Comparative Studies on the Degradability of Poly(ester amide)s Derived from L- and L,D-Alanine

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ABSTRACT: The hydrolytic degradation of two poly(ester amide)s derived from sebacic acid, dodecanediol, and alanine in both the quiral L configuration and the racemic L,D mixture has been studied. The degradation was monitored by following the changes in intrinsic viscosity, mass loss, chemical constitution, and mechanical properties. The results show that these poly(ester amide)s degrade slowly at 37°C through the ester linkage. Little changes in the Young's modulus of both samples were found at the beginning of the degradation process. Biodegradation has also been studied by using different enzymes. Papain was the most effective one, although the degradation rate was dependent on the stereochemical composition of the polymer. © 1999 John Wiley & Sons, Inc. J Appl Polym Sci 74: 2312–2320, 1999

Key words: hydrolytic degradation; enzymatic biodegradation; poly(ester amide)s; L-alanine; L,D-alanine

INTRODUCTION

Aliphatic copoly(ester amide)s have been suggested and recently investigated as a potential family of polymers for biomedical applications,[1,2] since they may have enhanced mechanical properties and processing facilities with respect to polyesters, which are the polymers more used[3] up to now. On the other hand, the inclusion of α-amino acids on a polymer chain is expected to lead to biodegradable materials as it has been demonstrated for polyamides.[4–6] However, the studies on poly(ester amide)s derived from α-amino acids are nowadays scarce. Saotome et al.[7,8] have reported the enzymatic degradation of polymers derived from 1,2-ethanediol, adipic acid, and different amino acids. In the same way, we have recently undertaken the study of a series of regular poly(ester amide)s (Scheme I) constituted from related compounds.[9–11] Our results confirm that this kind of polymers is enzymatically degradable. Furthermore, a detailed study[10] on biodegradation and biocompatibility was performed for the polymer constituted by L-alanine, dodecanediol, and sebacic acid (PADAS), since additional interesting characteristics as solubility in organic solvents and film-forming properties were found. We recall the notation used in the previous paper[10] where the *P*olymer is described by the sequence *A*lanine–*D*odecanediol–*A*lanine–*S*ebacic acid.

Scheme I

Physical and biodegradable properties can be changed by controlling the stereochemical compo-

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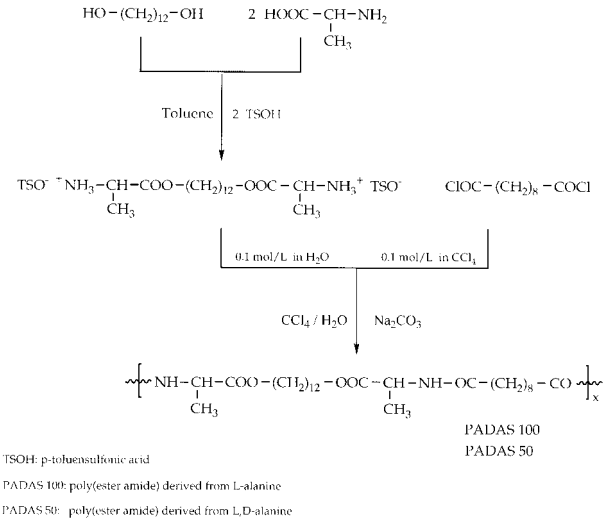
sition of a polymer chain, as it was found for polylactide[12] and poly(3-hydroxybutirate).[13] The proposal of this work is to get inside the characteristics of PADAS and to study how they vary when PADAS is derived from the quiral L-alanine (PADAS100) or from the racemic L,D mixture (PADAS50).

EXPERIMENTAL

Synthesis

The poly(ester amide)s were synthesized following the procedure outlined in Scheme II and previously reported for the quiral[10] polymer. However, the experimental conditions have been slightly modified in order to improve the molecular weights. Efforts have been focused to optimize the concentration of sebacoyl dichloride in the organic solvent. Thus, the highest intrinsic viscosity was attained for both polymerizations at a concentration of ca. 0.1 mol/L. Polymers were purified by pouring a chloroform polymer solution on acetone.



Scheme II

Characterization

The intrinsic viscosity of the polymers was determined by measurements with a Cannon-Ubbelhode microviscometer in dichloroacetic acid solutions at 25° ± 0.1°C. The chemical constitution was ascertained by IR and NMR spectroscopy. IR absorption spectra were recorded with a Perkin-Elmer 1600 FT-IR spectrometer in the 4000–500 cm^{-1} range from polymeric films obtained by evaporation of chloroform solutions. NMR spectra were registered from chloroform/trifluoroacetic acid polymer solutions using tetramethylsilane (TMS) as an internal standard. A Bruker AMX-300 spectrometer operating at 300.1 and 75.5 MHz was used for ^1H- and ^{13}C-NMR investigations, respectively.

Thermal analysis was performed by differential scanning calorimetry with a Perkin-Elmer DSC-PYRIS 1 to determine temperature and heat of fusion of the polymer samples. Indium metal was used for calibration purposes (T_m = 429.75 K, ΔH_f = 3267 kJ/mol). Thermogravimetric analysis was carried out with a Mettler TG50 thermobalance. All experiments were done under N_2 flow at heating or cooling rates of 10°C/min.

Powder X-ray patterns were recorded under vacuum at room temperature, and calcite (d_B = 3.035 A) was used for calibration. A modified Statton camera (W. H. Warhus, Wilmington, DE) with a nickel-filtered copper radiation of wavelength 1.542 Å was used for these experiments.

Mechanical properties were determined on a Minimat instrument from Polymer Laboratories. Rectangular shaped samples 30 mm in length, 3 mm in width, and 200 μm thick were used in stress–strain experiments, which were carried out at a deformation rate of 1 mm min^{-1}. The samples were cut off from a regular film (30 × 30 mm^2), which was prepared with 200 mg of polymer by melt pressing. Mechanical parameters were averaged from a minimum of 10 measurements for each polymer sample.

Degradation Studies

Hydrolytic degradation studies were carried out at 37°C in a pH 7.4 sodium phosphate buffer. The samples were prepared as indicated before for mechanical tests and placed in glass vials containing 30 mL of the buffer and sodium azide (0.03 wt %) to prevent microbial growth. After the prescribed times, the samples were removed from the vials and rinsed thoroughly with distilled water. Wet samples for mechanical measurements were obtained after absorption of superficial water with a filter paper. The remaining samples were dried in vacuum for several days to constant weight, and stored over $CaCl_2$ before analysis. Mass loss, intrinsic viscosity, changes in NMR and IR spectra, and mechanical properties were evaluated. Changes in the texture of samples after hydrolytic degradation experiments were observed with a Nikon Microflex AFX-DX optical microscope.

Table I Calorimetric Data for PADAS50 and PADAS100 Samples

	PADAS50		PADAS100	
	Film from Chloroform	Film by Melt Pressing	Film from Chloroform	Film by Melt Pressing
Melting peaks (°C)[a]	100	98	*101*, 120	97, *125*
Heat of fusion (kJ/mol)	22.0	20	31.9	24.8
Crystallinity (%)	24.9	22.6	36.0	28.1

[a] When different significative melting peaks are observed, the most intense is indicated by italics.

Enzymatic degradation studies were carried out at 37°C by using lipase from *Candida cylindracea* and proteolytic enzymes such as papain, trypsin, and α-chymotrypsin. Disks samples with a diameter of 13 mm were also prepared by melt pressing at 110°C. Those with a thickness of 200 μm and a weight of 40 mg were selected and placed in bottles with 10 mL of the enzymatic media. This consists on a sodium phosphate buffer (pH 6.0 for papain and 7.4 for the other enzymes) containing sodium azide (0.03%), and the appropriate enzyme. In the case of papain (30,000 USP-U mg^{-1}, No. 7144), the solution also contains L-cysteine (34 m*M*) and ethylenediaminetetraacetic disodium salt (30 m*M*) for activation.

All enzymatic solutions were renewed after every 72 h because of enzymatic activity loss. After the immersion time, the retrieved samples were washed and dried as indicated for hydrolytic experiments. In all cases, the mass loss and the intrinsic viscosity of the samples were evaluated.

RESULTS AND DISCUSSION

Synthesis and Characterization

The intrinsic viscosity of PADAS50 and PADAS100 ranged in the 0.80–1.00 and 0.55–0.83 dL/g intervals, respectively, depending on the concentration of sebacoyldichloride in the CCl_4 organic layer. In both cases, the maximum intrinsic viscosity was attained at concentrations near to 0.1 mol/L. Polymerizations of PADAS100 occurred with a significant higher yield than PADAS50. Thus, the averaged values after reprecipitation were 73 and 60%. These differences in the intrinsic viscosity and in the polymerization yield may be attributed to a major solubility for the low molecular weight fractions of the racemic PADAS50.

The NMR and IR spectra of PADAS50 were identical to that previously reported for PADAS100.[10] Both polymers were soluble in strong acids (formic, dichloroacetic, and trifluoroacetic acid), dimethylformamide, dimethylsulfoxide, *m*-cresol, and chlorinated polar solvents as chloroform and dichloromethane. Thus, transparent brittle films could be attained by evaporation from a low boiling point solvent as chloroform.

The crystallinity of samples was evaluated by using the heat of fusion for a 100% crystalline material (88.4 kJ/mol), which was estimated from the reported[14] group contribution of ester (−2.5 kJ/mol), amide (2.0 kJ/mol), $CH(CH_3)$ (4.7 kJ/mol), and methylene (4.0 kJ/mol).

Calorimetric data (Table I) were determined for samples prepared by slow evaporation of a $CHCl_3$ solution and also for the samples used in mechanical tests and degradation studies, which were prepared by melt pressing. As expected, the

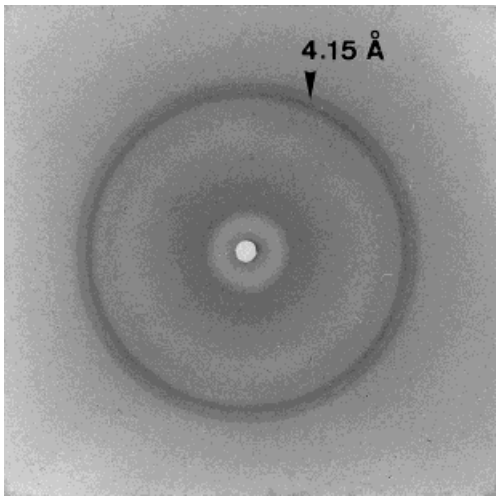


Figure 1 X-ray powder pattern of a film of PADAS50 prepared by melt pressing.

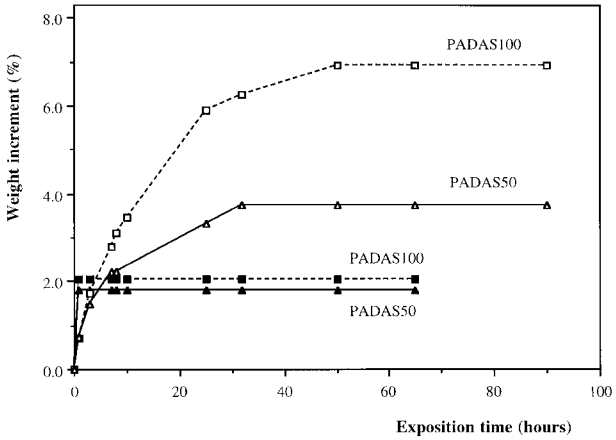


Figure 2 Water uptake of PADAS as a function of time from samples exposed to 100% humidity at 22°C (▲, ■) and from samples immersed in water at 22°C (△, □). All the measures were carried out with samples prepared by melt pressing at 110°C.

crystallinity varies according to the preparation conditions. Thus, a higher value was determined for the brittle samples prepared by slow evaporation than for the flexible films obtained by melt pressing. Moreover, it may be pointed out that the crystallinity of the racemic polymer is always lower than the stereoregular one. Two melting peaks associated to different lamellar crystals were found for PADAS100, whereas only the peak relative to the less organized crystals was observed for PADAS50. Few changes in the lower melting temperatures have been detected between the two polymers that differ in their stereochemical composition.

Thermogravimetric analysis of PADAS50 is in agreement with the reported one for PADAS100[10] and indicates the thermal stability of the sample. Thus, 50% of weight loss takes place at a temperature (of 310–330°C), 200°C higher than the melting point of the polymer.

X-ray powder patterns of the two samples were unfortunately similar and so differences on crystallinity could not be detected from this analysis. A strong ring at 4.15 Å characterizes the pattern (Fig. 1) together with other weak reflections at 6.0, 5.0, and 4.0 Å that are also related with the molecular packing.

The hygroscopicity of PADAS samples has been evaluated at 22°C by measuring the moisture sorption in disk samples exposed to 100% humidity or immersed in water. Figure 2 shows that both poly(ester amide)s have a similar and low capacity to absorb water from the ambient medium, as expected from their high methylene content. This sorption is produced in the first hour, and after that the water content remains constant for increasing exposure times. However, greater differences have been found when samples were immersed in water. Thus, in spite of the same chemical constitution, a greater hydrophilicity was found for PADAS100 (7% with respect to the 3.7% determined for PADAS50). A higher content of polar terminal groups (as deduced from intrinsic viscosity measurements) may contribute to enhance this hydrophilicity.

Stress–strain data show that PADAS is characterized by elastic and plastic deformation regions and that some differences can be detected in function of the stereochemical composition (Table II). Thus, although the tensile modulus and the elongation to break are similar, a greater tenacity is found for PADAS100 as a consequence of its higher tensile strength and elongation to break. Mechanical properties clearly depend on the previous treatment of samples. In this way, Table II also shows the dramatic changes observed in these properties when samples are immersed in an aqueous medium during a short time (1 day). In this short period, the degradation effects are negligible. The higher values found for the Young's modulus and the tensile strength of the

Table II Stress–Strain Parameters of Different PADAS Samples[a]

	PADAS50		PADAS100	
	Melt Pressed	After Buffer Immersion	Melt Pressed	After Buffer Immersion
Young's modulus (MPa)	388	1000	320	900
Tensile strength (MPa)	9	15	18	29
Elongation at break (%)	8	9	10	39

[a] All samples have been carefully dried at vacuum to constant weight before mechanical tests.

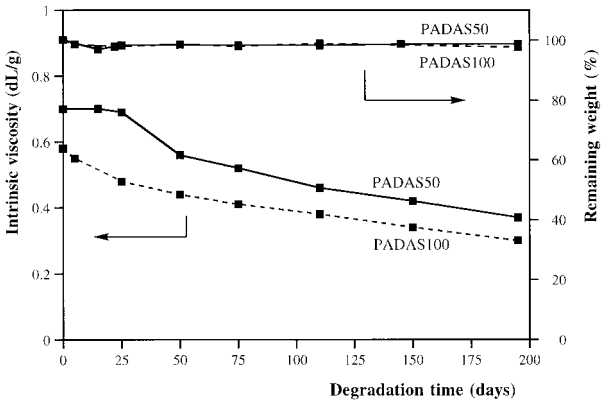


Figure 3 Changes in the intrinsic viscosity and the remaining weight vs hydrolytic degradation time for PADAS100 (- -) and PADAS50 (—).

immersed samples indicate that crystallinity has increased as a consequence of the water treatment and/or the solubilization of oligomers.

Hydrolytic Degradation

The evolution of the intrinsic viscosity of PADAS samples during incubation at 37°C in a pH 7.4 buffered solution is represented in Figure 3. It can be seen that both polymers degrade quite similarly, although the intrinsic viscosity of PADAS50 did not change during the first 25 days of exposure, probably due to its low hydrophilicity. However, after this initial induction period, the viscosity of PADAS50 clearly decreased (from 0.7 to 0.4 dL/g) in a way similar to PADAS100 (from 0.58 to 0.30 dL/g). Figure 3 also shows that the weight losses during degradation were minimal, which means that the products of degradation were still insoluble in the buffer solution. In fact, the change in size of the molecules must not be considerable, and in addition, the polymers are constituted by unities with a high content in hydrophobic methylene groups.

Inspection of IR spectra (Fig. 4) of samples exposed to the buffered solution revealed that degradation took place mainly through the ester linkages. Thus, the absorption band that corresponds to the ester group (1740 cm^{-1}) gradually decreases in intensity with degradation time, while amide bands (1650 and 1540 cm^{-1}) remain unchanged. Furthermore, ^{1}H-NMR spectra (Fig. 5) show that the signal at 4.16 ppm (—CH_2OCO—) decreases in intensity, while an additional signal at 3.65 ppm (—CH_2OH), indicative of unesterified groups, appears and increases in intensity with degradation time. However, note that no appreciable changes can be seen in the zone near 2.20 ppm, where the hydrolysis of amide groups should be detected.

Evolution of mechanical parameters such as Young's modulus, tensile strength, and maximum elongation during the degradation process is shown in Figure 6 and summarized in Table III. In order to simulate physiological conditions, the behavior of wet samples has been analyzed in addition to the carefully dried samples. Note that properties are measured with samples that have been immersed in an aqueous medium and so they must be compared with the initial state (Table II) that correspond to this sample treatment and not with the data of samples directly obtained by melt pressing.

In general, the degradation process greatly affects the mechanical properties of these poly(ester amide)s and thus the changes are more apparent than from intrinsic viscosity measures. Some significant features can be considered from the results displayed in Figure 6:

1. The Young's modulus decreases slowly during the first twenty days of degradation. Thus, the samples keep their elastic characteristics during an appreciable period of time. It is also important to note

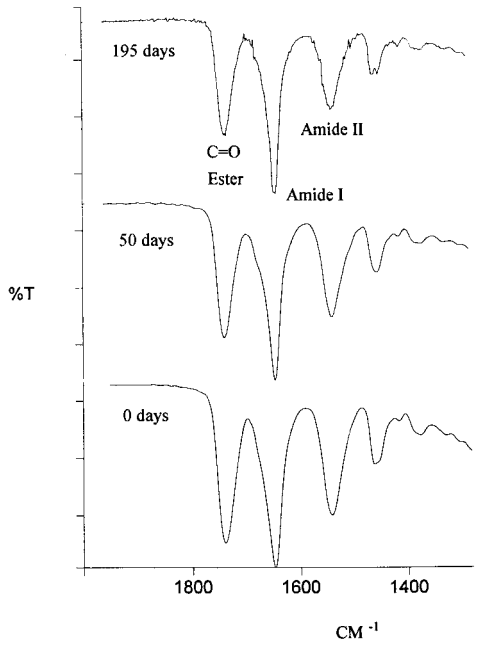


Figure 4 IR spectra of PADAS50 after the indicated hydrolytic degradation days.

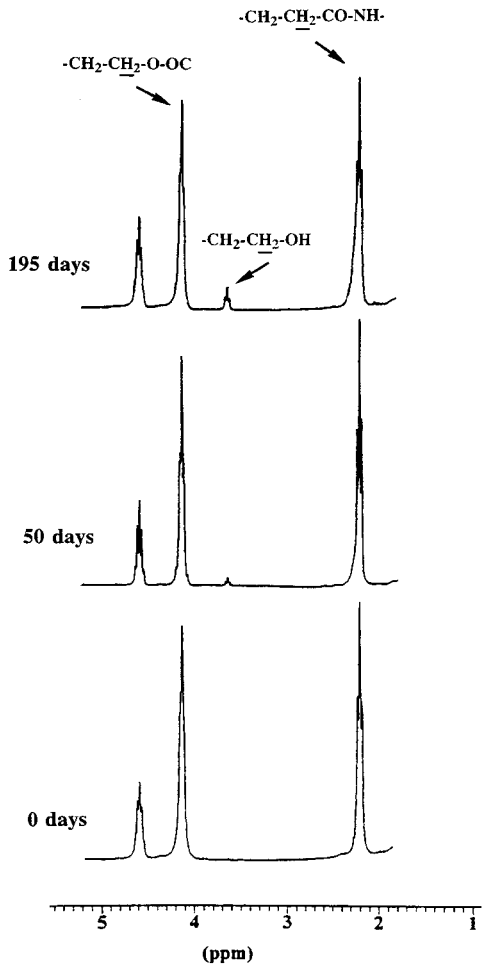


Figure 5 ^{1}H-NMR spectra of PADAS50 after the indicated hydrolytic degradation days.

that this behavior is also a characteristic of samples under simulated physiological conditions.

2. The properties of PADAS50 are generally worse than equivalent PADAS100 samples. Only the tensile modulus of the dry sample of PADAS50 is higher than PADAS100 during the first degradation steps, probably due to its initial lower degradability. However, the tensile modulus decreases to a considerable lower value than PADAS100 after twenty days. This behavior is consistent with the intrinsic viscosity measures that indicate a great degradation rate for PADAS50 after the initial induction time.
3. The elongation at break is the property more affected by degradation. Thus, a 75% decrease is observed during the first twenty degradation days for the dry sample of PADAS100. However, only 14 and 19% decreases are found for the tensile strength and tensile modulus, respectively.
4. The different characteristics found between dry and wet samples reflect the plasticier effect of water. Thus, wet samples show in the first degradation days a lower

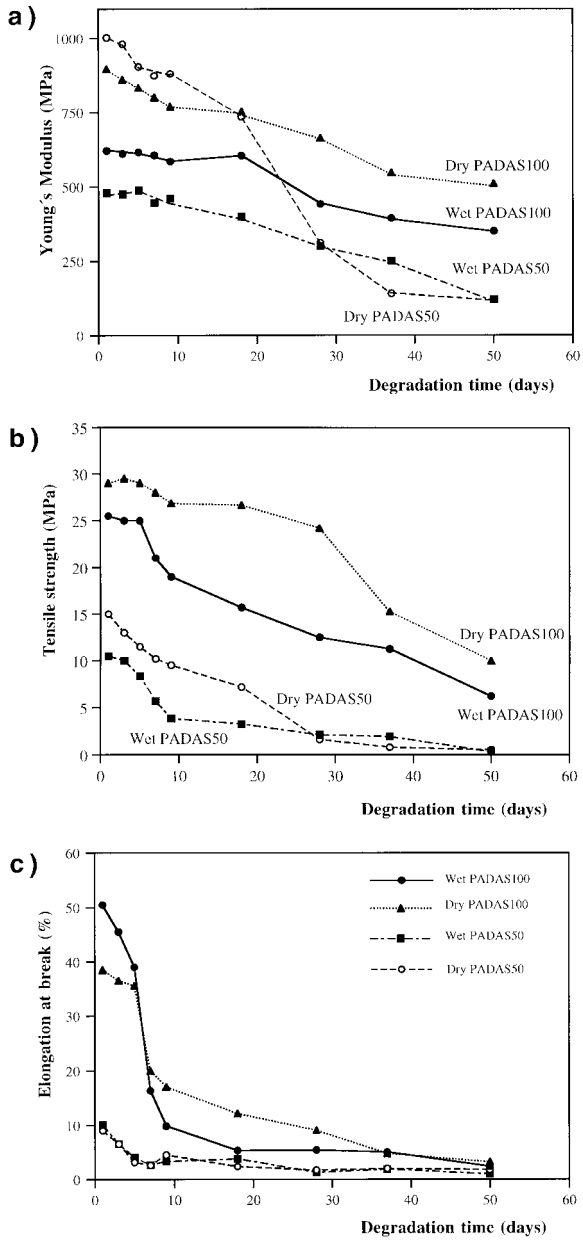


Figure 6 Changes in the mechanical parameters of PADAS100 (▲, ●) and PADAS50 (○, ■) vs hydrolytic degradation time for wet (●, ■) and dry samples (▲, ○). (a) Young's modulus, (b) tensile strength, and (c) elongation at break.

Table III Changes in Mechanical Parameters of PADAS After Degradation in a pH 7.4 Buffer at 37°C

Degradation (Days)	Young's Modulus (MPa)				Tensile Strength (MPa)				Elongation at Break (%)			
	Dry Samples		Wet Samples		Dry Samples		Wet Samples		Dry Samples		Wet Samples	
	PADAS50	PADAS100	PADAS50	PADAS100	PADAS50	PADAS100	PADAS50	PADAS100	PADAS50	PADAS100	PADAS50	PADAS100
1	1000	896	479	620	15.0	29.0	10.5	25.5	9.0	38.5	10.0	50.5
3	980	860	475	610	13.0	29.5	10.0	25.0	6.5	36.5	6.5	45.5
5	904	833	488	615	11.5	29.0	8.3	25.0	3.2	35.6	4.0	39.0
7	874	800	446	605	10.2	28.0	5.7	21.0	2.6	20.0	2.7	16.3
9	880	770	460	584	9.5	26.8	3.8	19.0	4.5	17.0	3.3	9.8
18	735	753	398	604	7.2	26.7	3.2	15.7	2.4	12.1	3.8	5.3
28	310	663	300	441	1.6	24.2	2.1	12.5	1.7	9.0	1.3	5.4
37	142	546	251	394	0.8	15.2	1.9	11.2	2.0	4.7	1.9	5.0
50	121	509	121	350	0.5	10.0	0.3	6.2	1.8	3.2	1.0	2.4

tensile modulus and a higher elongation to break than dry samples do.

The superficial texture of samples is also greatly affected during the hydrolytic degradation process. Figure 7 shows as the initially transparent samples become opaque and that appear numerous crevasses, which are responsible for the drop in mechanical properties.

Enzymatic Degradation

Changes in the texture of representative disk samples of PADAS are shown in Figure 8. Note that degradation clearly depend on the stereochemical composition of the polymer and on the enzyme used. Figure 9 shows the weight loss of PADAS50 disks samples in different enzymatic media at 37°C. Significant changes were mainly found by using papain. Thus, the weight loss after 16 days of incubation was 40% for papain and less than 7% for trypsin, α-chymotrypsin, and lipase. Controls in buffered solutions without enzymes during the equivalent exposure time indicate a weight loss of 6–2% and demonstrate the enzymatic degradation effect of papain. It is worth to note that the weight loss is an indication of degradation only when it has occurred in a sufficient extension to produce very small and soluble molecules. The previously reported data[10] on the degradation of PADAS100 are also included in Figure 9 for comparison purposes. The total solubilization reached in this case after 15 days of degradation clearly indicates that enzymatic degradation is stereospecific. Thus, papain fundamentally

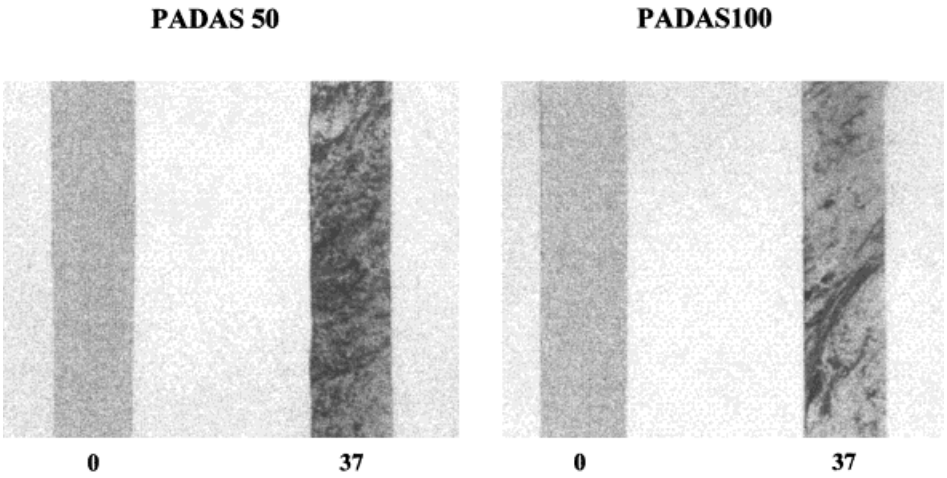


Figure 7 Optical micrographs of PADAS100 and PADAS50 samples as prepared and after 37 days of hydrolytic degradation. Magnification ×47.

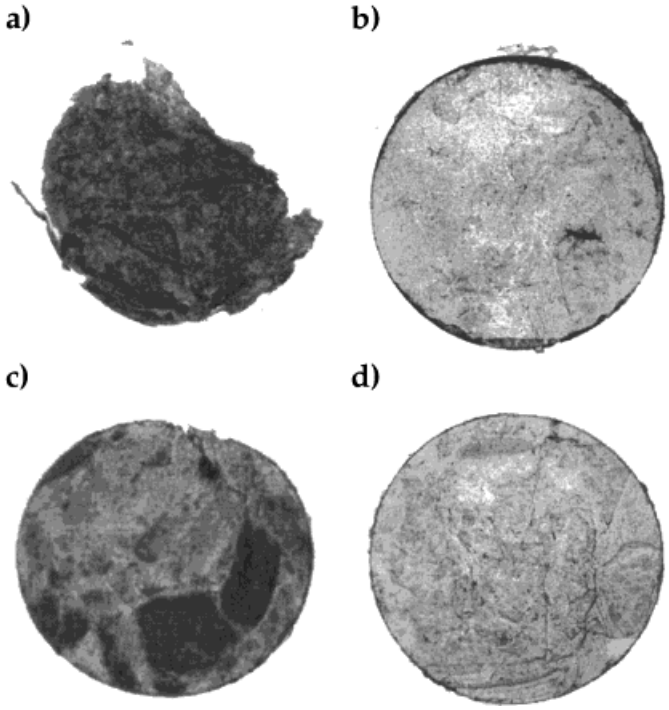


Figure 8 Optical micrographs of PADAS100 (a, c) and PADAS50 (b, d) disks after 7 days of enzymatic degradation with papain (a, b) and after 16 days of degradation on lipase (c, d). Magnification ×3.8.

degrades the linkages where the natural L-amino acid is involved. It is also obvious from the reported data that polymers with a different susceptibility to enzymatic degradation can be obtained by varying only their stereochemical composition.

CONCLUSIONS

The results reported in this work can be summarized as follows:

1. Slight but significant differences have been found between the interfacial polymerization of PADAS50 and PADAS100. The higher intrinsic viscosity and lower polymerization yield attained for PADAS50 may be a consequence of a major solubility for the low molecular weight fractions. Interfacial polymerizations of PADAS yielded maximum molecular weights when the concentration of the monomer in the CCl_4 organic layer was ca. 0.1 mol/L.
2. PADAS50 appears to be less crystalline than PADAS100 when samples prepared under the same conditions are compared. However, the hygroscopicity of PADAS50 is significatively lower than PADAS100. This contradictory result could be attributed to the lower content of terminal groups in PADAS100.
3. Both polymers undergo degradation in an aqueous medium by a simple hydrolytic

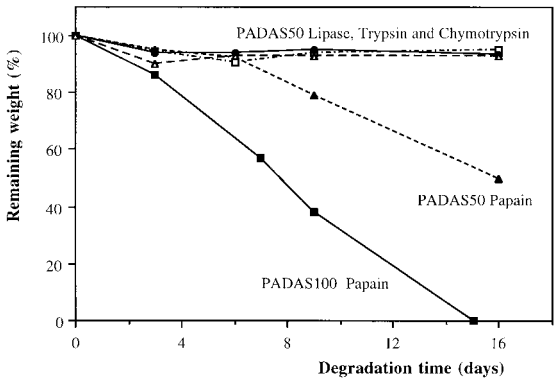


Figure 9 Percentage of weight remaining of PADAS50 during enzymatic degradation in a buffer with papain (▲), trypsin (□), α-chymotrysin (△), and lipase (●). Previous data on the degradation of PADAS100 with papain (■) are also represented.

mechanism that takes basically place through the ester linkages. Mechanical properties are highly affected by this degradation process, although the tensile modulus is practically constant during the first 20 days of degradation at 37°C. Moreover, the Young's modulus maintain a significant value after 50 days of degradation.

4. Both poly(ester amide)s are degraded at a very fast ratio by using a proteolytic enzyme as papain. However, enzymatic degradation is stereospecific and appreciable differences in the degradation rate are found between the stereoregular and the racemic polymers.

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