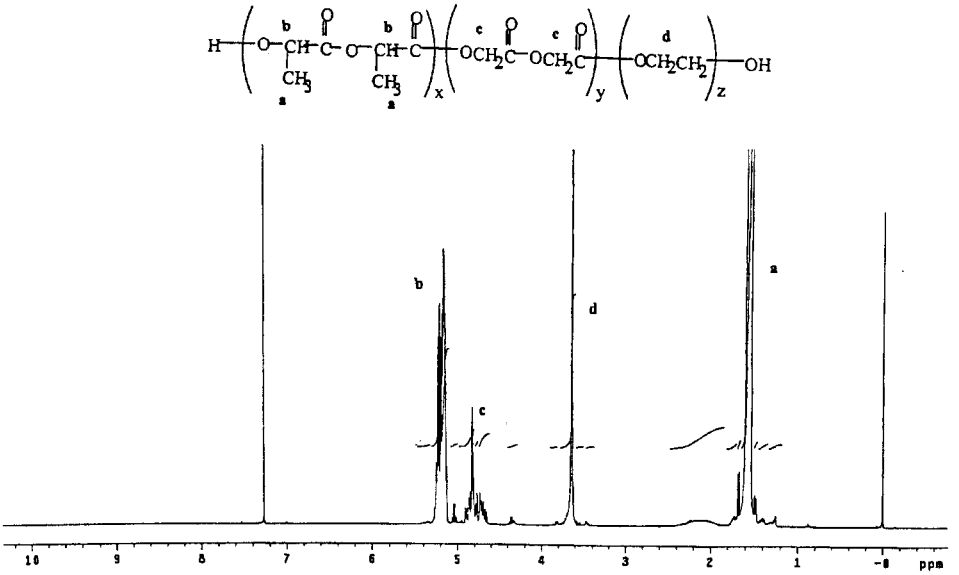


Figure 2 The ^{1}H NMR spectrum of PLGAE.

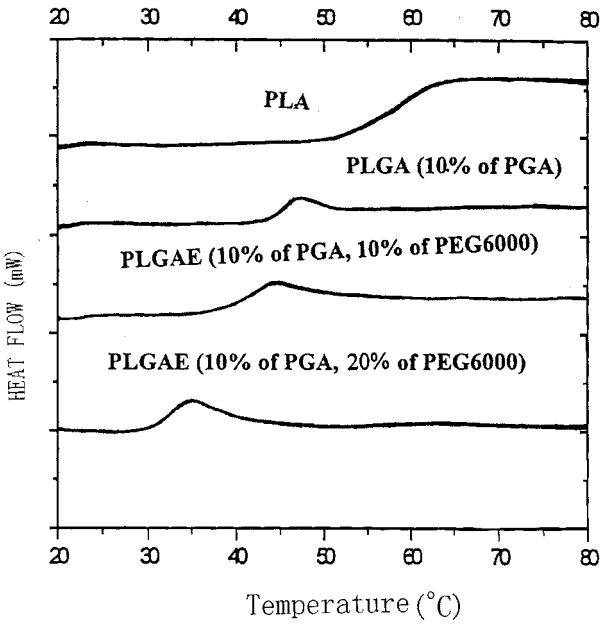


Figure 3 The DSC curves of polymers. (a) PLA; (b) PLGA (10% of GA); (c) PLGAE (10% of GA, 10% of PEG6000); (d) PLGAE (10% of GA, 20% of PEG6000).

reactor was purged with nitrogen and the copolymerization was carried out in an oven at a temperature above the melting point of DL-lactide and glycolide for 4 h. The products were extracted with a cold solvent mixture of ethyl acetate and *n*-heptane (2 : 1 v/v), and then washed with distilled warm water to remove the homopolymers. The crude product was recrystallized from acetone/absolute ethanol three times to give the pure product. The pure copolymer was dried in vacuum at 50°C for 3 days.

In vitro degradation study of the copolymer

Certain amounts of copolymers were suspended in 10.0 mL of 0.2 *M* phosphate-buffered saline (PBS, pH 7.4) in a series of vessels. The vessels were kept in a Thermostated Shaker Water Bath (Jiangsu Taicang Medical Apparatus Co., China) at 37°C at 60 rpm. At regular times, one of the vessels was taken out, and the degradation medium was removed from the vessel to detect the pH value. The remaining sample was rinsed with distilled water to remove any residual buffer salts and dried to constant weight in a vacuum desiccator at room temperature.

The degree of degradation was estimated from the reduction of molecular weight, from the loss-in-mass, and the pH value changes of the degradation medium.

The molecular weight of the polymers was determined by gel permeation chromatography (GPC) in tetrahydrofuran (THF) at 30°C with an Ultrastyragel lineal column (Waters, USA) and a refraction index detector (R401, Waters), and calibrated with polystyrene standards. Mass loss was calculated by comparing the net weights of samples at predetermined intervals with the initial net weight of the samples.

Preparation of microspheres

PLGAE microspheres were prepared by emulsion/evaporation procedure, which was based on the formation of a modified double emulsion $W_1/O/W_2$. Briefly, an aqueous solution of Human Serum Albumin (HAS) as the W_1 phase was dispersed into the organic solution (as oil phase), consisting of the polymer dissolved in methylene chloride. The mixture was emulsified with magnetic stirrer for 2 min at room temperature to form the primary W_1/O emulsion. This emulsion was then added to the external water phase containing stabilizers, and a high-speed homogenizer emulsified the mixture again. The organic solvent was extracted by adding 100 mL 6% isopropanol, then the mixture was stirred at a moderate speed at room temperature for 5–6 h. The solidified microspheres were collected by centrifugation (Tomy Seiko Co. Ltd) at 8000 rpm for 10 min. The obtained microspheres were rinsed with distilled water and centrifuged three more times, then lyophilized, and then stored at −20°C.

The loading efficiency of HSA-loaded microspheres was determined using a direct method. In detail, 200 mg HSA-loaded microspheres were dissolved in 1500 μL methylene chloride, and 500 μL of redistilled water was added to extract HSA. After centrifugation at 3000 rpm for 5 min, the aqueous layer was transferred into a fresh tube. The concentration of HAS was assessed by ultraviolet spectrophotometer (Shimadzu, UV2401, Japan).

RESULTS AND DISCUSSION

PLGAE has been synthesized for application in controlled drug delivery and targeting.[9] However, the hydrophilic domain, PEG, was only on the end group. We attempted to design a molecule with PEG segments distributing in any part of the main molecular chain. This kind of molecular structure rendered the copolymer degradation in aqueous more easily. The copolymer was synthesized by the copolymerization of DL-lactide, glycolide, and PEG being initiated by stannous octoate at 180°C. The molecular structure of the copolymer was analyzed by IR and ^{1}H NMR spectra. Figure 1 was the IR spectrum of PLGAE. The resonances at 2995, 2880, and 1384 cm^{-1} belonged to the methyl of poly (lactic acid); the resonances of carbonyl bond was at 1760, 1270, and 1189 cm^{-1}; the resonances at 1452 and 1362 cm^{-1} were assigned to methylidyne (CH═); the resonance at 2944 cm^{-1} corresponded to methylene; the resonances at 1093 and 1049 cm^{-1} belonged to methylene in PEG segments.

TABLE I
Characterizations of PLGAE[a] with Different Content of PEG6000

PEG%						
Feed	Found[b]	$M_n \times 10^4$ (Da)[c]	$M_w \times 10^4$ (Da)[c]	M_w/M_n	T_g (°C)	Yield (%)
5	7.5	7.84	10.9	1.39	45.2	86.4
10	17.3	3.13	4.49	1.43	43.5	89.7
15	19.9	2.89	4.26	1.47	42.1	91.8
20	22.6	2.54	3.89	1.54	38.7	93.5

[a] Copolymerized at 150°C for 3 h with 0.3% of stannous octoate, the content of PGA was 10%.
[b] Confirmed by ^{1}H - NMR.
[c] M_n is number-average molecular weight, and M_w is weight-average molecular weight. They were measured by GPC in THF with polystyrene as the reference.

Figure 2 was the ^{1}H NMR spectrum of PLGAE. The resonances in PLA segments were shown at 5.02 ppm (CH) and 1.6 ppm(CH_3); the resonances at $\delta = 4.8$ ppm was attributed to the methylene in PGA segments; the resonance of PEG segments was at 3.6 ppm.

The thermal behavior of the copolymer was shown in Figure 3. Poly (DL-lactic acid) was an amorphous polymer with the glass transition temperature (T_g) of 58°C. The T_g decreased to 46°C after introducing 10% of PGA into the polymer molecular chain. PEG6000 belonged to a crystalline polymer with a melting temperature at about 50°C. When 10% of PEG6000 was copolymerized with DL-lactide and 10% of glycolide, the T_g of the copolymer was 43.5°C, which was lower than the T_g of PLGA (PLA : PGA, 90 : 10) but higher than the T_g (34.6°C) of the PLGAE (20% of PEG6000, 10% of PGA). The decrease of T_g was because the flexibility of the molecular chain was improved by introducing PEG. But the copolymer of PLGAE showed no obvious melting temperature (T_m) in the DSC curves. It indicated that the copolymer, PLGAE, was the completely amorphous materials.

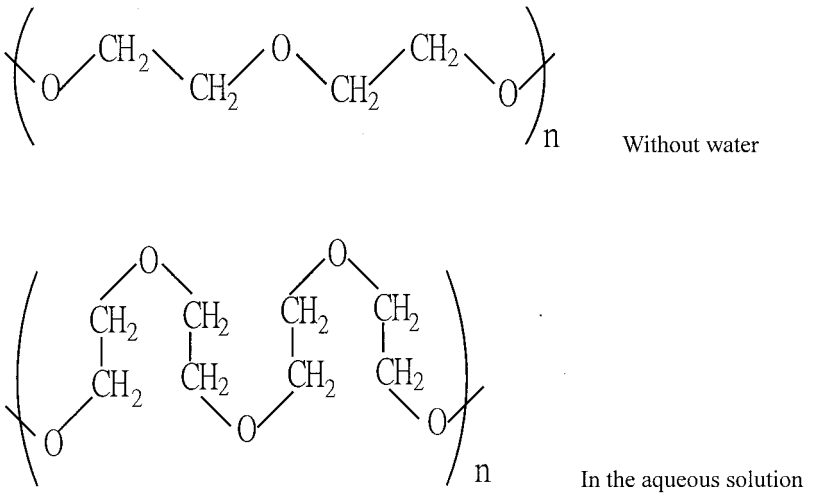
The characterizations of PLGAE with different PEG content were shown in Table I. The molecular weight of the copolymers decreased while the yield of the copolymerization increased with the increase of the PEG content. The measured molecular weight was smaller than the calculated molecular weight after 3 h copolymerization. The gap between measured values and calculated values decreased with increasing the PEG content. Analyzing the phenomena, it was inferred that the activity of DL-lactide was weaker than that of glycolide and PEG owing to the presence of an extra methyl group in DL-lactide. Thus, under the initiating the reactions of glycolide and PEG was faster than the reaction of DL-lactide. Before the copolymerization was completed, more PEG and PGA were found. But extending the reaction time, the content of DL-lactide in the molecular chain would increase generally, and the molecular weight and yield would increase also. Table II showed the influence of reaction time on the characterization of the copolymer. The results proved the hypothesis above. When the reaction time was 5 h, the found content of PEG was close to the feed values and the molecular weight and the yield were all increased.

Owing to the PLA and PEG contents, the crystalline region of PGA was eventually disrupted, producing an amorphous polymer. The introduction of PEG improved the hydrophilic property of the copolymer. The structure of PEG segments in the molecules of PLGAE was shown in Scheme 1. When the molecules were in the aqueous solution, the oxygen of the PEG segments would project towards the water. This kind of structure was of possible to change the degradation rate of the copolymer in aqueous solution and the

TABLE II
Influence of Reaction Time on the Characterization of PLGAE[a]

PEG%, Found[b]	Reaction time (h)	$M_n \times 10^4$(Da)[c]	$M_n \times 10^4$(Da)[d]	Yield (%)
26.4	1	1.46	2.27	46.7
20.2	2	2.09	2.97	68.5
17.3	3	3.13	3.47	89.7
12.2	4	5.28	4.92	91.4
10.5	5	6.23	5.71	93.0

[a]Copolymerized at 150°C for 3 h with 0.3% of stannous octoate, the content of PGA was 10%, the PEG content of feed was 10%.
[b] Confirmed by ^{1}H - NMR.
[c] M_n is number-average molecular weight, and M_w is weight-average molecular weight. They were measured by GPC in THF with polystyrene as the reference.
[d] Calculated according to the found PEG content.



Scheme 1 The structure of PEG.

affinity between hydrophilic drug and polymer matrix. This approach may be used to control the degradation rate of hydrophilic drug carriers made of lactic acid.

Several variables were investigated to characterize the degradation behaviors of PLGAE, including visual inspection, mass loss, molecular weight reduction, and pH changes of the degradation medium.

The appearance of the PLGA (10% of GA) or PLGAE (10% of GA, 10% of PEG6000) samples with the similar molecular weight (40–50 kDa) was an opaque white material at beginning. After 40 days in PBS buffer (pH7.4) at 37°C, the PLGA became brittle, while PLGAE was already brittle and broken at 30 days. The differences in the hydrophilic property were definitely responsible for these differences in physical strength reduction.

The mass loss and molecular weight reduction were shown in Figure 4. These results indicated that the degradation of PLGAE occurred in the bulk by random chain scission just like the degradation of PLGA. The bulk hydrolysis of samples led to the molecular weight reduction. Once the molecular weight of samples or the degradation fragments of samples reduced to a certain level, the samples or the fragments were soluble in PBS buffer and the mass loss was observed. That was why the mass loss lagged behind the reduction of molecular weight, but the lag was decreased with the increase of PEG content in the molecules. The degradation of PLGAE, however, was faster than that of PLGA (90 : 10, LA : GA), especially the mass loss. During 60 days, PLGAE (10% of GA, 10% of PEG6000) had lost 42.2% of the mass while PLGA had lost only 4.8%. The mass loss of PLGAE was 37.4% faster than that of PLGA, but the molecular weight reduction of PLGAE was just 20.7% faster than that of PLGA. The copolymer–PLGAE (with 20% of PEG6000 and 10% of GA) degraded the most rapidly due to the content of PEG. This indicated that the introduction of PEG, a soluble group in water, improved the affinity between water and polymer, which caused the promoted hydration and the hydrophilic property of the copolymer. On the other hand, PEG dispersed in the whole molecular chain of the copolymer, which caused the impossibility of crystallization for PEG and PGA segments in the molecular chain of copolymer so that the degradation of PLGAE was easier than that of PLGA, and more PEG contained the molecular chain more rapidly the copolymer degraded.

Figure 5 shows the pH changes of the degradation medium for PLGAE (10% of GA, 10% of PEG6000). From Figure 5, the pH values increased during the initial days and then decreased steadily. The reason might be that the degraded polymer could not be soluble in PBS during the initial days, but the negative end group that generated from the hydrolysis of the ester bond attracted the positive charges in the degradation medium. The results of that was the increase of pH values. Once the soluble acid products were generated, the pH values of the degradation medium were acidified generally.

The prominent advantage of PLGAE as a drug carrier over PLGA was its improved hydrophilic property and vested amphipathy. The increase of hydrophilic property of copolymer was proved again by the increased drug encapsulation efficiency. Human Serum Albumin (HSA) was chosen as the model drug in our investigations. HSA-loaded PLGAE or PLGA microspheres were elaborated by the solvent extraction method based on the formation of multiple $W_1/O/W_2$ emulsion. Under the same preparation conditions, the encapsulation efficiency of HSA-loaded PLGA microspheres was 52%, while that of HSA-loaded PLGAE (containing 10% of PEG6000) was 67%. In the preparation of microspheres, when the oil phase mixed with the inner water phase, a stable emulsion was obtained

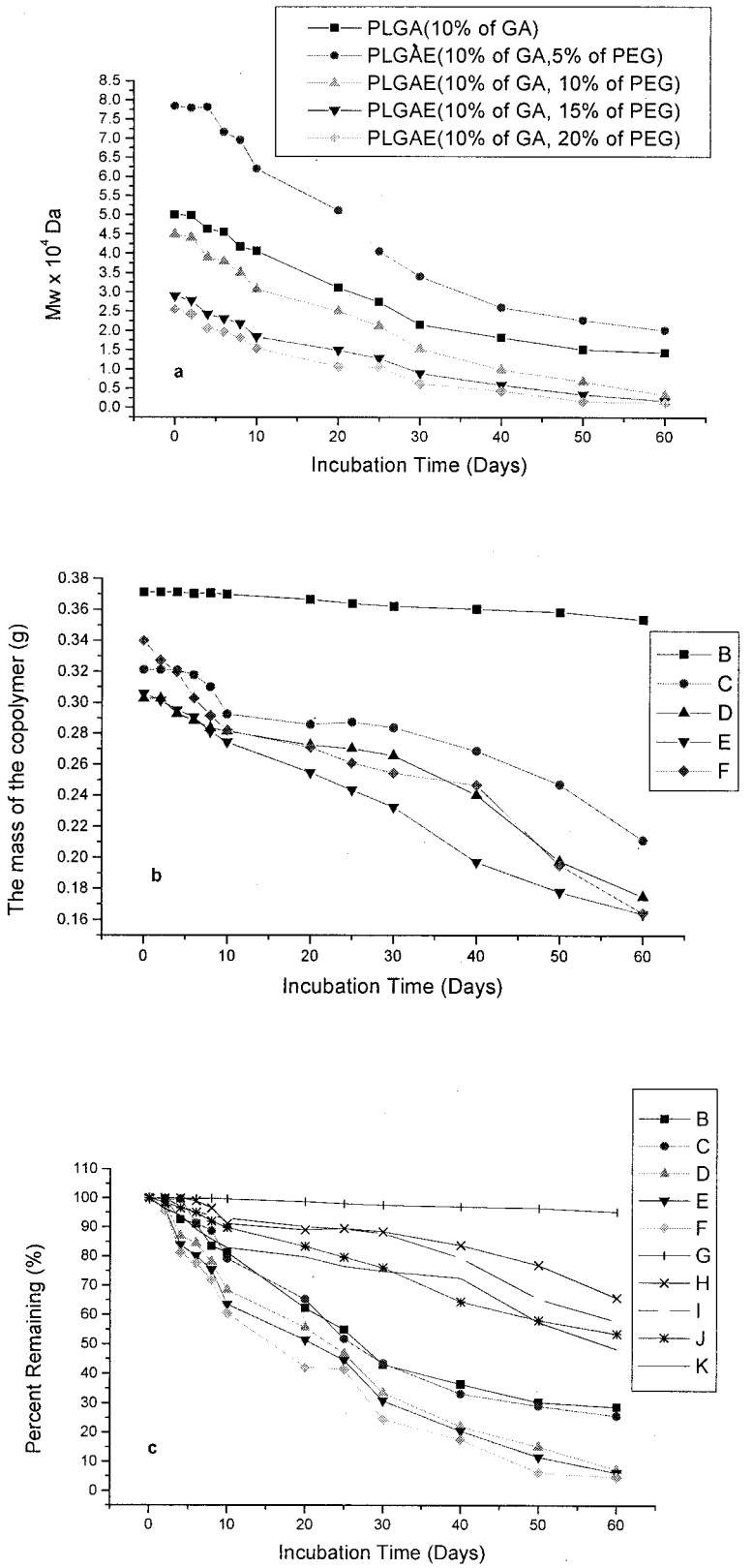


Figure 4 The molecular weight reduction and the mass loss of polymers. (a) The molecular weight reduction of copolymers with different PEG contents. (b) The mass loss of copolymers with different PEG contents (B: PLGA with 10% of GA; C: PLGAE with 5% of PEG6000 and 10% of GA; D: PLGAE with 10% of PEG6000 and 10% of GA; E: PLGAE with 15% of PEG6000 and 10% of GA; F: PLGAE with 20% of PEG6000 and 10% of GA); (c) Normalized mass and molecular weight (M_w) loss (B: M_w loss for PLGA with 10% of GA; C: M_w loss for PLGAE with 5% of PEG6000 and 10% of PLGAE; D: M_w loss for PLGAE with 10% of PEG6000 and 10% of GA; E: M_w loss for PLGAE with 15% of PEG6000 and 10% of GA; F: M_w loss for PLGAE with 20% of PEG6000 and 10% of GA; G: Mass loss for PLGA with 10% of GA; H: Mass loss for PLGAE with 5% of PEG6000 and 10% of GA; I: Mass loss for PLGAE with 10% of PEG6000 and 10% of GA; J: Mass loss for PLGAE with 15% of PEG6000 and 10% of GA; K: Mass loss for PLGAE with 20% of PEG6000 and 10% of GA).

without emulsifier, because the amphiphilic copolymer itself acted as the emulsifier. This property of PLGAE was even better than poly(DL-lactic acid)-*co*-poly(ethylene glycol) (PELA, 10% of PEG6000),[10] which should have acted as emulsifier in preparation PELA microspheres but the emulsifier was needed practically, especially the concentration of HSA in the inner water phase below 10%. It was indicated that the affinity between PLGAE and HSA was better than the affinity between PLGA and HSA, and the copolymer became the real amphiphilic material after introducing the PEG into the molecules. Another apparent proof of the increased hydrophilic property was the difference of PLGAE microspheres diameters measured by SEM and laser diffractometer, respectively. For example, the diameters measured by SEM were 0.9 and 4.9 μm for HSA-loaded PLGAE and PLGA microspheres, respectively, while the diameters measured by laser diffractometer were 1.3 and 5.1 μm. The increase rates were 44.4 and 4.1%. Because the microspheres were dispersed in water in the laser diffractometer, while microspheres were required in the vacuum-dried state for SEM measurement.

Figure 6 was the particle size distribution of HSA-loaded PLGA (LA : GA, 90 : 10) and PLGAE (10% of GA, 10% of PEG6000). It showed that the size distribution of PLGAE was narrower than that of PLGA. It was also caused by the hydrophilic property of PEG and the amphiphilic property of PLGAE, which rendered the emulsion more stable and the micelle more uniform.

CONCLUSIONS

The copolymer, PLGAE, was synthesized by bulk ring-opening polymerization DL-lactide, glycolide, and PEG6000 using stannous octoate as initiator at 180°C. Analyzing the structure, the copolymer was proven to be an amorphous material, which influenced the degradation behavior of the copolymer. The degradation of copolymer with different PEG contents was investigated. It was concluded that the crystalline structure of PGA was disrupted thoroughly and the hydrophilic property of the copolymer was improved. The more PEG that was contained in the copolymer, the more rapidly the copolymer degraded. The HSA-loaded PLGA and PLGAE microspheres were prepared by emulsion/evaporation method based on the formation of a modified double emulsion $W_1/O/W_2$. In the preparation, no emusifier was needed in the inner water phase because of the amphipaty of PLGAE. The average diameter of the HSA-loaded PLGAE microspheres was 1.3 μm measured by laser diffractometer, which was much smaller than that of HSA-loaded PLGA microspheres (5.1 μm). Moreover, the particle size distribution of PLGAE microspheres was more uniform than that of PLGA ones. The properties of PLGAE appeared to justify further investigation of the potentiality of the PLGAE for application in the drug controlled delivery system.

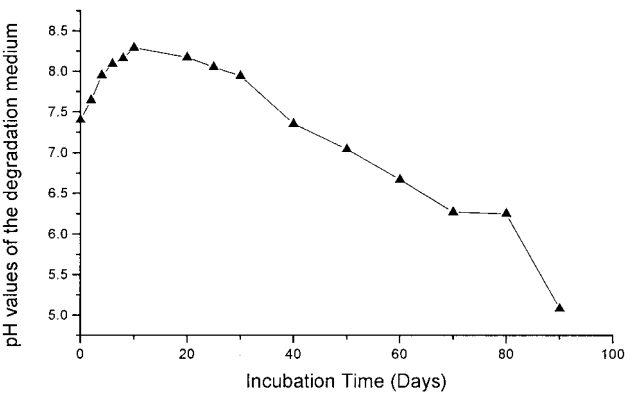


Figure 5 The pH value changes of the degradation medium.

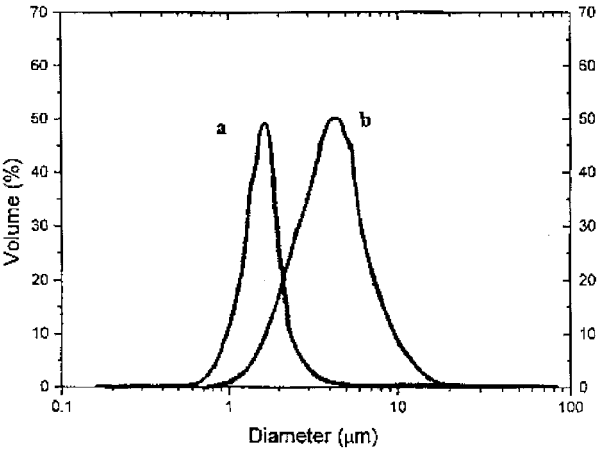


Figure 6 The particle size distribution of polymer microspheres. (a) HSA-loaded PLGAE (10% of GA, 10% of PEG6000) microspheres; (b) HSA-loaded PLGA (10% of GA) microspheres.

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