ORIGINAL CONTRIBUTION

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Synthesis, characterization and in vitro degradation of biodegradable polyesteramide based on lactic acid

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Abstract A new type of biodegradable polyesteramide P(LA/AU) based on lactic acid and aminoundecanoic acid was synthesized by the melt polycondensation method. The copolymers obtained were characterized by FTIR, ^{1}H-NMR, DSC, and XRD. The in vitro degradation behavior of the copolymers was studied using weighing, ^{1}H-NMR, FTIR, DSC, and SEM. With the increase in aminoundecanoic acid units, the melting temperature and crystallinity increased, but the water absorption and degradation rate decreased accordingly. During degradation in vitro, the ester moiety decreased due to ester cleavage along the macromolecular main chain. As degradation proceeded, the melting temperature and crystallinity increased at first, then started to decrease because the crystalline phase was destroyed.

Keywords Polyesteramide · Biodegradable polymer · Hydrolytic degradation · Aminoundecanoic acid · Surface morphology

Introduction

Aliphatic polyester and its copolymers are the most important biodegradable polymers for biomedical applications because of their good biocompatibility and biodegradability [1, 2]. But, they still lack certain optimum properties such as thermal, mechanical, and processing properties. So a new kind of aliphatic polyesteramide copolymer was proposed to overcome these shortcomings [3, 4, 5, 6]. In this copolymer, the ester and amide bonds were randomly distributed along the macromolecular main chain. Due to the polar nature of amide groups, these polyamide segments may form intra- as well as inter-molecular hydrogen bonding. Thus, such a polyesteramide copolymer would have great thermal and mechanical properties even at relatively low molecular weight. On the other hand, the hydrolytic degradable ester bond in the main chain imparts good degradability to the copolymer. In this paper, a new kind of biodegradable polyesteramide copolymer based on lactic acid and aminoundecanoic acid was synthesized by the melt polycondensation method. This P(LA/AU) copolymer has never been reported before.

Experimental

Materials

Aminoundecanoic acid was CP grade, and all the others were AR grade. The materials were used without purification.

Synthesis of P(LA/AU)x/y copolymers

P(LA/AU)x/y copolymers were synthesized by the melt polycondensation method according to Scheme 1. The typical P(LA/AU)50/50 copolymer was prepared as follows: 31.5 g (0.15 mol) of aminoundecanoic acid, 13.5 g (0.15 mol) of d,l-lactic acid, 0.1 g of titanium dioxide, 0.1 g of Irganox1010, and 0.05 g of tetrabutyl titanate were added into a reaction vessel under nitrogen atmosphere. The mixture was kept at 110 °C for 1 h. Later, the temperature was gradually elevated to 160 °C over 30 min. Then, the mixture was rapidly heated to 240 °C under vacuum conditions for another 1 h. At the end, the resultant melt was poured out into a
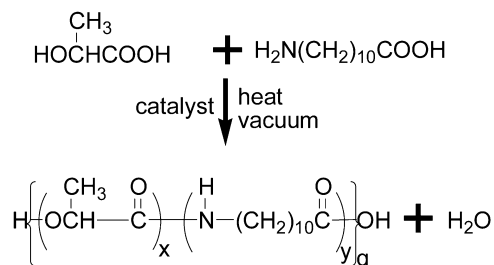
Scheme 1 Synthesis of P(LA/AU)x/y copolymer

steel plate. Thus, P(LA/AU)50/50 copolymer was obtained. The copolymer film was prepared by pouring the hot melt into a steel mold (100×100×1 mm). The samples for XRD test, water absorption, and in vitro degradation were cut from the film. The copolymers prepared in this work are shown in Table 1.

Water absorption of P(LA/AU)x/y copolymers

The copolymer films were immersed in distilled water at 30 °C for a predetermined period, then they were taken out and the surplus surface water removed by a filter paper. The value was calculated according to the following equation:

$$\text{Water absorption\%} = \frac{W_{ht} - W_d}{W_d} \times 100 \qquad (1)$$

where W_d is the weight of dry sample, and W_{ht} is the weight of humid sample at time t.

In vitro degradation of P(LA/AU)x/y copolymers

The films were placed in a small bottle containing 10 mL of phosphate buffer saline (PBS, pH = 7.2) at 37 °C. PBS was refreshed every 2 weeks. The samples were removed from the bottles at the predetermined time, rinsed thoroughly with distilled water and vacuum dried for 12 h at 63 °C. The degree of degradation was denoted as weight loss:

$$\text{Weight loss(\%)} = \frac{W_0 - W_t}{W_0} \times 100 \qquad (2)$$

where W_0 is the dry weight before degradation and W_t is the dry weight at time t.

Analytical methods

FTIR spectra

FTIR spectra (KBr) were recorded on NICOLET 200SXV meter.

Table 1 The composition, intrinsic viscosity, and thermal properties of polyesteramide copolymers

P(LA/AU)x/y	LA/AU (mol/mol)	$[\eta]$ (dl/g)	T_g (°C)	T_m (°C)	ΔH_m (J/g)	X_c (%)
P(LA/AU)25/75	25/75	0.46	40.49	159.94	27.58	14.0
P(LA/AU)40/60	40/60	0.45	39.29	122.22	22.12	11.2
P(LA/AU)50/50	50/50	0.43	40.49	88.85	18.91	9.6
P(LA/AU)60/40	60/40	0.39	–	75.45	2.8	1.4

Differential scanning calorimetry (DSC)

Non-isothermal crystallization behavior of these copolymers was characterized on DSC TA2910 (TA Instruments, Germany). The specimens were heated from 40 °C to 250 °C under nitrogen atmosphere at a heating rate of 10 °C/min, and cooling rate of 10 °C/min. The crystallinity (X_c %) was calculated from the heat of fusion.

$$X_c(\%) = \frac{\Delta h_m}{\Delta h_m^0} \times 100 \qquad (3)$$

where Δh_m is the heat of fusion of polyesteramide, Δh_m^0 is the heat of fusion of the 100% crystalline poly(aminoundecanoic acid) sample (nylon11, $\Delta h_m^0 = 47$ cal/g = 196.6 J/g) [7].

X-Ray diffraction (XRD)

XRD patterns were performed on an X-ray diffractometer (DMAX1400, Rigaku, Japan) with CuK$_\alpha$ radiation.

Scanning electron microscopy (SEM)

The surface morphology was observed on SEM (JSM-5900LV, 20KV, JEOL, Japan; and AMRAY, 20KV, America). The film surface was sputtered with gold.

^{1}H-nuclear magnetic resonance (^{1}H-NMR)

^{1}H-NMR spectrum (in D_2O or $CF_3COOD/CDCl_3$) was recorded on a Bruker 300 spectrometer (Bruker, Rheinstetten, Germany) at 300 MHz using trimethylsilane (TMS) as internal reference standard.

Results and discussion

Synthesis and characteristics

FTIR spectra of P(LA/AU)x/y copolymers are shown in Fig. 1. The major characteristic absorptions identified in FTIR spectra were: amide A (3304 cm^{-1}) and amide B (3087 cm^{-1}), amide I (1644 cm^{-1}) and amide II (1548 cm^{-1}), and aliphatic ester groups $\nu_{C=O}$ (1741 cm^{-1}).
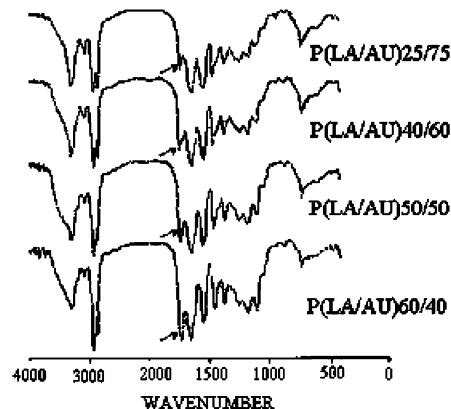
Fig. 1 FTIR spectra of P(LA/AU)x/y copolymers after reaction. The *arrows* indicate the absorption due to ester moiety
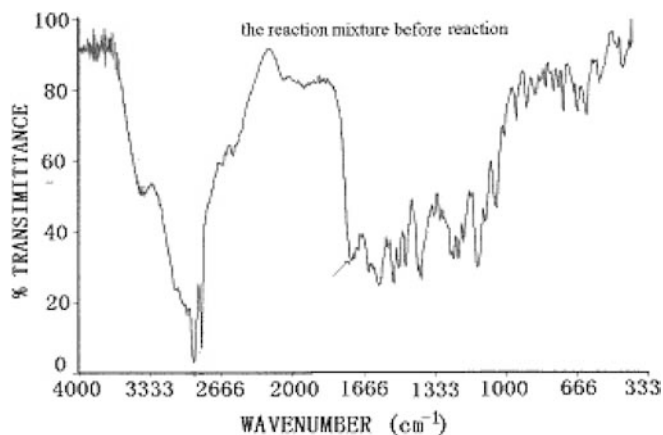
Fig. 2 FTIR spectrum of the reaction mixture before reaction. The *arrow* indicates the absorption due to the end carboxyl group of lactic acid and aminoundecanoic acid
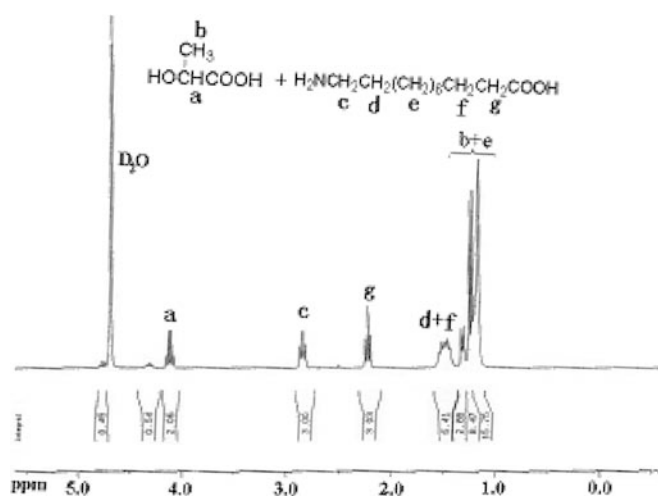
Fig. 3 ^{1}H-NMR spectrum of lactic acid and aminoundecanoic acid before reaction (in D_2O)

With the increase in lactic acid content, the absorption intensity at 1741 cm^{-1} increased. Figure 2 presents the FTIR spectrum of the mixture before reaction. The absorption at 1710 cm^{-1} was due to the end carboxyl group of lactic acid and aminoundecanoic acid. After reaction, the absorption at 1710 cm^{-1} disappeared, but the absorption due to ester and amide moieties came into being because of acylation. Further evidence for the molecular architecture of this polyesteramide copolymer is also provided by the analysis of ^{1}H-NMR spectra. The spectrum of the reaction mixture of *d,l*-lactic acid (LA) and 11-aminoundecanoic acid (AU) before polycondensation is shown in Fig. 3. The chemical shift of CH (peak a) of LA was found at 4.1 ppm, and the chemical shift of CH$_2$ (peak c) of AU connected to the nitrogen atom was at 2.8 ppm. The main evidence for the macromolecular architecture is the chemical shifts of peak a and peak c.
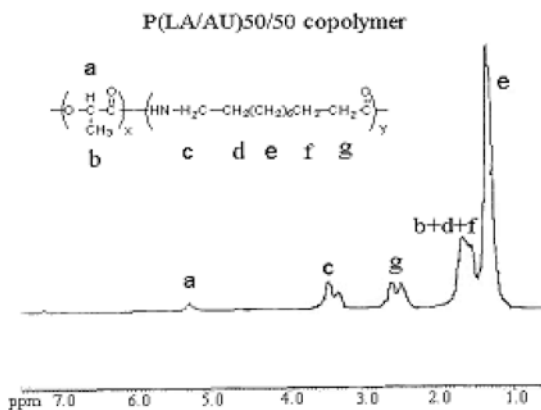

Fig. 4 ^{1}H-NMR spectrum of P(LA/AU)50/50 copolymer after reaction (in $CDCl_3$/CF_3COOD)

After melt polycondensation, the chemical shifts of these two peaks switched to other positions. The typical ^{1}H-NMR spectrum of P(LA/AU)50/50 copolymer is shown in Fig. 4. The chemical shift of CH (peak a) in poly(lactic acid) segments main chain increased to be at 5.3 ppm, and the chemical shift of CH$_2$ (peak c) in poly(aminoundecanoic acid) segments connected to the nitrogen atom increased to be at 3.5 ppm, because of the formation of ester bond and amide bond along the macromolecular main chain. According to Figs. 1, 2, 3, and 4, a conclusion could be made that P(LA/AU) copolymer was really produced by the melt polycondensation method.

Crystallization behavior of P(LA/AU)x/y copolymers

For this semicrystalline P(LA/AU) copolymer, there are crystalline regions and amorphous regions, which could be observed from XRD patterns shown in Fig. 5. With the increase in aminoundecanoic acid content, the diffraction peak become sharper and higher, and the crystallinity increased. P(LA/AU)25/75 copolymer showed two strong reflections at the diffraction angle 2θ of 20.2̊ (d = 0.439 nm) and 22.2̊ (d = 0.401 nm). This diffraction pattern was very similar to the α-form crystal of nylon 11 homopolymer [8, 9, 10]. But P(LA/AU)40/60, P(LA/AU)50/50, and P(LA/AU)60/40 copolymers just showed one strong reflection peak at the diffraction angle 2θ of 20.0∼21.2̊ (d = 0.443∼0.418 nm), which was very similar to the δ′-form structure of nylon 11 homopolymer [10]. The δ′-form structure of nylon 11 homopolymer was "smectic phase". This depression of the crystallinity and the change in crystal form with increase in lactic acid content might be due to the insertion of lactic acid units into the nylon 11 lattices. Further information on the crystallization behavior of these copolymers was obtained by means of differential scanning calorimeter. As shown in Table 1 and Fig. 6,

Fig. 5 XRD patterns of P(LA/AU)x/y copolymers at room temperature
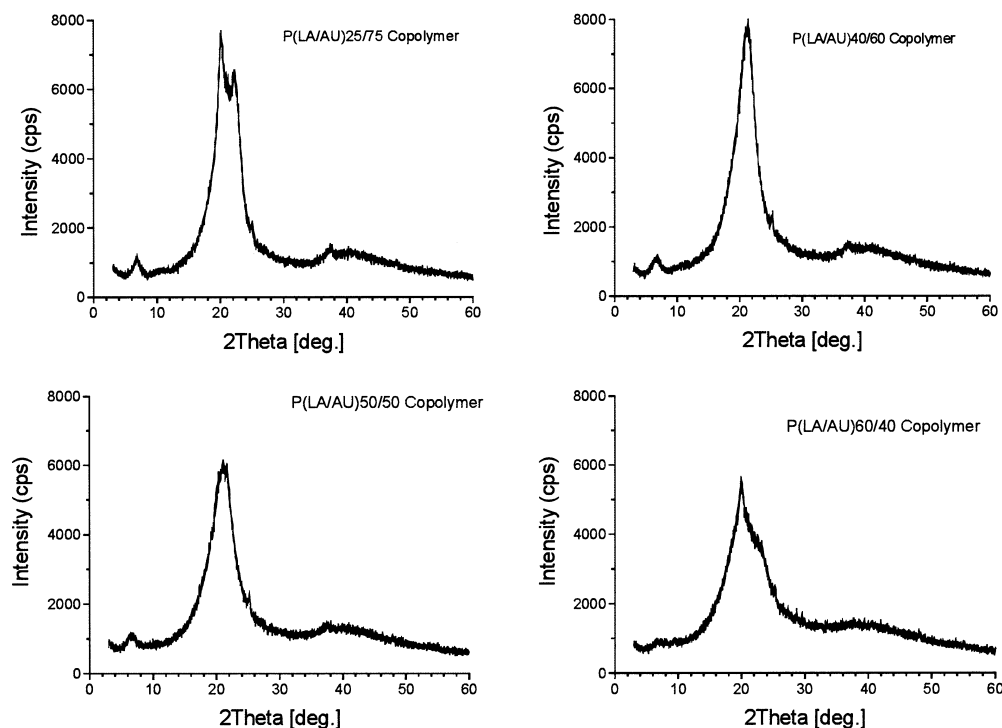
the melting temperature, heat of fusion, and crystallinity of these copolymers increased with the increase in aminoundecanoic acid content.
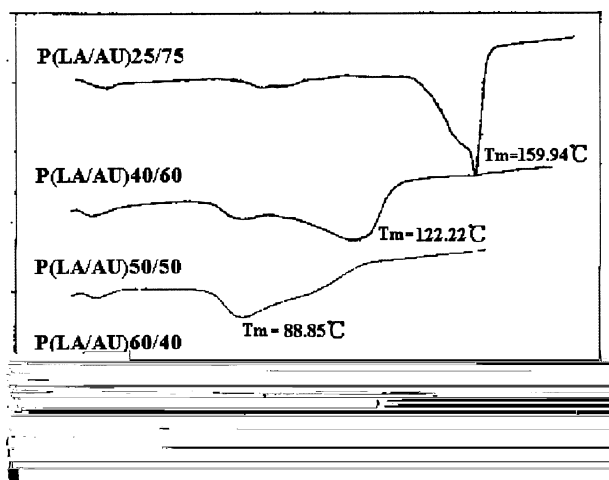
Water absorption of P(LA/AU)x/y copolymers

P(LA/AU) copolymers are semi-crystalline. In the crystalline region, the macromolecules were in ordered conformation. The water could not penetrate into this region. When P(LA/AU) copolymer was incubated in aqueous medium, the amorphous region would absorb water because of its random macromolecular conformation. Water absorption behavior was determined by the amorphous region of P(LA/AU) copolymers. With the increase in lactic acid, the crystallinity decreased, which can be seen from Table 1. As a result, water absorption increased with the increase of lactic acid as shown in Fig. 7.

In vitro degradation of P(LA/AU)x/y copolymers

For these semi-crystalline P(LA/AU) copolymers, the crystalline regions were composed of chain sequences in ordered or preferred conformation, and the amorphous regions contained chain folds, chain ends, and tie-chain segments in characteristically disordered conformations. The great difference between the crystalline and amorphous regions provides the basis for understanding the in vitro degradation behavior of P(LA/AU) copolymers. It was proposed that hydrolytic degradation of semi-crystalline materials proceeds through three main stages. At first, the little molecule (here it was water), would diffuse into the amorphous region, where water acted as plasticizer. In this period, the crystallinity would increase, and the weight loss would be very little. But, as more and more water was adsorbed into amorphous regions, the labile ester bond would be attacked and hydrolytically degraded. So, the whole weight would start to decrease. Finally, the crystalline regions would also be attacked by the water molecules. The weight would decrease quickly in this period.
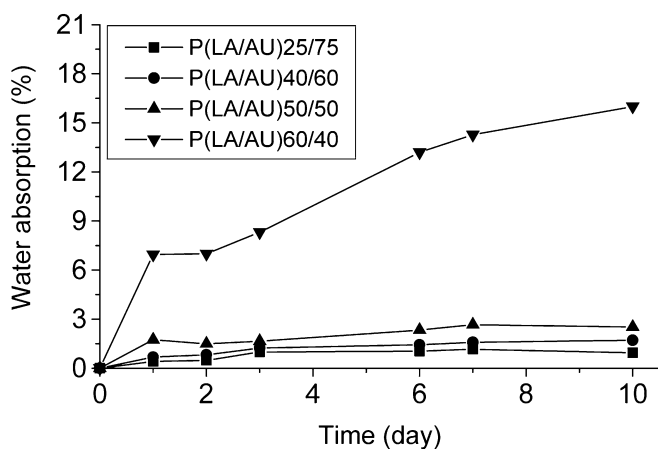

Fig. 6 DSC curves of P(LA/AU)x/y copolymers

Fig. 7 Water absorption behavior of P(LA/AU)x/y copolymers with various LA/AU molar ratios

Fig. 9 FTIR spectra of P(LA/AU) 50/50 copolymer during degradation in PBS (37 °C, pH = 7.2; The arrows indicate the absorption due to ester moiety)
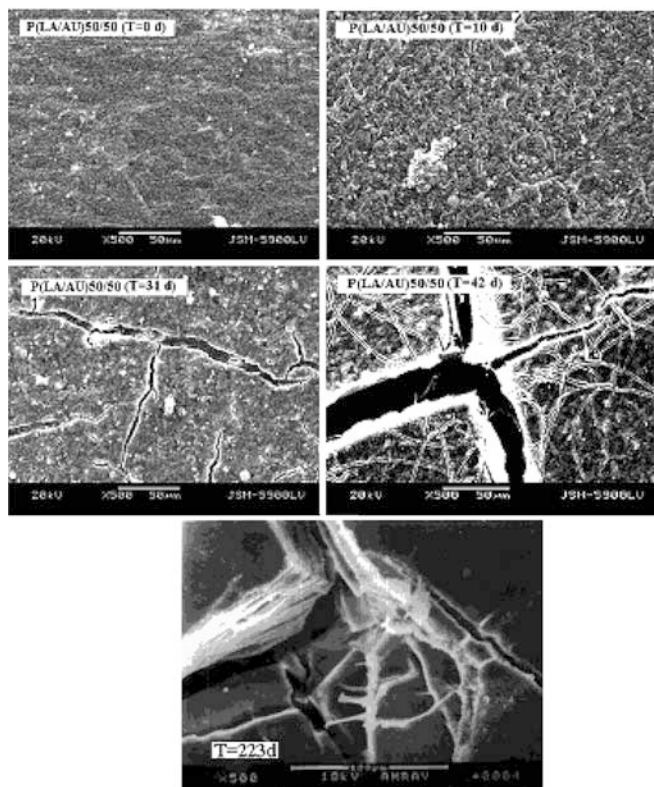

Fig. 8 Surface morphology of P(LA/AU) 50/50 copolymer during degradation in PBS (37 °C, pH = 7.2)

The surface morphology of P(LA/AU)50/50 copolymer films during degradation in vitro is shown in Fig. 8. As degradation proceeded, the surface became rugged, and then cracks appeared. The cracks became larger with degradation.

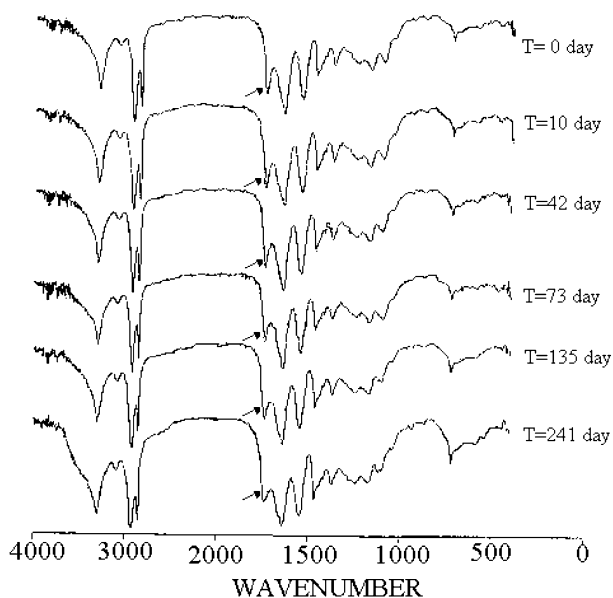FTIR spectra of P(LA/AU) 50/50 copolymer during degradation in vitro are shown in Fig. 9. As degradation proceeded, the area of absorption at 1741 cm^{-1} decreased. From this figure, a conclusion could be made that the ester content decreased quickly, but amide content decreased slowly. So, the amide fraction (compared to ester bond) would increase during the degradation process. This was due to the macromolecular structure. In the main chain, the ester bond was more labile than the amide bond. So the chemical composition of P(LA/AU)50/50 copolymer would change during degradation. This idea could be confirmed by ^{1}H-NMR spectra shown in Fig. 10. The ester molar fraction could be calculated from the integral area of peak a and peak c in this figure. For this P(LA/AU)50/50 copolymer, the ester molar fraction before degradation (T = 0 day) was 1.0/4.72. When this copolymer was incubated in PBS saline for 73 days, the ester fraction changed to 1.0/6.05, and decreased to 1.0/7.64 when it was incubated in PBS saline for 223 days.

The thermal properties of P(LA/AU)50/50 copolymer during degradation were also studied. The results are presented in Fig. 11 and Table 2. As degradation proceeded, the melting temperature and crystallinity increased at first, then started to decrease after 135 days. This might be due to the chain scission of ester bond in the macromolecular backbone. After degradation, the molar fraction of amide moiety increased. So, the melting temperature and crystallinity increased at first. But, when the molecular weight decreased to an extent, the crystallinity domain was destroyed and, as a result, the melting temperature and crystallinity then decreased.
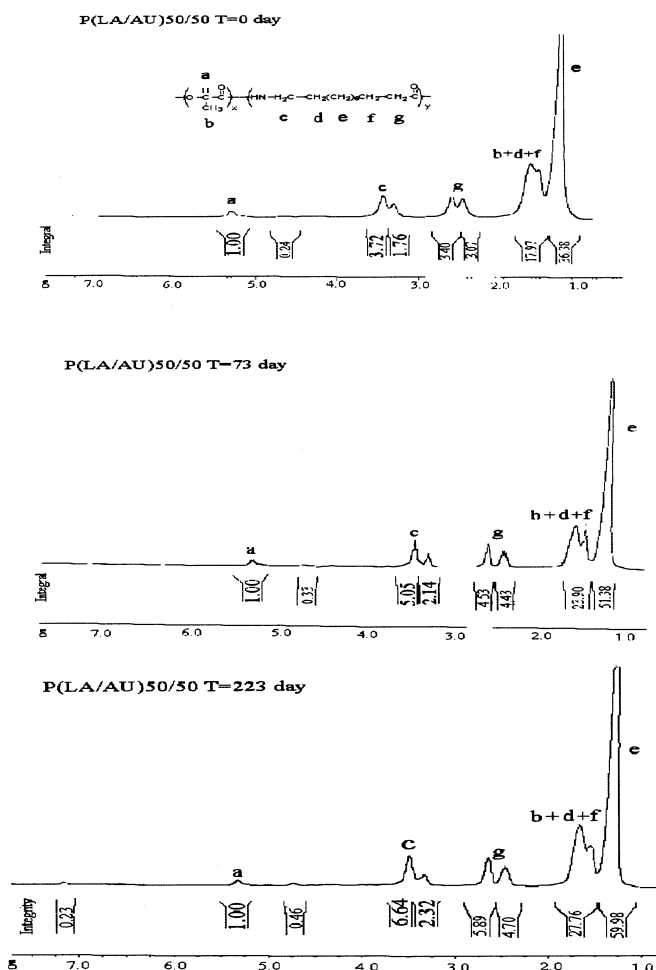
P(LA/AU)50/50 T=0 day

P(LA/AU)50/50 T=73 day

P(LA/AU)50/50 T=223 day

Fig. 10 ^{1}H-NMR spectra of P(LA/AU)50/50 copolymer during degradation in PBS (37 °C, pH = 7.2)

The in vitro degradation behavior of P(LA/AU)x/y copolymers was determined by the aminoundecanoic acid content, that is to say, by the amide content in this copolymer. With the increase in amide content, the degradation rate decreased. In the case of P(LA/AU)25/75 copolymer, it did not show obvious weight loss in 240 days. The lowest degradation rate out of the three copolymers might be due to its highest amide content and crystallinity . In the experimental period, the whole weight increased to some extent because this co-polymer adsorbed water due to the strong polarity of the amide moiety. These results are shown in Fig. 12, which was consistent with the water absorption behavior of these copolymers.

Conclusions

Biodegradable P(LA/AU) copolymers were produced by the melt polycondensation method from lactic acid and aminoundecanoic acid. With the increase in aminoun-
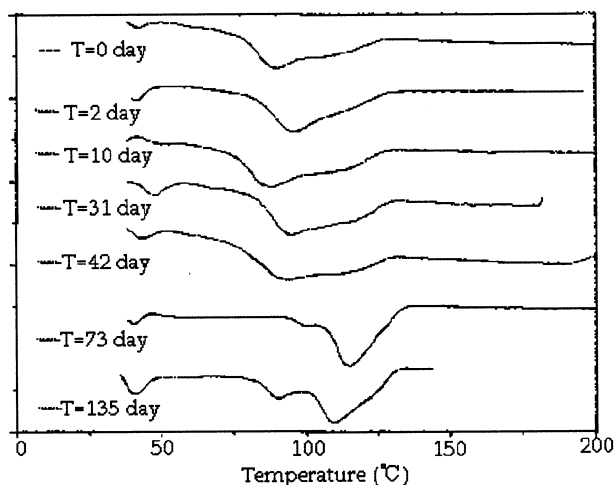

Fig. 11 DSC curves of the P(LA/AU)50/50 copolymer during degradation in PBS (37 °C, pH = 7.2)

Table 2 Thermal properties of P(LA/AU)50/50 after degradation in PBS saline (pH = 7.2, 37 °C)

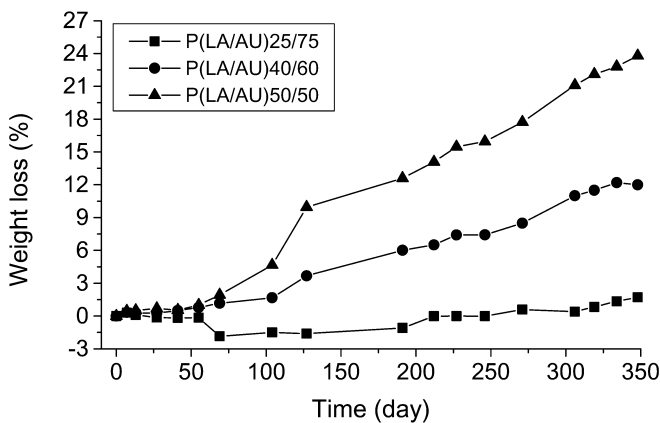| Time (day)	0	2	10	31	42	73	135	223
T_m (°C)	88.85	93.08	86.08	92.75	89.52	112.09	109.82	117.02
ΔH_m (J/g)	18.91	19.95	22.73	22.28	32.96	20.57	26.79	19.30
X_c (%)	9.6	10.1	11.6	11.3	16.8	10.5	13.6	9.8

Fig. 12 In vitro degradation behavior of P(LA/AU)x/y copolymers in PBS (37 °C, pH = 7.2)

decanoic acid, the crystallinity increased, but water absorption and in vitro degradation rate decreased. When the polyesteramide was incubated in phosphate buffer saline, the labile ester moiety was attacked by water. Degradation behavior depends on the ester content in the copolymer. During degradation in vitro, the ester content decreased gradually.

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