Synthesis and Characterization of a Novel Biodegradable Polymer Poly(lactic acid–glycolic acid-4-hydroxyproline)

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ABSTRACT: The effect of certain preparative variables, such as the composition of the feeds, the reaction time, catalyst concentration, degrees Centigrade (°C), and the reaction temperature on the properties of prepared polymer poly(lactic acid–glycolic acid-4-hydroxyproline) (PLGA-Hpr), was investigated via direct melt polymerization with stannous chloride as a catalyst activated by a proton acid. The new polymer had pendant amine functional groups along the polymer backbone chain. The results with regard to the inherent viscosity and yield of PLGA-Hpr are discussed in relation to a recently proposed polymerization mechanism. The content of lactic acid, glycolic acid, and 4-hydroxyproline (Hpr) in the copolymer was found to affect the surface and bulk hydrophilicity of various PLGA-Hpr copolymers. The inherent viscosity of the copolymer and the yield of the reaction depended on the reaction temperature and varied with the reaction time. The higher the 4-hydroxyproline content of the feedzaq, the lower the inherent viscosity of the copolymer and the yield of the reaction. When the glycolic acid content was more than 70% or the content of HPr was more than 10%, the polymer changed from hemicrystalline to amorphous. The in vitro degradation rate of the PLGA-HPr copolymers is dependent on the feed ratios of lactic acid and glycolic acid in the polymer chain. Lactic acid-rich polymers are more hydrophobic; subsequently they degrade more slowly. The structure of this polymer was verified by infrared (IR) spectroscopy, proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy, X-ray diffractometry (XRD), and differential scanning calorimetry (DSC). © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 103: 3585–3590, 2007

Key words: lactic acid; 4-hydroxyproline; biodegradable; copolymerization; differential scanning calorimetry

INTRODUCTION

Biodegradable polymers have raised increasing interest over the past two decades in fundamental research and in the chemical industry. Polylactide and its copolymer is a type of biodegradable material with low toxicity, excellent biocompatibility, and bioabsorption in vivo, which have been widely used as sutures, sustained drug carriers, implants for orthopedic devices, and absorbable fibers to ensure temporary mechanical or therapeutic function, as well as cell scaffolds in tissue engineering.[1–6]

However, the copolymers of lactic acid with pseudo-poly(amino acid) began to attract considerable interest only in recent years; this kind of material has the advantage of being nontoxic, biodegradable, biocompatible, and fitted with pendant functional groups on the backbone.[7] Some studies have focused on the synthesis of biodegradable polymers bearing pendant functional groups available for the attachment of bioactive peptides or other further chemistry by the polymerization or copolymerization of functional monomers; for example, Elisseeff et al.[8] prepared the polymer of poly(lactic acid-co-aspartic acid), and Lee and Yang[9] prepared polymers based on lactic acid and *trans*-4-hydroxyl-proline (Hpr).

The polymerization of suitable comonomers is the oldest and most successful strategy for varying the properties of a polymer systematically over a broad range. The properties and potential applications of polylactide can be varied over a broad range as well. The incorporation of glycolide enhances the rate of hydrolytic degradation and is usually desired, when the polylactide is designed for controlled drug release. However, few people have considered the poly(lactide-co-glycolide) (PLGA)/Hpr copolymer. In our research, we choose 4-hydroxyproline to introduce functional groups on PLGA because 4-hydroxyproline is a kind of amino acid needs must by human body and has the advantage of being nontoxic, biodegradable, biocompatible and fitted with pendant functional groups on the backbone, after polymerization with other polymers it still retaining some active groups. The present work was aimed at the synthesis and properties of poly(lactic acid–glycolic acid-4-hydroxyproline (PLGA-Hpr), which prepared by the melt polycondensation of lactic acid, glycolic acid,

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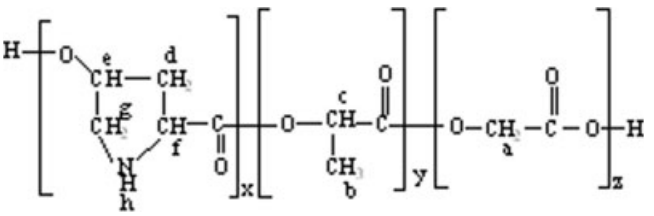


Scheme 1 Structure of poly(lactic acid–glycolic acid-4-hydroxyproline).

and Hpr. The resulting copolymers were characterized by various analytical techniques. The structural formula of targeting polymer is as shown in Scheme 1.

EXPERIMENTAL

Materials

Lactic acid was purchased as 90% aqueous solution from Shanghai Chemical Industries. Stannum chloride dihydrate and *p*-tolulenesulfonic acid monohydrate (TSA) were purchased from Guangzhou Chemical Industries, and all these materials were used without further purification. All other chemicals were purchased in analytical grade made in the People's Republic of China.

Characterization

Infrared (IR) spectra were measured on a NICOLET-20DXB IR spectrophotometer. Samples were pressed into KBr pellets. ^{1}H NMR spectra were measured on a Varian INOVA 400-MHz spectrometer in DMSO containing 1 vol% of tetramethylsilane (TMS) as the internal reference. The crystallinity of the polymer was carried out on a XD3-A wide-angle X-ray diffraction (WAXD) apparatus. Differential scanning calorimetry (DSC) measurements were carried out at a heating rate of 10°C/min on a 910S thermal analyzer with N_2 gas protection. Contact angle of polymer films were measured statically on a JY-82 contact angle meter (made in the People's Republic of China); water sorption was evaluated by immersing polymer films in distilled water for 24 h at room temperature and then weighted at regular intervals for 3 h until constant weight. Water sorption was calculated as follows:

$$\text{Water sorption } (\%) = \{[W_{wet} - W_{dry}]/W_{dry}\} \times 100\%, \quad (1)$$

where W_{wet} is the weight of the polymer film just taken out of the water after removal of the surface water by filter paper, and W_{dry} is the weight of the wet film after rigor drying in vacuum at room temperature. The final value is the average of three samples of each polymer.

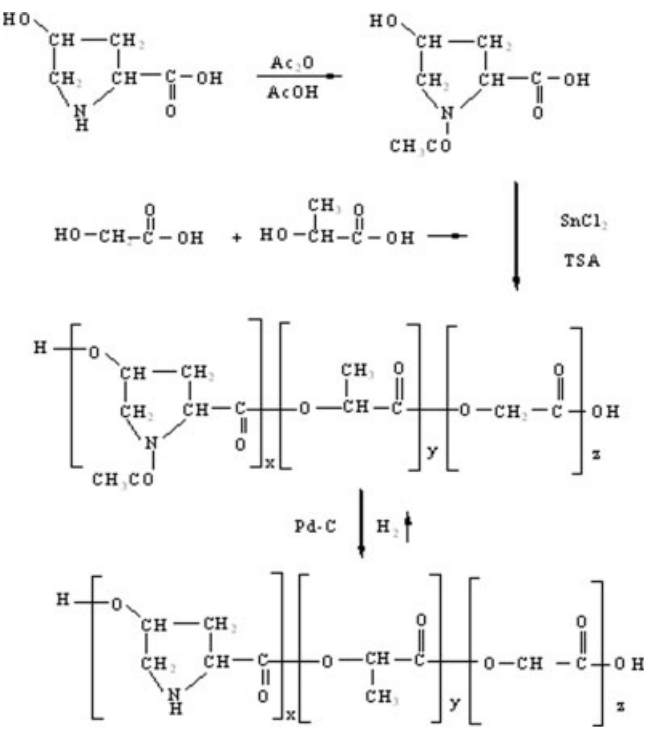
Method

Synthesis of the PLGA-N-Ace-Hpr (Scheme 2)

Lactic acid (0.4 mmol) and glycolic acid (0.4 mmol) were dehydrated at 150°C, at a pressure of 5 mm Hg for 6 h. The oligomer was crushed into power, and then put into a 250-mL flask, mixed with 4 wt % (N-Ace-Hpr)[7], and 2.5 wt % Tin(II) chloride dihydrate and TSA. The mixture was heated at 180°C under mechanical stirring at 5 mm Hg for 5 h. Under the same pressure, the react temperature was controlled at 150°C for 9 h. At the end of the reaction, the flask was cooled down. The product was dissolved in acetone and subsequently precipitated in ether. The resulting solids were filtered and dried in vacuum. At last, the protected amino group was reduced by a 5 wt % palladium-on-charcoal catalyst. The composition in the copolymers was analyzed by IR spectroscopy (Fig. 1) and ^{1}H- NMR spectroscopy (Fig. 2), at a yield of 70%. In the region of methylene, protons of PLGA-Hpr signals are observed: the absorption peaks (δ4.85 ppm) belong to (O=C—CH_2—C—O—) methylene protons. The absorption peak (δ5.25 ppm) is the methane (C_4—H) of the proline in PLGA-Hpr and with the absorption peaks (δ1.49 ppm) is the methyl (C—CH_3) of the lactic acid in PLGA-Hpr.

Degradation of the PLGA-Hpr polymer

PLGA-Hpr polymer films with a thickness of 0.5 mm were cast from a tetrafluoroethylene mold after melt. Degradation experiments were carried in vitro



Scheme 2 Synthetic route of the PLGA-Hpr.

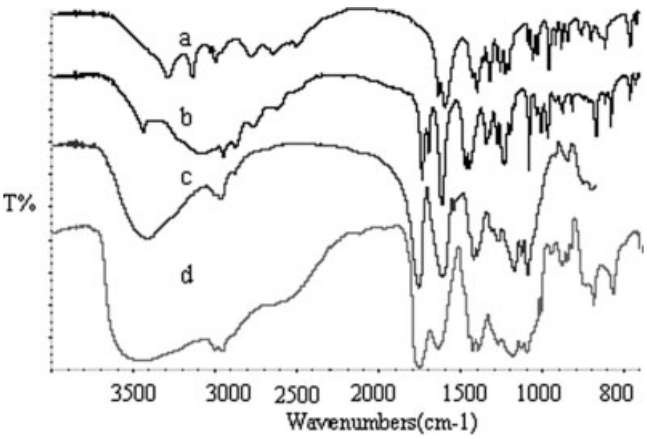


Figure 1 IR spectrum of (a) Hpr, (b) N-Ace-Hpr, (c) PLGA- N-Ace-Hpr (C), (d) deprotected PLGA-N-Ace-Hpr.

out by immersing the specimens of the PLGA-Hpr polymer samples in phosphate buffer solution (pH 7.4) at 37°C. At preset time intervals, the samples were taken out and dried under vacuum at room temperature to constant weight; the buffer solution was then renewed. The changes of weight loss of the degraded samples were determined as described in Figure 3.

RESULTS AND DISCUSSION

Synthesis of the PLGA-Hpr polymer

Polymers were obtained by polymerization of a mixture of lactic acid, glycolic acid, 4-hydroxyproline with stannum chloride dihydrate as a catalyst activated by a proton acid TSA. Stannum chloride dihydrate activated by TSA is a well-known co-catalyst with medium activity, often used in promoting the polymerization of lactic acid.[10]

Structural characterization

Compared with IR spectra of the Hpr [Fig. 1 (a)], N-Ace-Hpr [Fig. 1 (b)] has absorption peaks of C=O of amide at 1730 cm^{-1}. N-Ace-Hpr was prepared successfully. The representative IR spectra of the PLGA-Hpr polymers are shown in Figure 1 (c). Characteristic absorption bands related to both Hpr and PLGA can be observed. The IR spectra of the protected copolymers C [Fig. 1 (c)] and deprotected PLGA-Hpr [Fig. 1 (d)] exhibit strong ester carbonyl bands at 1750 and 1745 cm^{-1}, respectively. The most distinctive features of the deprotected PLGA-Hpr polymer were the presence of a broad amine band (-NH-) at 2400–3100 cm^{-1} superimposed with aliphatic —C—OH stretching).

To gain insight into their chemical structure, the various copolymerization products were subjected to ^{1}H-NMR measurement. A typical spectrum of PLGA-Hpr polymers (C) is shown in Figure 2. The ^{1}H-NMR spectra of copolymer are consistent with the IR data. The characteristic absorption peaks at δ4.85 ppm [due to the methylene protons of the (—CH_2^*—)], δ5.25 ppm (due to the methylene (C_4—H) of the proline), δ4.35–4.22 ppm (due to the C_2—H methine proton of the Hpr), δ3.64–3.48 ppm (due to the C_5—H methylene protons of the Hpr), and δ2.37–2.09 ppm (due to the C_3—H methylene protons of the Hpr) are exhibited; δ5.15 ppm (due to the methine proton of the lactic acid) and δ1.45–1.53 ppm (due to the methyl proton of the lactic acid) are exhibited. The H-NMR spectrum of the protected copolymer [Fig. 2 (A)] and deprotected PLGA-Hpr [Fig. 2 (B)] is shown in

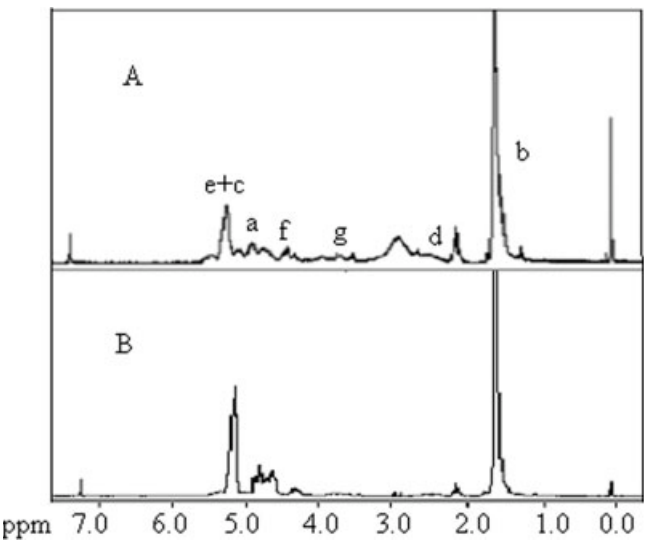


Figure 2 ^{1}H-NMR spectrum of PLGA-N-Ace-HPr (A) and PLGA-HPr (B) [sample (C)].

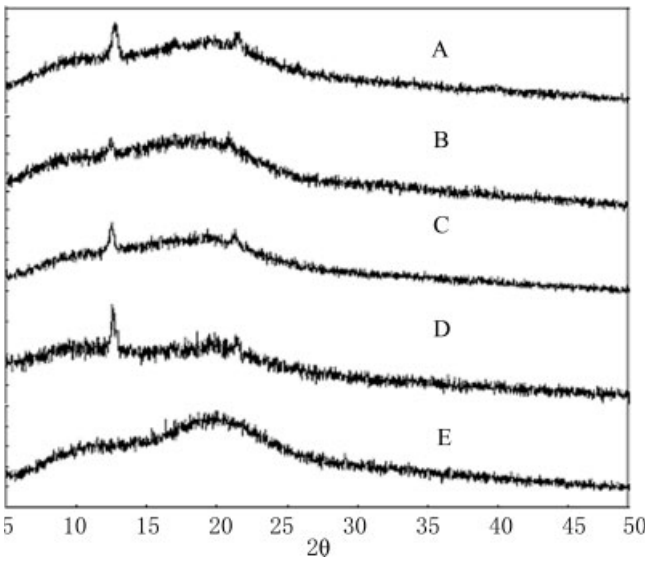


Figure 3 X-ray diffraction of series poly(lactic acid–glycolic acid-4-hydroxyproline).

TABLE I
Effect of the Amount of Catalyst on the Inherent Viscosity and Yield on PLGA-HPr Prepared by Polymerization of Lactic Acid, Glylactic Acid and 4-Hydroxyproline

Run	Catalyst[a] (wt%)	[η] (dL/g)[b]	Yield (%)
1	0.4	0.1709	71
2	1	0.1806	76
3	2	0.3474	75
4	3	0.3488	78
5	4	0.2764	70

[a] At 180°C for 5 h at < 10 mm Hg and then 150°C for 9 h.
[b] Measured at a concentration of 0.125 g/dL in DMF at 25°C.

Figure 2. The most distinctive features of the deprotected PLGA-Hpr polymer were the vanishing peaks at δ2.01 ppm (due to the methyl proton of the acetyl protecting group).

The composition of the copolymer was analyzed by ^{1}H-NMR. The amounts of comonomer incorporated into the copolymer could be calculated by comparing the integrated areas of the absorption peaks at δ5.25 ppm of the methine proton (C_4—H) of the proline with the absorption peaks at δ1.5 ppm (due to the methyl proton of the lactic acid) and 4.8 ppm (due to the methylene proton of the glycolic acid). The mole ratio percentages of the comonomers incorporated into the copolymers are shown in Table IV. These results show that the degree of polymerization of the monomers is close to that of the corresponding feeds. However, Hpr units in the copolymer were higher than that in the monomer feeds, which may result from the loss in the processing of dehydration of the lactic acid and glycolic acid at the first stage.

Effect of preparative variables on the yield and inherent viscosity of the reaction

The yield and inherent viscosity depended on polymerization time, polymerization temperature, and catalyst. The polymerization of Hpr, lactic acid, and glycolic acid was investigated by melt polymerization over a wide range of compositions. The polymerization was performed in bulk using $SnCl_2$ and TSA as the catalyst; the results of polymerization are listed in Tables I–IV To find the optimum polymerization conditions, the effects of catalyst level, polymerization temperature, and polymerization time on η and yield were investigated.

Initially, PLGA-Hpr was polymerized with lactic acid, glycolic acid, and N-Ace-Hpr at a ratio of 50 : 50 : 10 (w/w/w) with various amounts of catalyst ranging from 0.4 wt % to 4 wt % [$SnCl_2$: TSA = 1 : 1(w/w)]. The results are shown in Table I. When the content of the catalyst kept rising, the η of the copolymers reached a maximum value 0.3488 dL/g at 3%, after which the η began to fall. This is may be the low proportion of catalyst playing an important role in PLGA-Hpr polymerization, in a range in which the higher the catalyst proportion, the larger the polymer molecular weight. There exists a balance point, after exceeding the balance point, would lead to a low η because of the too active center. The yield of the copolymers was greater than 60%.

The effect of polymerization temperature on the inherent viscosity and yield was investigated. Lactic acid, glycolic acid, and Hpr were reacted at a ratio of 70 : 30 : 10 (w/w/w) at different reaction temperatures of 140–190°C, respectively, using 3 wt % [$SnCl_2$: TSA = 1 : 1 (w/w)] for 10 h. The results are shown in Table II. As the polymerization temperature was increased from 140 to 180°C, the η increased from 0.2238 to 0.2577 dL/g. However, when the reaction temperature was further increased from 180 to 190°C, the η decreased from 0.2577 to 0.1557 dL/g. Thus, the optimal reaction temperature for the polymerization was 180°C. When the reaction temperature continued to rise after the balance point was reached, the speed of catalyzing polymerization was less than that for degradation and oxidization, which could be responsible for the lower molecular weight of the polymer. For this reason, the yield of these polymers was decreased as well. The color of the products changed

TABLE II
Effect of Reaction Temperature on the Inherent Viscosity and Yield Prepared by Polymerization of Lactic Acid, Glylactic Acid, and 4-Hydroxyproline

Run	*T* (°C)	[η] (dL/g)[a]	Yield (%)
1	150	0.2239	73
2	160	0.2240	75
3	170	0.2342	73
4	180	0.2577	70
5	190	0.1557	69

[a] Measured at a concentration of 0.125 g/dL in DMF at 25°C.

TABLE III
Effect of Reaction Time on the Inherent Viscosity and Yield Prepared by Polymerization of Lactic Acid, Glylactic Acid, and 4-Hydroxyproline

Run	Reaction time[a] (h)	*T* (°C)	[η] (dL/g)[b]
1	11	180	0.1425
2	12	150	0.1306
3	14	150	0.1391
4	16	150	0.2624
5	18	150	0.2500
6	20	150	0.4932
7	22	150	0.3113

[a] Cumulate reaction time.
[b] Measured at a concentration of 0.125 g/dL in DMF at 25°C.

TABLE IV
Main Properties of PLGA-Hpr with Different Compositions

Sample	m(GA)/m(LA)/m(HPr) Feeding dose ratios	m(GA)/m(LA)/m(HPr) Product[b]	[η] (dL/g)[a]	Contact angle (°)	T_g[c] (°C)	Yield (%)	Water uptake (%)
A	10/90/10	9/87/9	0.3183	56.5	42.71	73	16.8
B	30/70/10	32/65/8	0.2779	55.6	34.60	74	22.9
C	50/50/10	49/47/9	0.2096	45.7	32.33	77	34.8
D	70/30/10	73/36/9	0.2280	34.1	25.5	71	42.1
E	90/10/10	94/8/9	0.2122	26.6	25.07	73	49.7
F	30/70/30	23/55/28	0.2575	21.7	20.07	69	—[d]
G	30/70/50	26/59/47	0.2120	16.5	22.12	75	—
H	30/70/70	27/55/67	0.1821	15.1	39.89	74	—
I	30/70/90	24/56/88	0.1788	12.6	52.5	67	—

[a] Determined using DMF as solvent at 25°C with a solution concentration of 0.125 g/dL.
[b] Determined by ^{1}H-NMR.
[c] Determined by DSC.
[d] Dissolvable in water.

from white to brown and then black, which could be attributable to the carbonization and oxidization of the reactant caused by the high temperature.

To study the effect of polymerization time on the inherent viscosity and yield of PLGA-Hpr, polymers were prepared at different stages, as described in the Experiment section. The results are shown in Table III. The first sample was taken after the reaction reacted at 180°C for 5 h, and then one sample per 2 h at the following 10 h. The largest η is 0.4932 dL/g after 20-h reaction. On the basis of these findings, the highest η and yield of PLGA-Hpr [η = 0.3488 dL/g) were obtained at 3 wt % ($SnCl_2$: TSA = 1 : 1(w/w)] as the catalyst for 20 h.

Crystallinity

The crystalline behavior of PLGA-Hpr polymers was carried out by X-ray diffraction (XRD) measurements. The crystallinity of the PLGA copolymer is dependent on the type and the molar ratio of the individual monomer components (lactic acid and glycolic acid) in the copolymer chain. Also, the crystallinity of the PLGA-Hpr polymers was affected by the molar ratio of the individual monomer components (lactic acid, glycolic acid, and 4-hydroxyproline) in the copolymer chain. The XRD spectrum of PLGA-Hpr polymers with different compositions is shown in Figure 4. There are two continuous intensity distribution ''obtuse peaks,'' the typical diffraction curve of the amorphous polymer. According to XRD, with an Hpr feed ratio of more than 10%, or glycolic acid with a feed ratio of glycolic acid more than 70%, the copolymers were amorphous. Keeping the amount of Hpr constant, with the increase of the rate of glycolic acid from 10% to 90% the intensity of diffraction peak at 2θ = 12.64 was gradually increased and then appears the maximum value at 70%, when it continue arouse to 90% the diffraction peak at 2θ = 12.64 was disappeared [copolymers A, B, C, D, and E (A, B, C, D, and E refer to the polymers in Table IV)]. Thus, in the series copolymers, when the content of glycolic acid was more than 70% or more than 10% for Hpr, the polymer changed from hemicrystalline to amorphous.

Thermal properties

The thermal behavior of PLGA-Hpr polymers was detected by DSC equipment. The glass transition temperatures (T_g) of PLGA-Hpr polymers with different feed ratios are shown in Table IV. The T_g of the PLGA-Hpr copolymers changed from 20.07 to 52.5°C when the feed ratio of Hpr increased from 10% to 90%, respectively [copolymers F, G, H, and I (F, G, H, and I refer to the polymers in Table IV)]. Hpr profoundly affected the polymer thermal properties, and similar results were observed by Lee and Yang.[11,12]

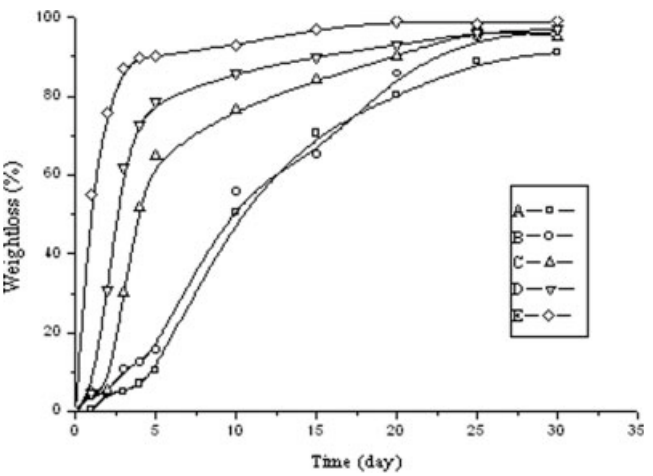


Figure 4 Weight loss of PLGA-Hpr samples A, B, C, D, and E in phosphate buffer solution (pH 7.4) at 37°C.

This is because Hpr is a hard component in comparison with lactic acid and glycolic acid. Therefore, when more rigid linkages, such as cyclic proline groups, were incorporated into the macromolecular backbone, the rotation of the copolymer chain was restricted and led to an increase in T_g.

Hydrophilicity of PLGA-Hpr multiblock copolymers

The surface and bulk hydrophilicity of various PLGA-Hpr polymers with different feed ratios were determined by contact angle and water uptake; the data are presented in Table IV. It can be seen that the contact angles of the polymers were smaller when the feed ratios of glycolic acid and Hpr were higher. This meant that glycolic acid and 4-hydroxyproline are more hydrophilic than lactic acid. The bulk hydrophilicity of the copolymer was detected by water uptake; the results are shown in Table IV.

Preliminary *in vitro* degradation study

The *in vitro* degradation of PLGA-Hpr was evaluated at 37°C under physiological conditions (pH 7.4) from the weight loss of the sample. When the feed ratio of the protected 4-hydroxyproline was more than 30%, the polymer could dissolve in water. So when it was used as drug carries, the content of Hpr should be controlled below 30%. The degradation profile behavior of PLGA-Hpr as the feed ratios of lactic acid and glycolic acid changed from A to E is shown in Figure 4. This trend is in line with the crystallinity of corresponding polymers.

The biodegradation speed of the PLGA-Hpr polymers is dependent on the feed ratios of lactic acid and glycolic acid in the polymer chain. Lactic acid-rich PLGA-Hpr polymers are more hydrophobic and subsequently degrade more slowly. Thus, the degradation rate of the PLGA-Hpr copolymers could be well controlled by changing the ratio of lactic acid and glycolic acid in the polymer chain.

CONCLUSION

PLGA-Hpr polymers with pendant functional groups available for attachment of bioactive peptides or other further chemistry can be obtained by melting polymerization in the presence of $SnCl_2$ and TSA as the catalyst. On the basis of these findings, the optimum polymerization conditions was produced: D,L-lactic acid and glycolic acid were dehydrated for 6 h at 150°C, after which 4-hydroxyproline was added to the oligomer reacted at 3 wt % ($m(SnCl_2) : m(TSA) = 1 : 1$) as the catalyst at 180°C for 5 h, and then reacted for 9 h under 150°C; the highest η (0.3488 dL/g) and yield (64%) of PLGA-HPr was obtained. The degradation speed of PLGA-Hpr could be adjusted by varying the ratio of lactic acid and glycolic acid in the polymer chain. Further investigations are under way to examine the drug release behavior from these copolymers.

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