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Polymer 41 (2000) 5967–5970

polymer

Studies on the degradability of a poly(ester amide) derived from L-alanine, 1,12-dodecanediol and 1,12-dodecanedioic acid

A. Rodríguez-Galán, L. Fuentes, J. Puiggalí*

Departament d'Enginyeria Química, Universitat Politècnica de Catalunya, Av. Diagonal 647, E-08028, Barcelona, Spain

Received 3 August 1999; received in revised form 6 October 1999; accepted 14 October 1999

Abstract

A new aliphatic poly(ester amide) derived from L-alanine has been synthesized and characterized. Degradability in different media has been studied and compared with BIOPOL, a well-known biodegradable polymer. The new poly(ester amide) shows a hydrolytic degradation that takes place through the ester linkage and an enzymatic degradation that strongly depends on the type of enzyme. Thus, proteolytic enzymes such as papain and proteinase K are the most effective ones. Biodegradation by microorganisms from soils and activated sludges has also been evaluated. Results indicate that BIOPOL degrades faster with microorganisms than the new polymer does. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Hydrolytic degradation; Enzymatic degradation; Biodegradability

1. Introduction

There is an on-going research effort to develop biodegradable polymers due to their specific applications both for biomedical and pharmaceutical uses and the environmental problems related to the plastic materials. A great interest has lately been focused on the study of poly(ester amide)s, since they combine amide groups responsible for hydrogen bond interactions that improve mechanical properties and highly hydrolyzable ester groups [1]. In particular, a series of regular polymers (Scheme 1) that also includes α-amino acids into the main chain appear promising due to their expected degradability with proteolytic enzymes [2–4]. Further, their synthesis (Scheme 2) is relatively simple, easy to scale up and proceeds with high yield. Recently, we have given attention to the characterization, structure and properties of some glycine [5,7] and alanine derivatives [6,8,9]. Among them, the polymer described by the sequence alanine–dodecanediol–alanine–sebacic acid (PADAS) showed interesting properties as solubility in organic chlorinated solvents [6], thermal stability [6], biocompatibility [6] and a susceptibility to the enzymatic degradation that can be controlled by varying the L- and D-alanine ratio [9]. The purpose of this work is to extend the characterization and degradation studies to a related poly(ester amide) derived from 1,12-dodecanedioic acid (PADAD), since there is a trend to substitute sebacic acid in commercial nylons by 1,12-dodecanedioic acid due to the improved raw material cost [10].

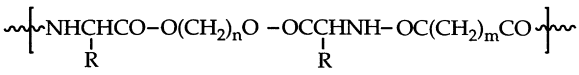
2. Experimental

The poly(ester amide) PADAD was synthesized by interfacial polymerization following the procedure outlined in Scheme 1 with the experimental conditions optimized for the sebacoyl derivative [9]. The polymer was purified by pouring a chloroform polymer solution into acetone. The intrinsic viscosity was determined with a Cannon-Ubbelhode microviscometer in dichloroacetic solutions at 25 ± 0.1°C. Infrared absorption spectra were recorded with a Perkin–Elmer 1600 FT-IR spectrometer in the 4000–500 cm^{-1} range from films obtained by evaporation of chloroform solutions. NMR spectra were registered from chloroform/trifluoroacetic acid solutions using tetramethylsilane as an internal standard. A Bruker AMX-300 spectrometer operating at 300.1 and 75.5 MHz was used for ^{1}H and ^{13}C NMR investigations, respectively. Thermal analysis was performed by differential scanning calorimetry with a Perkin–Elmer DSC-PYRIS 1, using indium metal for calibration. Thermogravimetric analysis was carried out with a Mettler TG50 thermobalance.

A BIOPOL sample with a comonomer ratio of 9:1 ((*R*)-3-hydroxybutyric acid: (*R*)-3-hydroxyvaleric acid) was

* Corresponding author. Fax: +34-934017150.
E-mail address: puiggali@eq.upc.es (J. Puiggalí).

PII: S0032-3861(99)00756-9



n	m	R	References
2	4	H, $CH_2CH(CH_3)_2$, CH_2Ph	2,3
6	2 - 8	H, CH_3, CH_2Ph	4,5
12	8	CH_3	6

Scheme 1.

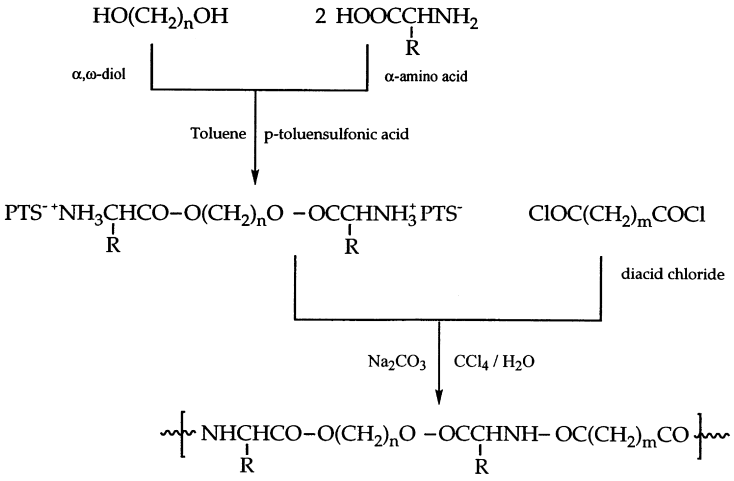
supplied by Fluka (No. 27826) and used for comparative purposes, since it is a well-known biodegradable polymer. Plates of 1.5 × 1.5 cm × 200 μm were cut off from films prepared by melt pressing 200 mg of PADAD or BIOPOL powder samples. Thicker plates of 1500 μm were also prepared, but only used for evaluating the degradation with papain. Hydrolytic degradation studies were carried out in two different conditions: (a) pH 7.4 sodium phosphate buffer at 37°C; and (b) distilled water at 55°C. Enzymatic degradation studies were performed at 37°C by using lipases from *Candida cylindracea* (943 units/mg) and *Pseudomona cepaya* (1500 units/mg), and proteolytic enzymes such as papain (30,000 units/mg, No. 7144), proteinase K (*Tritirachium album*, 13 units/mg), trypsin (*Bovine-pancreas*, 10,600 units/mg) and α-chymiotrypsin (*Bovine-pancreas*, 50 units/mg). The media consisted of a sodium phosphate buffer (pH 6.0 for papain and 7.4 for the other enzymes) containing sodium azide (0.03%) to prevent microbial growth and the appropriate enzyme (0.5 mg/ml). In the case of papain the solution also contained L-cysteine (34 mM) and ethylenediaminetetracetic disodium salt (30 mM) for activation. Solutions were renewed every 72 h because of enzymatic activity loss. Degradation was also evaluated in activated sludges from a purifying plant, which were renewed every 15 days. In all cases, the plates were placed in glass vials containing 30 ml of the degradation media (hydrolytic, enzymatic or sludges) and removed after the prescribed times. Polymer plates were also buried in soils rich in microorganisms. Mass loss, intrinsic viscosity, and changes in NMR and IR spectra were evaluated in all these different degradation experiments.

3. Results and discussion

3.1. Synthesis and characterization

PADAD was obtained in a 71% yield and 0.7–0.8 dl/g intrinsic viscosity after purification by precipitation in acetone. The polymer was soluble in strong acids (formic, dichloroacetic and trifluoroacetic acid) and chlorinated polar solvents such as chloroform and dichloromethane. The IR spectra showed the characteristic absorption bands of methylene, amide and ester groups: 3301 (amide A), 3066 (amide B), 2920 and 2850 (CH_2), 1734 (C=O, ester), 1648 (amide I), 1540 (amide B), 1205 and 1161 (ester), 668 (amide V) and 592 cm^{-1} (amide VI). Data from NMR spectroscopy were fully consistent with the anticipated chemical constitution (Table 1). Further, no signals corresponding to terminal groups could be detected in agreement with viscosimetric data. The hygroscopicity of PADAD was evaluated at 22°C by measuring the moisture sorption of plate samples exposed to 100% humidity or immersed in water. The respective water absorption values of 1.1 and 2.4% indicate the low hydrophilicity of this polymer.

A melting temperature of 98.1°C and a heat of fusion of 30 kJ/mol were measured for the sample recovered from polymerization. A low crystallinity of approximately 30% could be estimated taking into account the reported group contributions to the heat of fusion [11]. After crystallization from the melt (T_c = 65.1°C) two melting temperatures at 76 and 97.5°C were observed, whereas the total heat of fusion decreased to 15.4 kJ/mol. These melting temperatures are lower, as expected, than that previously reported for PADAS [6] (122 and 89°C). A glass transition temperature



Scheme 2.

Table 1
Assignment of the ^{1}H and ^{13}C NMR spectra from deuterated chloroform solutions of PADAD (δ,ppm)

	....-$(CH_2)_3$-CH_2-CH_2-CH_2-OOC-CH (CH_3)-NH-OC-CH_2-CH_2-$(CH_2)_3$-....											
^{1}H	1.28	1.28	1.63	4.13	—	4.60	1.43	6.21	—	2.21	1.63	1.28
^{13}C	29.2 - 29.5	25.76	28.47	65.58	172.70	47.92	18.64	—	173.4	36.51	25.56	29.2 - 29.5

in the 10–13°C interval also characterized the sample. Thermogravimetric analysis demonstrated that PADAD is thermally stable through fusion, since decomposition begins at 340°C, more than 240°C after fusion. Total decomposition of the sample (more than 97%) is achieved at 490°C following a three step process.

3.2. Degradability

The change of the intrinsic viscosity of PADAD and BIOPOL samples during incubation at 37 and 55°C is represented in Fig. 1. It can be seen that both polymers degrade quite similarly, although the intrinsic viscosity decrease is more pronounced for the PADAD sample. Thus, in the accelerated conditions of 55°C the viscosity of PADAD falls from 0.73 to 0.15 dl/g, whereas BIOPOL viscosity only changes from 1.3 to 0.68 dl/g. Inspection of IR and NMR spectra of PADAD samples exposed to the solutions revealed that degradation took place mainly through the ester linkages. Thus, the relative intensity of the carbonyl ester absorption band at 1734 cm^{-1} decreases with the exposure time. In the same way, the ^{1}H NMR spectra show that the signal at 4.13 ppm ($-CH_2OCO-$) decreases in intensity, while only one additional signal at 3.67 ppm ($-CH_2OH$), indicative of unesterified groups, appears and increases in intensity with degradation time. Thus, the number of bond cleavages that occurred during degradation can be estimated from measuring the intensity of both signals ($100 \times I_{3.67}/(I_{3.67} + I_{4.13})$). The values corresponding to different degradation times are summarized in Table 2 together with the weight loss of PADAD and BIOPOL samples. This is minimal for BIOPOL, which means that its degradation products are still insoluble in the degradation media. However, the weight loss of the PADAD sample is more prominent (20%) in the degradation at 55°C that gives a lower molecular weight ($[\eta] = 0.15$ dl/g). In conclusion, the weight loss measurements appear only significant in the last stages of degradation where small and soluble fragments could be produced.

Fig. 2 shows the weight loss of PADAD samples in different enzymatic media. The most significant changes are found in papain where 43% of the material is solubilized after only 21 days of exposure. Similar results were reported in the degradation studies of PADAS samples [6,9]. Note also that degradation evidence could not be detected by using enzymes that only have an estearase activity as

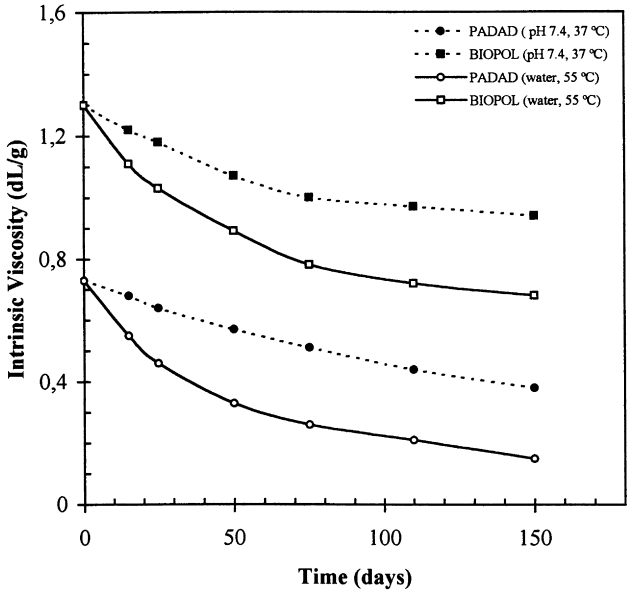


Fig. 1. Changes in the intrinsic viscosity of PADAD and BIOPOL during hydrolytic degradation under different conditions.

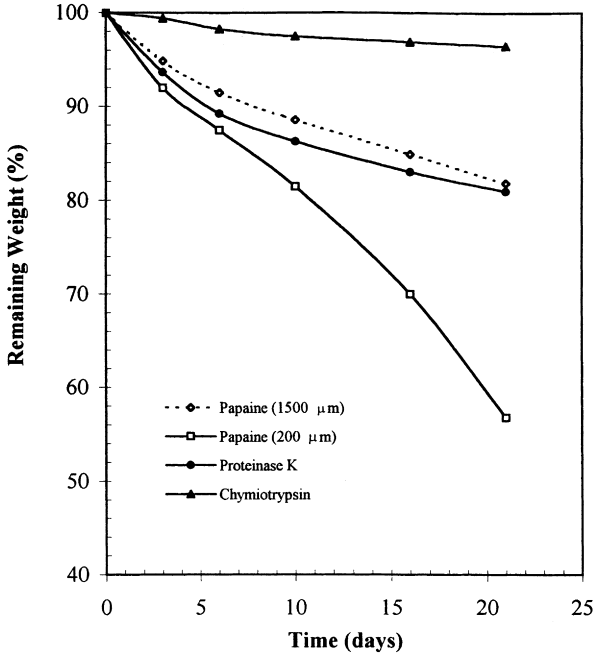


Fig. 2. Plot of the remaining weight of PADAD samples versus degradation time with different enzymes.

Table 2
Hydrolytic degradation data for PADAD and BIOPOL at different temperatures

Time (days)	PADAD				BIOPOL	
	Remaining weight (%)		Number of bond cleavages (%)		Remaining weight (%)	
	pH 7.4 (37°C)	Distilled water (55°C)	pH 7.4 (37°C)	Distilled water (55°C)	pH 7.4 (37°C)	Distilled water (55°C)
0	100	100	0	0	100	100
15	100	99.5	–	–	99.2	96.7
25	99.8	96.0	1.8	3.2	98.9	98.0
50	99.5	94.5	1.9	8.0	98.7	98.3
75	96.0	93.8	2.4	12.0	98.6	97.9
110	95.0	91.1	2.9	14.0	98.2	97.0
150	94.0	80.0	6.5	20.1	97.0	96.0

lipases. Intrinsic viscosity remains practically invariable for all samples during the degradation process, suggesting that it mainly takes place on the polymer surface. The degradation of a thicker plate and so with a lower surface/volume ratio was also followed in the most active medium (papain) in order to corroborate this assertion. The results (Fig. 2) clearly show a decrease in the degradation rate due to the physical hindrance for the enzyme to penetrate inside the sample.

Fig. 3 shows the weight loss measurements of PADAD samples after treatment with two different media rich in microorganisms (activated sludges and soil). Data on the bacterial BIOPOL samples are also included for comparison. The results indicate that the natural polymer degrades faster by microorganisms than PADAD in contrast to the hydrolytic degradation observations. It is also remarkable that PADAD only degrades in soil, since the slight weight loss found in the sludge immersed samples can be attributed to a hydrolytic degradation. No significant changes in the intrinsic viscosity of the samples buried in the soil could be observed, since the degradation by microorganisms is again a process that takes place on the sample surface.

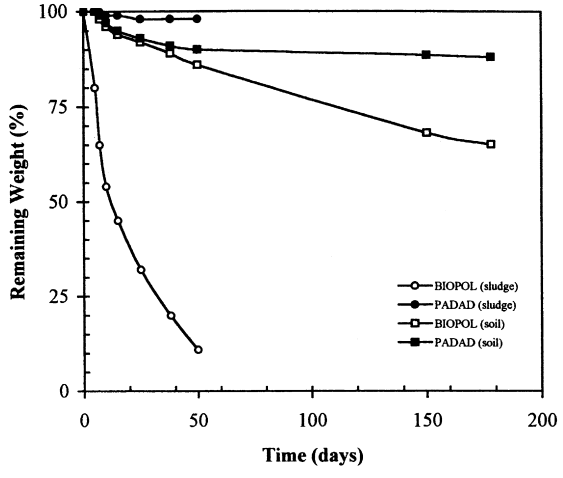


Fig. 3. Plot of the remaining weight of PADAD and BIOPOL samples after different times of exposure in soils or sludges.

In summary, PADAD shows a high hydrolytic degradability, at least faster than the polyester BIOPOL samples, and a very fast enzymatic degradability in proteolytic enzymes as papain and proteinase K. However, degradation by microorganisms proceeds slower than BIOPOL.

Acknowledgements

This research has been supported by a research grant from CICYT (MAT97-10113).

References

[1] Gonsalves KE, Mungara PM. Trends Polym Sci 1996;4:25.
[2] Saotome Y, Miyazawa T, Endo T. Chem Lett 1991:21.
[3] Saotome Y, Tashiro M, Miyazawa T, Endo T. Chem Lett 1991:153.
[4] Ho LH, Huang S. J Polym Prepr Am Chem Soc Div Polym Chem 1992;33(2):94.
[5] Paredes N, Rodríguez-Galán A, Puiggalí J. J Polym Sci, Polym Chem Ed 1998;36:1271.
[6] Paredes N, Rodríguez-Galán A, Puiggalí J, Peraire C. J Appl Polym Sci 1998;69:1537.
[7] Paredes N, Casas MT, Puiggalí J, Lotz B. J Polym Sci, Polym Phys Ed 1999;37:2521.
[8] Puiggalí J, Aceituno JE, Paredes N, Rodríguez-Galán A, Pelfort M, Subirana JA. Polym Prepr Am Chem Div Polym Mater 1998;79:60.
[9] Rodríguez-Galán A, Pelfort M, Aceituno JE, Puiggalí J. J Appl Polym Sci 1999;74:2312.
[10] Kohan MI. Nylon plastics handbook, Munich: Hanser, 1995.
[11] Van Krevelen DW. Properties of polymers, 3. Amsterdam: Elsevier, 1990.