Polymer Bulletin 60, 323–330 (2008)
DOI 10.1007/s00289-007-0869-3

Polymer Bulletin

# Synthesis and characterization of biodegradable copolymers based on 6-aminocaproic acid and α-L-alanine

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Received: 4 July 2007 / Revised version: 22 October 2007 / Accepted: 22 November 2007

## Summary

Random copolymers based on 6-aminocaproic acid and α-L-alanine were prepared by melt phase polycondensation method and characterized by FTIR, $^1$H NMR, DSC and WAXD. The density, water absorptivity and enzymatic degradation of copolymers were measured. The results show that with increasing of alanine content in comonomers, the density, water absorptivity and rate of enzymatic degradation of copolymers increase, but the intrinsic viscosity and the degree of crystallization decrease. When the molar percentage of alanine increases to 40% in comonomers, copolymer is amorphous.

## Introduction

The continuously increasing extent of pollution of environment is attracting much and much attention. Biodegradable polymers, used for package materials, have urgent demands. In medicine area, tissue engineer materials and drugs releasing materials should be biodegradable.

α-L-amino acids and glycine are basic unit composing proteins. Poly(α-L-amino acids) degrade to basic amino acids composing organism, which are less toxic, biocompatible, and can be absorbed and metabolized as nutriments.

However, polyamino acids have many unfavorable properties. Firstly, polyamino acids have high melting temperature, and decompose before its melting temperature. It is difficult to process them. Secondly, raw materials and products of polyamino acids are insoluble in common organic solvents. Syntheses of polyamino acid need toxic phosgene, diphosgene or triphosgene, and often require formation, isolation and storage high reactive and sensible to water N-carboxyanhydrides, it is extremely difficult to scale up. Thirdly, polymers with more than three different α-L-amino acids often show immunogenicity. Finally, cost is high with active ester method, which limits its usage ranges in many fields. For these reasons, except polyglutamic acid, polyaspartic acid and polylysine, other polyamino acids seldom were taken into account.

To improve the disadvantages, α-L-amino acids often were copolymerized with other monomers, forming copolymers such as polyesteramide with hydroxyl acids or diacids and diols[1-4], copolyamides with ω–amino acids or diacids and diamine[5-7]. Many results showed that polyamino acids and random copolymers made from nonbiodegradable polymers and appropriate quantity α-L-amino acid(s) are biodegradable, and enzymolysis play an important role[8-14], copolymers based on α-D-amino acids have not enzymolysis sensibility[15,16]. Considering biocompatibility, degradation products of polymers from hydroxyl acids and diacids are acidic, which could make hosts inflammatory response [17].

ω–amino acids possess similar structures to α-L-amino acids, some of them are beneficial to organism[18-21]. Poly(ω–amino acids) such as nylon 6,7,8,9,10,11,12, have good mechanical, processing and biocompatible properties, and widely used in some areas. It is expected that copolyamides based on α-L-amino acids and ω–amino acids should have prospective fields. Some results showed that this kind of copolymers could enzymatic degrade controllablely according to ratio of α-L-amino acids in copolymers[5,22].

α-L-alanine is a kind of natural amino acid, which can be used by body to build blocks of proteins. We prepared and characterized copolymers from 6-aminocaproic acid and α-L-alanine, tested some properties of them, and hope to discuss the possibility of them for practicality.

## Experimental

### *Materials*

6-aminocaproic acid, pharmaceutical grade, was provided by the Haixin Pharmaceutical Co.( Xi'an, China). α-L-alanine, pharmaceutical grade, were acquired from the Donboo Amino acids Co.(Nantong, China). Papain(from Carica papaya, 30000 USP-U/mg for biochemistry, EC 3.4.22.2) was purchased from Merck (German). All reagents are used without further purification.

### *Polymerization*

Copolymers were prepared by melt polycondensation. 6-Aminocaproic acid and α-L-alanine were weighed exactly and mixed well, then loaded to flask. $N_2$ was input to protect reactive products. After 2 hours, reaction continued at 3mm Hg pressure for another 2 hours, and target polymers were acquired. N6 indicates pure nylon 6, and N6A10 indicates copolymer with 10% α-L-alanine in molar ratio, and so on.

### *Characterization*

The molecular weight of the copolyamides was expressed by their intrinsic viscosity, which was determined in solutions of the copolymers (c=0.4g/100ml in 96% $H_2SO_4$) with Ubbelohde viscometer at 25°C.

The density of copolymers was measured on weight of samples in air and in insoluble liquids such as ethyl acetate, equations as follow:

$$m_1=\rho Vg \qquad \textbf{(1)}$$

$$m_2=\rho Vg-\rho_l Vg \tag{2}$$

$$\rho=\rho_l/(1-m_2/m_1) \tag{3}$$

where, $m_1$–weight of samples in air; $m_2$–weight of samples in insoluble liquids; $\rho$–density of samples; $\rho_l$–density of insoluble liquids; $V$–volume of samples; $g$–acceleration of gravity.

Infrared spectra were recorded on a Nicolet Nexus 870 FTIR spectrometer(USA) using KBr pellets technique.

The $^1$H NMR spectra were characterized using a Bruker AV500 MHz superconducting NMR spectrometer (Switzerland), $D_2SO_4$ as solvent.

The thermal behaviour of samples was investigated using a differential scanning calorimeter (DSC 2910, USA). The heating rate used was 10°C/min from 0 to 250°C. All DSC measurements were performed under a nitrogen atmosphere.

D/MAX-2400 (Japan) diffractometer was used to observe crystallization behaviors. The operation was $CuK_\alpha$ target(K = 0.154 nm). Scan rate, 10°/min; scan range, 3° ~ 90°.

The samples used to measure the water absorptivity had same dimensions, which were 10mm length, with width of 10mm and thickness of 0.5mm. Samples were dried to constant weight under vacuum before use, and then immerged in distilled water for 48 hours at 37°C. Water at surface of samples was absorbed using filter paper and weighed. The ratio of water absorptivity was calculated by Eq. (4).

$$Q=(W_2-W_1)/W_1\times 100\% \tag{4}$$

Where, $Q$–ratio of water absorptivity; $W_1$–weight of samples before immerged in water; $W_2$–weight of samples after immerged in water.

The enzymatic degradation tests were carried out in a 0.05M phosphate buffer solution (pH 6.0) with papain (0.05 wt %) at 37°C. The enzyme was activated in the buffered solution with 30mM EDTA and 34mM cysteine. Sodium azide (0.03 wt %) was used to prevent microbial growth. N6 and copolymers were dissolved in formic acid, cast in glass to form films about 0.1mm in thickness after formic acid was volatilized. The films were immerged in distilled water for 48 hours at 50°C to remove soluble components, and then were dried in vacuum to constant weight at 50°C. After weighed and recorded ($G_1$), the samples of the films were immerged to a phosphate buffer solution (pH 6.0) with papain. One week later, samples were dried to constant weight in vacuum at 50°C, the weights of them were recorded ($G_2$), and the weight retained ratio of samples are calculated. Buffer solution was renewed per week.

$$L=(G_1-G_2)/G_1\times 100\% \tag{5}$$

## Results and discussion

### *Intrinsic viscosity*

Intrinsic viscosities are shown in Table 1. With increasing the alanine content, the intrinsic viscosity of copolymers decrease rapidly, showing that reactivity of 6-aminocaproic acid and α-L-alanine is different in the reaction condition. Molecular weight of polymers is close relevant to mechanical, thermal and processing properties, polymers with larger molecular weight possess better mechanical property. Therefore, on the premise of ensuring suitable degradation rate, the content of alanine in copolymers should decrease as much as possible. According to results of Andreoni[23], ligomers comprising of eight monomeric units or below of polyamide 6 could be utilized by bacteria, and the lower was molecular weight of polyamide, the higher utilization extent was. It is speculated that when molar ratio of alanine to 6-aminocaproic acid is above 1:8, copolymers are biodegradable.

**Table 1.** Basic properties of polymers

| No. | Molar ratio of feed (6-amino caproic acid:alanine) | Intrinsic viscosity [η] (dl/g) | Molar percentage of alanine in polymers (%, based $^1$HNMR) | Density ρ (g/cm$^3$) | Ratio of water absorption in weight (%) |
|---|---|---|---|---|---|
| N6 | 100:0 | 1.70 | 0 | 1.14 | 8.6 |
| N6A10 | 90:10 | 1.66 | 10.4 | 1.16 | 8.9 |
| N6A20 | 80:20 | 1.37 | 19.6 | 1.18 | 10.6 |
| N6A30 | 70:30 | 1.20 | 27.7 | 1.19 | 11.0 |
| N6A40 | 60:40 | 1.07 | 38.0 | 1.21 | 11.5 |

### *Density*

Density values of copolymers are shown in Table 1. With increasing the alanine content, density of copolymers increases, which could contribute to increase of density of amide bonds in copolymers.

### *FTIR characterization*

The FTIR spectrum of copolyamides with different molar percentage of alanine is shown in Figure1. Except copolymers have an unclear peak at 1713 cm$^{-1}$(at arrows), which was taken for amino acid moiety[22,24,25], there are almost no any difference otherwhere. They showed the typical polyamide peaks at: 3307cm$^{-1}$ (N–H stretching), 1640cm cm$^{-1}$ (C=O stretching, amide I), 1541cm cm$^{-1}$ (N–H bending, amide II) and 1467cm cm$^{-1}$ ($CH_2$ bending).

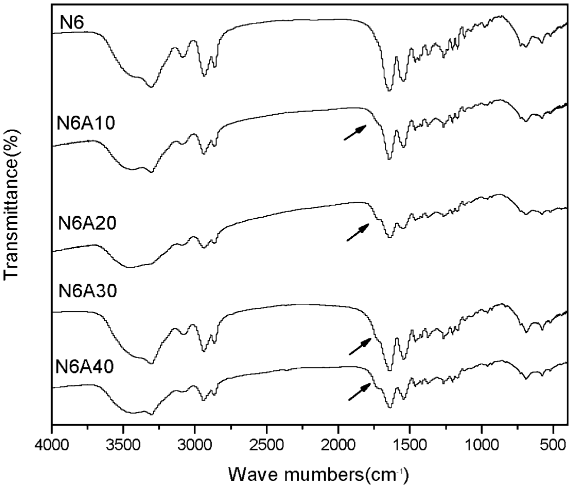

**Figure 1.** FTIR spectra of polymers with different alanine content

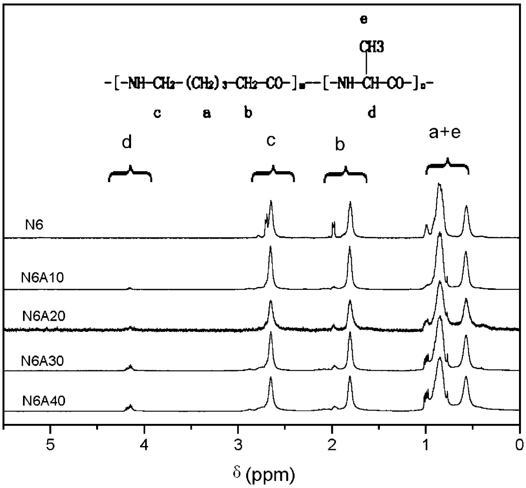

**Figure 2.** $^{1}$HNMR spectra of polymers with different alanine content

### *$^{1}$H NMR characterization*

The $^{1}$H NMR spectra of copolyamides with different molar percentage of alanine are shown in Figure2, which indicated peaks attributed. With increasing the alanine content, the peaks at 4.1ppm increase. At the same time, the peak at 4.1ppm divided to many little peaks, indicating polymers are random copolymers, not block copolymers. Ratios of alanine in copolymers are shown in Table 1. During reacting, a part of 6-aminocaproic acid and α-L-alanine volatilized, and the ratios of them fluctuated.

### *DSC thermal analysis*

The DSC curves of copolyamides are shown in Figure3. With increasing the alanine content, the melting point and melting enthalpy decrease. When molar percentage of

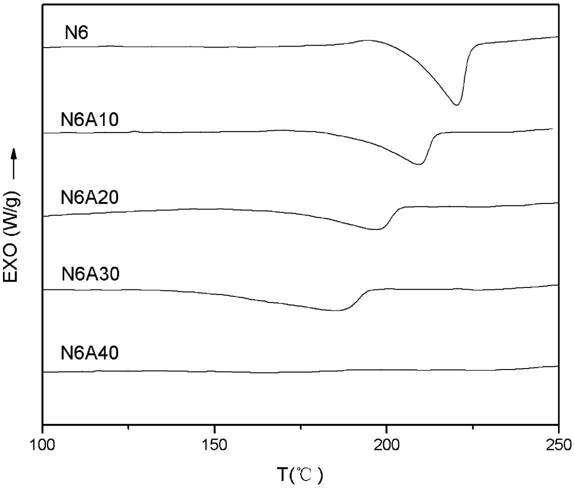

**Figure 3.** DSC curves of polymers with different alanine content

alanine in copolymer was 40%, almost no melting peak was seen. The decrease of melting enthalpy indicates that the degree of crystallization of copolymer decreases. Every thermogram showed single Tm, indicated that the copolymerization formed random copolymers, and only one crystal phase. WAXD showed polymers existing as α phase. Melting enthalpy of nylon 6 α-crystal is 203.5J/g, which is base for calculating the degree of crystallization. Melting point($T_m$), initial decomposition temperature ($T_{m,onset}$), melting enthalpy( $\Delta H_f$) and degree of crystallization of polymers are shown in Table 2.

**Table 2.** Characteristic values of polymers related to DSC curves

| No. | $T_m$ (°C) | $T_{m,onset}$ (°C) | ΔH ($J \cdot g^{-1}$) | Degree of crystallization (%) |
|---|---|---|---|---|
| N6 | 220.4 | 205.6 | 78.15 | 38.4 |
| N6A10 | 209.4 | 191.0 | 63.12 | 31.0 |
| N6A20 | 197.2 | 173.1 | 52.24 | 25.7 |
| N6A30 | 185.3 | 146.7 | 45.83 | 22.5 |
| N6A40 | | | 0 | 0 |

*WAXD*

Diffraction spectra are shown in Figure4. 2θ=24.0° was indexed as α crystal 002 and 202 reflection. $2\theta$=20.2° was indexed as α crystal 200 reflection. Obviously, with increasing the alanine content, intensity of $2\theta$=24.0° decreased, and $2\theta$=20.2° increased. The peak of crystal decreased markedly, suggesting the degree of crystallization decreased, which is accordant to the results of DSC curves. In the range of $2\theta$=15–30°, no new peak occurred. In other experiments, we found that copolymers of 6-aminocaproic acid and α-L-proline, 6-aminocaproic acid and α-L-leucine had similar WAXD peaks shapes, suggesting it is possible that no new crystal phase formed.

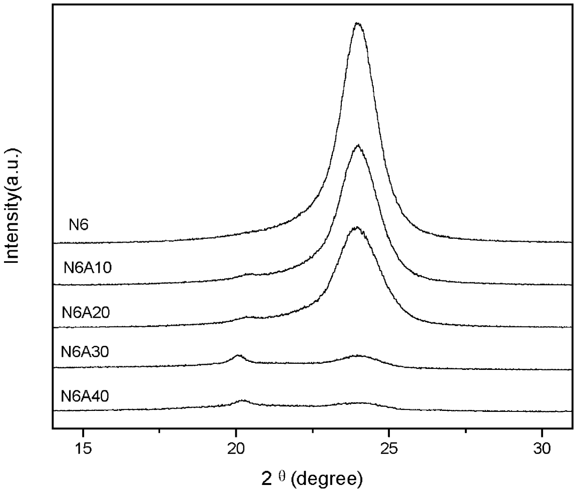

**Figure 4.** WAXD spectra of polymers with different alanine content

### *Water absorptivity*

Water absorption results of polymers are shown in Table 1, indicating that with increasing the alanine content, ratio of water absorption of copolymer increases. Amorphous polymer has loose structure, which is beneficial to absorb water for hydrophilic polymers. The results are correspondent with the degree of crystallization of polymers.

### *Degradation test*

Figure5 shows the extent of enzymatic hydrolysis as a function of degradation time. The degree of degradation was estimated on retained mass. Pure N6 had shown no any degradation property in the whole test time. With increasing the alanine content, degradation rates increase. Obviously, the reason of degradation is that α-L-alanine was introduced, which is sensible to enzymatic attack.

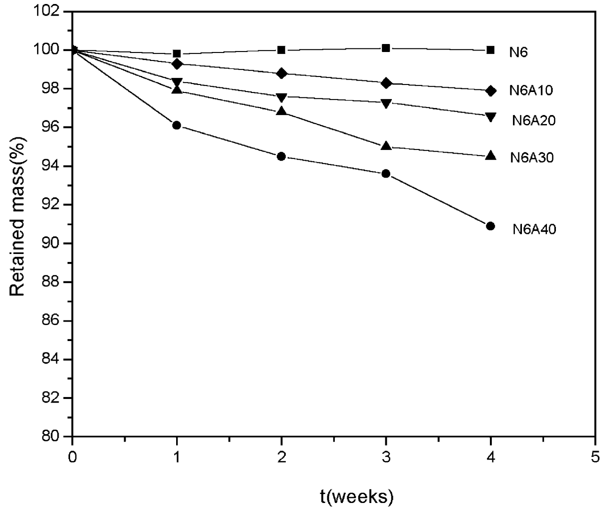

**Figure 5.** Retained mass of polymers with different alanine content

## Conclusions

Random copolyamides based on 6-aminocaproic acid and α-L-alanine were synthesized and characterized by FTIR and $^1$H NMR. Thermal properties and crystallization behaviors of polymers were tested by DSC and WAXD. Density, water absorptivity and enzymatic degradation of them were measured. Results of above tests show that copolymers possess good water absorptivity, and are enzymatic degradable. Copolymers could be hopeful to be biodegradable package materials, biomedical materials, or used in other fields.

*Acknowledgements.* The authors acknowledge the support from the Natural Science Foundation (China, No.30572140).

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