ELSEVIER

Polymer 41 (2000) 1481–1486

polymer

Synthesis and characterization of ε-caprolactam–L-phenylalanine copolymers

A. Demicheli[a], S. Russo[a,*], A. Mariani[b]

[a]Dipartimento di Chimica e Chimica Industriale, Università di Genova, via Dodecaneso 31, 16146 Genoa, Italy
[b]Dipartimento di Chimica, Università di Sassari, via Vienna 2, 07100 Sassari, Italy

Received 8 March 1999; accepted 21 April 1999

Abstract

A series of copolyamides has been prepared by hydrolytic polymerization of ε-caprolactam (CL) in the presence of various amounts of L-phenylalanine (Phe). The solution viscosity of the obtained copolymers decreases by increasing comonomer content, [η] values ranging from 1.27 dl/g for neat poly(ε-CL) (PCL) to 0.33 dl/g for the copolymer obtained, when 16.2 mol % of Phe is present in the reaction medium. A similar, less pronounced trend is shown by copolymer thermal transitions (both T_g and T_m are lowered by ca. 8 and 19°C, respectively, in the copolyamide originated from 12.6 mol % of Phe present in the monomer pair). The environmental degradability has been ascertained by testing the resistance of CL–Phe copolymers to both alkaline hydrolysis and composting conditions. The degradation rate and the extent of hydrolysis of the above copolyamides have been evaluated by using the mass loss and thermogravimetric analysis for the former, and solution viscosity measurements for the latter. The CL–Phe copolymers could be envisaged as suitable film-forming materials for environmentally degradable applications in various fields, such as agricultural coverage and food packaging, when time-limited performances are requested.

Keywords: Hydrolytic copolymerization; ε-Caprolactam–L-phenylalanine copolymers; Environmental degradability

1. Introduction

During the last two decades, specific research has been oriented toward the design of suitable polymeric materials for several time-limited applications in various fields, e.g. biomedical, pharmaceutical, agricultural and packaging ones [1–8]. Besides few specific characteristics, such as easy processability, dimensional stability, film orientation suitability and other relevant properties, the various polymer structures have been matched with the capability of being hydrolysed into small metabolizable species, in order to generate a series of useful bioerodible or biodegradable materials. As recently reviewed [9], the emphasis has been initially focused on the synthesis of novel aliphatic polyesters, because of their high susceptibility to biodegradation; unfortunately, this class of polymers presents several disadvantages (e.g. rather low glass transitions and melting points), which strongly reduce their technological applications. Conversely, synthetic polyamides, being structurally close to natural polypeptides, have often attracted the interest of several research groups, as they already contain nitrogen essential for life growth, and show excellent hydrophilic character, reasonably high melting points and good mechanical properties, even at relatively low molecular weights. In the present paper, we add some new experimental data to the well-known approach of introducing α-amino acids in the main chain of industrially relevant polyamides with the aim to increase their low environmental- and bio-degradability. Indeed, copolymers of aliphatic polyamides, containing α-amino acids, have been synthesized by several groups by melt and interfacial polycondensation [10–15] and properties of the resultant materials have been found dependent on both the nature and the content of amino acid.

In the present work, L-phenylalanine (Phe) has been used as ε-caprolactam (CL) comonomer to build up a predetermined fraction of amide linkages (containing an adjacent phenyl ring) enzymatically cleavable, e.g. by α-chymotrypsin [13]. The aim of our study is to obtain a series of potentially biodegradable (or at least environmentally degradable) copolyamides by hydrolytic melt polymerisation of CL in the presence of different amounts of Phe. Synthetic procedures, as well as copolyamide characterization and properties, will be given in the following sections.

---

* Corresponding author. Tel.: +39-00-3536198; fax: +39-00-3536199.

PII: S0032-3861(99)00312-2

2. Experimental

2.1. Materials

CL has been generously provided by DSM (Geleen, The Netherlands) and used as received. 6-amino hexanoic acid (ACA) and Phe, purchased from Fluka, have been utilized without further purification.

2.2. Polymerization runs

Copolyamides have been synthesized by the hydrolytic melt polymerization, initiated by ACA, typically at the polymerization temperature (T_p) of 270°C. Heat supply and control have been provided by an aluminium block heated by electric resistors connected to a rheostat. Mixtures of CL, ACA and Phe have been introduced into the glass polymerisation tube at room temperature, heated at the rate of 3°C $\min^{-1}$ under a stream of dry nitrogen and held at 270°C, usually for a polymerization time (t_p) of 4 h. For a few samples, the polymerization conditions have been modified in order to investigate the influence of T_p and added vacuum on molecular weight of the resultant copolyamide. At the end of the reaction, the polymerization tube has been rapidly cooled to room temperature under a continuous stream of nitrogen, and the reaction products removed and turned into fine shavings. The low molecular weight compounds (mostly made of higher cyclic oligomers) and residual monomers have been extracted by deionized water in a Soxhlet apparatus for 12 h.

2.3. Copolyamide characterization

2.3.1. Fourier-transform infrared analysis (FTIR)

FTIR spectra have been recorded by a Bruker IFS 66 spectrophotometer using KBr discs, in which ca. 1% (w/w) of copolyamide was finely dispersed.

2.3.2. Ultraviolet analysis

UV spectra of copolyamide solutions in anhydrous formic acid (0.1% w/w) were recorded in the 250–400 nm range by a Perkin–Elmer UV/VIS/NIR Spectrophotometer, mod. Lambda [9]. Optical path length of 1 cm was adopted.

2.3.3. Potentiometric titration of end groups

The copolyamide (0.5–0.8 g), cut into small pieces, was suspended in a 15–25 ml of an azeotropic mixture of 2,2,2-trifluoroethanol and water (88/12 v/v) at pH = 4.3–4.7. The suspension was heated at 150°C for 30–60 min up to a complete dissolution and then cooled to room temperature. The pH meter (Hanna HI 9321) was calibrated with buffer solutions at pH 4.0 and 7.0. The solution was moderately stirred and titrated with N/50 aqueous hydrochloric acid, until a sharp mV increase is reached. This point corresponds to full neutralization of amino groups. Additional hydrochloric acid was subsequently added, up to an excess of 0.8–1 ml. This solution was counter-titrated with N/50 sodium hydroxide up to a second sharp mV increase, which corresponds to the neutralization of the excess hydrochloric acid. The titration was carried out further, until the third point of equivalence, corresponding to the neutralization of carboxylic groups, is reached.

2.3.4. Viscosity measurements

Viscosities were determined at 30°C on copolyamide solutions at different concentrations in 96% H_2SO_4, using a Ubbelhode-type viscometer.

2.3.5. Differential scanning calorimetry (DSC) analysis

DSC analysis of copolyamide samples (5–10 mg) was carried out by a Mettler TC10A calorimeter. Heating rate was 20 and cooling rate 10°C $\min^{-1}$. The temperature range chosen was −20 to +280°C. Polymer melting points (T_m) were taken in correspondence to the enthalpy peak maxima. Glass transition temperatures (T_g) were evaluated as midpoints of step changes of heat flow. From the enthalpy peak area, the crystallinity content (x_c) was determined by comparison with the $\Delta H°_f$ value pertaining to the α form of crystalline PCL. A standard sample of indium was used for the temperature scale calibration and as a reference for the enthalpy evaluation from the peak areas.

2.3.6. Thermogravimetric analysis (TGA)

TGA measurements were performed in a nitrogen stream on 2–11 mg of copolyamide by a Perkin–Elmer TGA7 calorimeter interfaced to a thermal control system TAC7/DX, using a scanning rate of 20°C $\min^{-1}$. T_{onset} (initial temperature of degradation) was evaluated from the intercept between the initial temperature profile and the inflection point straight line.

2.3.7. Wide-angle X-ray diffraction (WAXD) patterns

WAXD ($2\theta = 5–35°$) patterns of our copolyamides were recorded using a Philips PW 3020/00 diffractometer.

2.3.8. Mechanical properties

Tensile tests were carried out at room temperature on heat-pressed specimens, using an Instron 5565 apparatus at an extension rate of 20 mm $\min^{-1}$. From the stress–strain curves, Young's modulus, fracture strength and elongation at break were calculated. The specimens used had the following dimensions: 4.25 cm long; 0.91–0.96 cm wide; and 0.07–1 mm thick.

2.4. Degradability experiments

2.4.1. Alkaline hydrolysis

Alkaline hydrolysis was carried out at 80°C (4 h. under stirring) on a suspension of copolyamide film (cast from formic acid) in 10% (w/w) NaOH aqueous solution. Molecular weight decrease of the copolyamide samples was determined by intrinsic viscosity measurements. Structural changes due to hydrolysis were evaluated by DSC.

Table 1
The synthesis of Phe-containing copolyamides

Polymer sample	CL (mol%)	ACA (mol%)	Phe (mol%)	T_p (°C)	t_p (h)
1a	96.5	3.5	0.0	270	4
1b	94.0	3.3	2.7	270	4
1c	91.8	3.3	4.9	270	4
1d	88.0	3.1	8.9	270	4
1e	84.4	3.0	12.6	270	4
1f	80.9	2.9	16.2	270	4
2b	94.0	3.3	2.7	230	4
3c	91.8	3.3	4.9	270	5[a]

[a] Additional hour of reaction under dinamic vacuum.

2.4.2. Biodegradation under controlled composting conditions

Biodegradability of copolymer films under composting conditions was determined using a method derived from the standard test method described in the Italian UNIPLAST E13.21.717.0 Norm [16]. The samples were exposed to an active composting environment and stored in an incubator at 52°C. The mass loss was determined by weighing the polymer films after 45 and 115 days of incubation. For comparison, a sample of neat PCL was submitted to the same characterization procedures, as described above.

3. Results and discussion

The specific experimental conditions, chosen for the various copolyamide syntheses, are listed in Table 1. Each sample was identified by a double code, i.e. a number referring to the experimental conditions of its synthesis, and a letter linked to the Phe content in the initial mixture. As mentioned in Section 2, the polymerization conditions were modified for a couple of syntheses, i.e. for samples 2b (T_p of 230°C) and 3c (applied vacuum in the additional fifth hour of the reaction).

Characterization data of the various copolyamides are hereinafter presented and compared to those of neat PCL. Indeed, in their IR spectra, besides the absorption bands due to the carbonyl stretching at 1640 cm^{-1} (amide band I) and N–H bending near 1540–1550 cm^{-1} (amide band II), which give an evidence of the presence of amide group in all structures, an additional absorption band due to the α-amino acid moiety is present at 1715 cm^{-1}, thus supporting the insertion of Phe units in CL sequences, as already reported in literature [11,13]. Table 2 shows some additional characterization data of the copolyamides. Solution viscosities are given in columns 2 and 3, in terms of both relative viscosity (η_r), typically used in the PCL molecular characterization, and intrinsic viscosity ($[\eta]$), respectively. For the copolymers, both series of values decrease by increasing the comonomer content. This result is presumably due in part to the reduced reactivity of Phe moiety and, more probably, to some degradative processes undergone by the amide linkage formed by the α-amino acid, that leads to far less reactive structures. Samples 2b and 3c, prepared at a lower T_p (230°C) or under vacuum in the terminal part of its synthesis, respectively, show viscosity values very close to those samples containing the same amount of Phe and synthesized under standard conditions (samples 1b and 1c, respectively). The above result reveals that both the applied vacuum and the lower reaction temperatures do not significantly affect the copolyamide molecular weight. In other words, the degradative process, if present, is not very sensitive to temperature and applied vacuum in the explored conditions.

The role of Phe units present in the copolyamide on

Table 2
The characterization data and properties of PCL and Phe-containing copolyamides

Polymer sample	η_r[a]	$[\eta]$ (dl g^{-1})	TGA T_{onset} (°C)	Young's modulus[b] (MPa)	Elongation at break[b] (%)
1a	2.81	1.27	438	1740	2.05
1b	1.99	0.77	418	1670	1.50
1c	1.76	0.59	430	2015	1.22
1d	1.58	0.46	417	—	—
1e	1.46	0.38	414	—	—
1f	1.39	0.33	396	—	—
2b	2.04	0.80	—	—	—
3c	1.77	0.59	—	—	—

[a] Measured at the concentration of 1 g/100 ml.

[b] Five measurements per sample have been taken and the results, given here, represent their mean values.

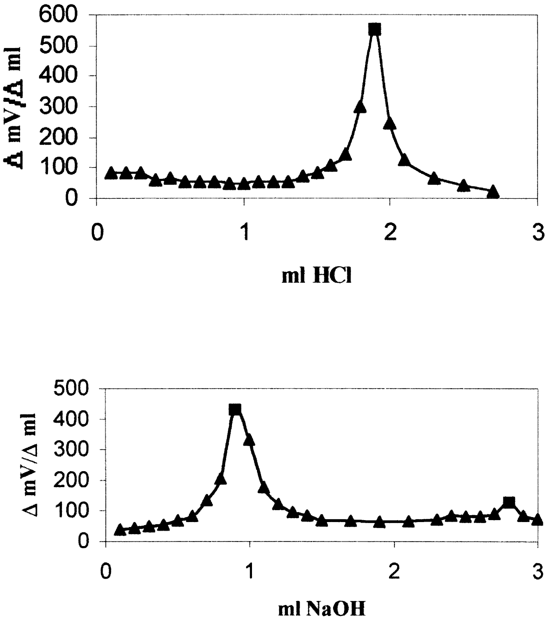

Fig. 1. The titration curves of neat PCL (sample 1a).

molecular characteristics of the latter is underlined by the end group titration results, too. As shown in Fig. 1, the end group titration is quite easily performed for neat PCL, and leads to a M_n value of ca. 18,200. On the contrary, titration of all Phe-containing copolymers shows contradictory data without a sharp equivalence point (Fig. 2: sample 1d). This result is probably due to some side reactions that occur during the synthesis and able to substantially modify the end portion of the chain. The aforementioned degradative process may support these findings. Modification of the end portion of copolyamide chains can also be hypothesized on the basis of UV absorption measurements. The UV spectra of samples 1b and 2b are given in Fig. 3, and reveal a broad absorption band between 300 and 400 nm, stronger for the sample prepared at a higher temperature. On the contrary, neat PCL does not show any UV absorption, whatsoever, in that spectral region. The above absorption band is characteristic of π-conjugated electron systems. Most probably the copolymers undergo some degradation near the amide linkages formed by the α-amino acid, with subsequent formation of π-conjugated electron systems in which Phe units play a significant role. In conclusion, T_p is a variable process which controls degradation, as suggested by the spectra in Fig. 3. The relatively small variation of η_r and $[\eta]$ when T_p is lowered (see Table 2), may seem to be in a contradiction with the above statement. However, if the strong UV absorption is substantially linked to chain end modifications, evidenced by titration data too, the overall effect on molecular weight may result rather low or masked by other counterbalancing phenomena.

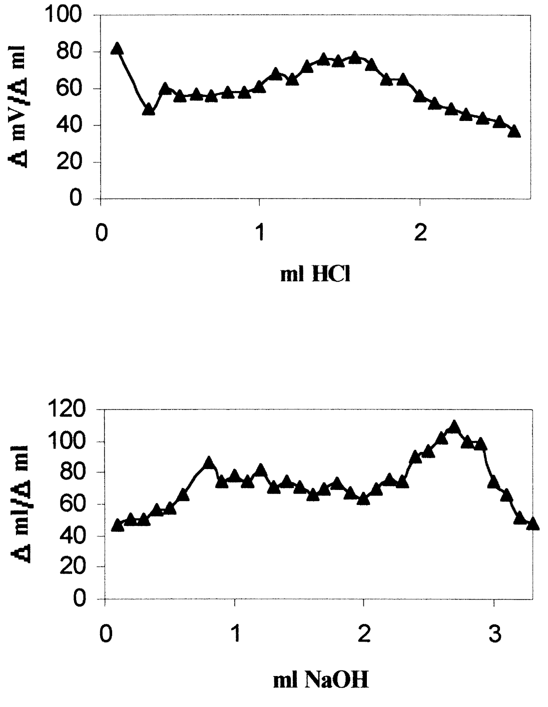

Fig. 2. The titration curves of the copolyamide containing 8.9 mol% Phe (sample 1d).

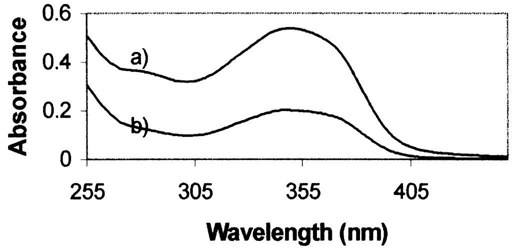

Fig. 3. A comparison between UV spectra of copolyamides synthesized at different temperatures: (a) (sample 1b, T_p: 270°C); and (b) (sample 2b, T_p: 230°C).

Fig. 4 shows T_m and T_g curves, as measured by DSC traces vs. Phe content in the copolymer. It is clear that the insertion of Phe in the polymer chain decreases both T_g and T_m values. The above effect may be attributed to the presence of Phe bulky side groups, which interfere with the hydrogen bonding formation, thus preventing (at least

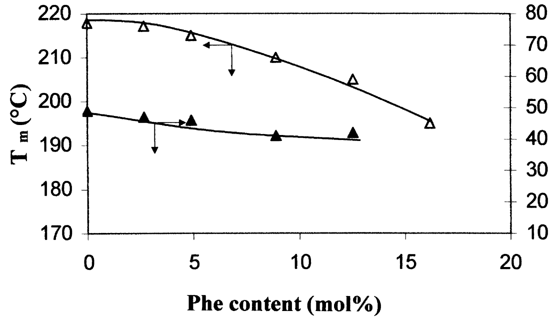

Fig. 4. Decrease of T_m (△) and T_g (▲) as functions of Phe content in the copolyamide.

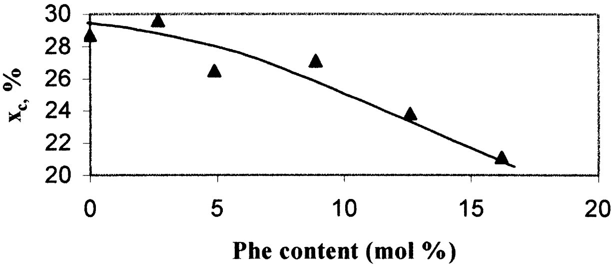

Fig. 5. Decrease of crystallinity (x_c,%) as a function of Phe content in the copolyamide (note: measured on second heating).

in part) crystallization and increasing free volume of amorphous regions. From the T_g data, the chain stiffening, due to the increased rotational energy barriers caused by bulky side groups, seems to have less relevant effects.

The crystallinity data, obtained by the DSC analysis (Fig. 5), show a decreasing trend probably due to the chain defects caused by the presence of Phe units in the main chain.

The semicrystalline nature of the copolyamides was confirmed by the WAXD analysis with a crystallinity content (x_c), calculated following Refs. [17,18], ranging between 31 and 27%. These data correspond to those achieved by the first heating of the DSC analysis, confirming that the approximations used to calculate the percentage crystallinity are correct.

TGA analysis (Table 2: column 4) enables to evaluate thermal stability of the copolymers. TGA data are given as T_{onset} vs. Phe content in the copolymer. As expected, by increasing the content of the latter, a corresponding more marked decrease of T_{onset} is observed, thus emphasizing the reduced thermal stability of the copolyamides. Tensile properties, e.g. Young's moduli and elongations at the break of samples 1a, 1b and 1c, are also shown in Table 2 (columns 5 and 6). Tensile properties have been measured only for those polymers having a low comonomer content, since it had been rather difficult to prepare suitable films from all others. The slight increase in Young's modulus is probably due to some stiffening of the polymer chain, caused by α-amino acid insertion; the corresponding decrease of elongation at break is presumably related to the lowering of copolymer molecular weight, too.

3.1. Degradability experiments: alkaline hydrolysis

Change of copolymer molecular weight after treatment with aqueous NaOH has been followed by [η] measurements. A sharp decrease of [η], most probably due to hydrolysis of amide bonds close to Phe units along the copolymer chain, is observed (Table 3: columns 2–4). The effect seems stronger for copolyamides with a lower Phe content, i.e. characterized by higher [η], and reaches a relative decrease of ca. 14%.

Structural modifications after alkaline hydrolysis have been investigated by the DSC analysis. Table 3 (columns 5–7) shows the regular increase of x_c (in relative terms) for the NaOH-insoluble fraction of copolyamide, up to a plateau value. The above result could be explained in terms of a stepwise bond disruption starting from the amorphous regions of the chains. Indeed, it is generally accepted in literature [19,20] that the degradation process of biodegradable polymers often occurs in two stages. For our system, in the first stage, there is the cleavage of amide bonds in the amorphous regions. This chain scission results in a recrystallization process, made easier by the lowering of entanglements content and, as a consequence, by the increased mobility of polymer chains. In the second stage, quite obviously, there is a slower increase of crystallinity due to the progressively lower content of residual tie segments.

3.2. Biodegradability experiments: microbial–bacterial attack

The microbial–bacterial attack test has been carried out following the method described in Ref. [16] on two samples characterized by a low Phe content (1b and 2b). The above copolymers could be considered as the most promising ones, in terms of the retention of original physical and chemical properties, but they did not exhibit any greater tendency to biodegradation, at least in the chosen experimental conditions, since after 115 days their films showed a weight loss of only ca. 3%.

4. Conclusion

A series of copolyamides, originated by the insertion of Phe in the main chain of PCL, was synthesized and characterized. The copolymer properties (x_c, T_g, $T_{m,}$) were found directly to be dependent on the content of α-amino acid added to the polymerization mixture.

The susceptibility to degradation of the above

Table 3
Viscosity and crystallinity changes in PCL and Phe-containing copolyamides, after alkaline hydrolysis (4 h)

Polymer sample	[η] (dl g^{-1})	[η] after hydrolysis (dl g^{-1})	$\Delta[\eta]/[\eta]$ (%)	x_c[a] (%)	x_c after hydrolysis [a] (%)	$\Delta x_c/x_c$ (%)
1a	1.27	1.25	−1.6	32.2	31.6	−1.9
1b	0.77	0.66	−14.3	31.3	33.9	+8.3
1c	0.59	0.51	−13.6	30.6	36.1	+18.0
1d	0.46	0.41	−10.9	27.2	32.0	+17.6

[a] Calculated on the first heating (−20 → 280°C).

copolyamides has been proven by alkaline hydrolysis but not confirmed by microbial–bacterial tests. Our results show an easy chemical cleavage of amide linkages formed by Phe, with a subsequent degradation of the polymer chain and the formation of low-molecular weight structures, without a real biodegradation sustained by a considerable weight loss.

Acknowledgements

This work has been carried out in the framework of a grant related to the “Programma Nazionale di Ricerca sulla Chimica-II fase”, assigned by MURST—Ministero dell’Università e della Ricerca Scientifica e Tecnologica—to Novamont S.p.A., Novara. WAXD measurements have been performed by Dr. F. Riva (IRTeMP, CNR, Arco Felice), TGA evaluations by Mr. V. Trefiletti (IMAG CNR, Genoa), and biodegradability tests by Dr. F. Degli Innocenti (Laboratorio di Biodegradazione, Novamont, Novara); to all of them we express our sincere thanks. The authors are grateful to Drs. E. Dossi and L. Ricco for their collaboration to the present work.

References

[1] Baker R. Controlled release of biologically active agents, New York: Wiley-Interscience, 1987.
[2] Glass JE, Swift G. Agricultural and synthetic polymers: biodegradability and utilization, ACS Symposium Series, 433. Washington, DC: American Chemical Society, 1990.
[3] Chasin M, Langer R. Biodegradable polymers as drug delivery systems, New York, NY: Marcel Dekker, 1990.
[4] Vert M, Feijen J, Albertson AC, Scott G, Chiellini E. Biodegradable polymers and plastics, Cambridge: Royal Society of Chemistry, 1992.
[5] Shalaby SW, Ikada Y, Langer R, Williams J. Polymers of biological and biomedical significance, ACS Symposium Series, 540. Washington, DC: American Chemical Society, 1994.
[6] Shalaby SW. Biomedical polymers: designed to degrade systems, Munich: Carl Hanser, 1994.
[7] Griffin GJL. Chemistry and technology of biodegradable polymers, London: Chapman and Hall, 1994.
[8] Timmins MR, Lenz RW. Trends Polym Sci 1994;2:15.
[9] Mayer JM, Kaplan DL. Trends Polym Sci 1994;2:227.
[10] Saotome K, Schulz RC. Makromol Chem 1967;109:239.
[11] Nagata M, Kiyotsukuri T. Eur Polym J 1992;28:1069.
[12] Arvanitoyannis I, Nikolaou E, Yamamoto N. Angew Makromol Chem 1994;221:67.
[13] Arvanitoyannis I, Nikolaou E, Yamamoto N. Polymer 1994;35:4678.
[14] Arvanitoyannis I, Psomiadou E, Yamamoto N, Nikolaou E, Blanshard JMV. Polymer 1995;36:2957.
[15] Arvanitoyannis I, Nikolaou E, Yamamoto N. Macromol Chem Phys 1995;196:1129.
[16] Tosin M, Degli Innocenti F, Bastioli C. J Envir Polym Degr 1996;4:55.
[17] Alexander LE. X-ray diffraction methods in polymer science, New York: Wiley, 1969.
[18] Chu CC. J Appl Polym Sci 1981;26:1727.
[19] Pitt GG, Chasalow FJ, Hibionada YM, Klimas DM, Schindler A. J Appl Polym Sci 1981;26:3779.
[20] Leeshlag JW, Pennings AJ, Bos RR, Rozema FR, Boering G. Biomaterials 1987;8:311.