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Hydrolytic and fungal degradation of polyamides derived from tartaric acid and hexamethylenediamine

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Abstract

The hydrolytic and fungal degradability of a set of polyamides made from D and L-tartaric acids and 1,6-hexanediamine was investigated as a function of the enantiomeric composition of the polymers. Optically pure enantiomorphs (100% D and 100% L) as well as a series of stereocopolymers with D:L ratios varying from 1:9 to 1:1 were examined. Hygroscopicity of these polyamides was found to increase from 8 to 25% when the D:L ratio changed from 0 to 1. Degradation in water under physiological conditions (pH 7.4 and 37°C) took place slowly and also increased with the D:L ratio of the stereocopolymer. Weight loss between 10 and 30% and decays in the molecular weight down to nearly 50% were observed after 2 months of incubation. Degradation by *Aspergillus niger* was tested on cast films in the presence of Sabouraud's dextrose broth medium. Fungal attack was evaluated by mycelial coverage and invasion based on standards. Extensive surface growing and a considerable pervading of the bulk material by the fungi was observed for stereocopolyamides with D:L ratios 1:4 and 1:1. On the contrary, the microorganism appeared to be completely inactive in cultures of either of the two optically pure polymers. These results indicated that the dominant factor determining the biodegradability of poly(tartaramide)s is water affinity rather than the configurational nature of the polyamide.

Keywords: Poly(ester amide)s; Poly(tartarester amide)s; Hydrolytic degradation

1. Introduction

Polyamides derived from carbohydrates are the object of much current attention [1–5]. The interest arises not only from the naturally occurring character of the feedstocks, but also from the potential that such polymers offer as biodegradable and biocompatible materials. In the last few years our group has devoted great efforts to the study of poly(tartaramide)s [5–9] i.e. polyamides obtained from diamines and 2,3-dihydroxy-butanodioic acid, more commonly known as tartaric acid. Among the different classes of poly(tartaramide)s that have been explored by us, those obtained from 2,3-di-*O*-methyl-L-tartaric acid and linear 1,*n*-alkanediamines, abbreviated as P*n*DMLT, are remarkable because they are unique in combining an overall good pattern of general properties with a relatively easy accessibility [7]. P6DMLT is the member of this family that has been studied in most detail. This polyamide is a stereoregular nylon 6,4 disubstituted with two methoxy groups at the two respective carbon atoms of the diacid moiety. The material is highly crystalline, melts at 230°C and may absorb up to 10% of water when exposed to a humid atmosphere. Its crystal structure has been determined to consist of a layered arrangement of hydrogen bonded chains with features similar to those of the α-form of nylon 6,6 [10]. The hydrolytic degradability of P6DMLT has been measured for a variety of pH and temperatures. The resistance of this polymer to hydrolysis at pH 7.4 and 37°C appears to be rather high, showing a weight loss of fewer than 10% of the initial sample, after 1 year of incubation [11].

It is widely known that chain tacticity affects the crystallinity and as a consequence it also modifies the sensitivity of the polymer to chemical hydrolysis [12]. Random stereocopoly(tartaramide)s made of mixtures of D and L-tartaric acid, abbreviated as P6DM(D,L)T, were synthesized a few years ago, and their crystalline structure and properties examined in detail [13,14]. All of them were found to be highly crystalline irrespective of the enantiomeric composition [13]. Exploratory investigations carried out with the (1:9) stereocopolymer indicated that their response to water attack was not very different from that displayed by the parent optically pure poly(tartaramide) P6DMLT. The

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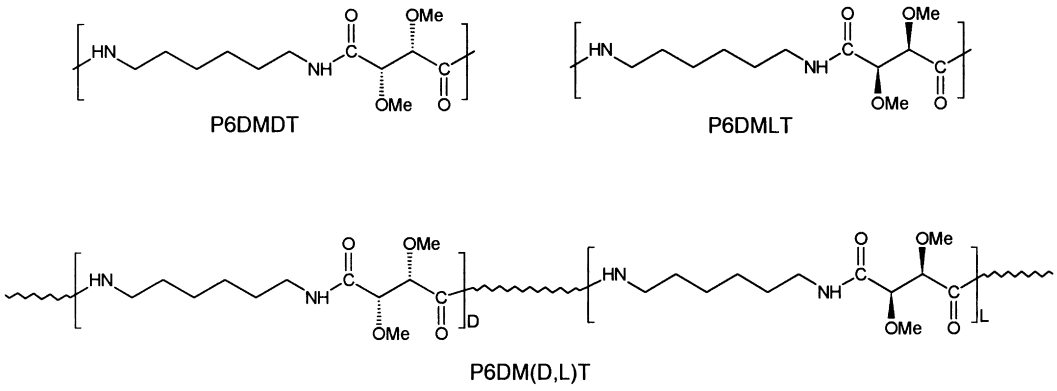

Fig. 1. Chemical structures of polyamides examined in this work.

ability of D- and L-tartaric units to share the same crystal structure was considered to be the reason for such unexpected behavior.

Since enzymes are stereospecific catalysts, tacticity also affects polymer biodegradation. Whereas polypeptides, polyamides and poly(ester amide)s made from naturally occurring α-L-amino acids are readily degraded by enzymes, their D-isomer counterparts are not [15]. On the contrary, complete biodegradation of poly(3-hydroxyalkanoate)s seems to be limited to optically pure polymers, stereocopolymers leading to non-biodegradable residues [16]. The sensitivity of synthetic chiral polymers to enzymatic hydrolysis provoked by filamentous fungi has been examined only in a few cases with dissimilar results. Poly(D,L-lactic acid) oligomers were shown to be biodegradable and bioassimilable by several strains regardless of the polymer configuration, although no similar conclusions could be drawn for high molecular systems [17,18]. In contrast, depolymerization of poly(3-hydroxybutyrate) by fungal enzymes has been proved to show a marked tacticity dependence [19], and some polyesters based on naturally occurring polyols are known to undergo fungal degradation that varies significantly depending on their molecular structure [20].

The present work was undertaken to examine the effect of the enantiomeric composition on the chemical hydrolysis and biodegradability of poly(tartaramide)s. For this purpose, a set of polyamides P6DM(D,L)T with D:L compositions varying from 0 to 1 were prepared, and then subjected to degradation tests including water hydrolysis and biocolonization by the filamentous fungi *Aspergillus niger*. This strain has proved to be effective in the biodegradation of certain polyurethanes derived from L-tartaric acid [21] and also in the biodegradation of nylon 2/6 [22]. Changes taking place on the substrate were evaluated by GPC, DSC and optical and electron microscopy. The chemical structures of the polyamides examined in the present work are shown in Fig. 1, and a selection of their properties with more significance to the study carried out here is given in Table 1.

2. Materials and methods

2.1. Synthesis and characterization of polymers

Polyamides were obtained by polycondensation in solution from mixtures of 2,3-di-*O*-methyl-D and L-tartaric acids and 1,6-hexanediamine using the active ester method. The diacid was activated as bis(pentachlorophenyl) ester and the diamine as its *N*,*N*′-bis(trimethylsilyl) derivative. The polymers were characterized by FTIR and $^{1}H/^{13}C$ NMR spectroscopy, and their average molecular weights determined by GPC. A detailed account of the synthesis of all the polymers

Table 1
Data of poly(tartaramide)s examined in this work

Polyamide	[η][a] (dl g^{-1})	Mn[b]	PD	T_m[c] (°C)	(ΔH_m[c] (calories g^{-1})	T_g[c] (°C)
P6DMLT	1.35	42 400	2.1	230	14.8	106
P6DMDT	0.48	19 300	1.9	—	—	—
P6DM(D,L)T(1:9)	1.49	47 600	2.6	226	9.9	95
P6DM(D,L)T(1:4)	0.69	18 400	2.1	225	11.2	74
P6DM(D,L)T(1:1)	1.19	37 800	2.2	223	12.8	68

[a] Intrinsic viscosity measured in formic acid.
[b] Determined by GPC in chloroform/*o*-chlorophenol (95:5).
[c] T_m, T_g and ΔH_m measured by DSC.

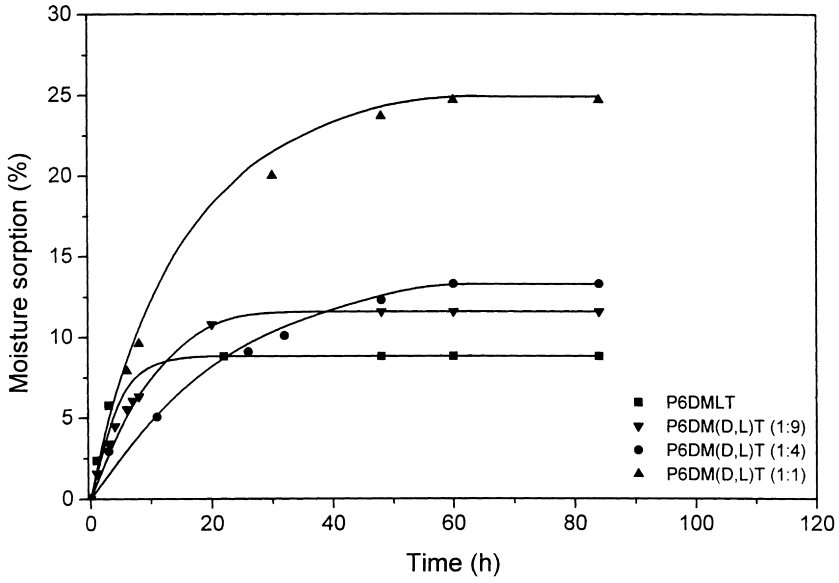

Fig. 2. Moisture uptake of polymer discs exposed to 100% R.H. at 18°C as a function of time.

investigated in this work has been published elsewhere [7,13].

Gel permeation chromatography was performed on a 510 Water Assoc. instrument fitted with two columns with respective exclusion limits at 10^3 and 10^4 nm, and a refraction index detector. A (95:5) chloroform:*o*-chlorophenol mixture was used as eluent and molecular weights were estimated against polystyrene standards (Polysciences) using the Maxima 820 computer program. Hygroscopicities were evaluated by measuring the moisture sorption in a 100% relative humidity atmosphere at 18°C. Discs of 12 mm diameter and about 250 μm thick were cut from films prepared by casting from chloroform at room temperature. The discs were exposed to test conditions and water uptake was monitored by weighing until a constant value was achieved.

2.2. Hydrolysis and biodegradation assays

For hydrolytic degradation experiments, the discs were dipped in phosphate buffer at pH 7.4 at 37°C containing 0.3% of sodium azide for periods of time ranging from 12 h to 3 months. After incubation, specimens were weighed, and then subjected to GPC and DSC measurements. DSC experiments were performed with 1–3 mg samples at heating rates of 20°C min^{-1} on a Pyris Perkin–Elmer instrument calibrated with indium.

The filamentous fungus *A. niger* ATCC 9642 was chosen according to ASTM G21-70 standard recommended practice for determining resistance of synthetic polymeric materials to fungi. Fungus was grown on 2% Sabouraud dextrose agar slants (Bio-Mérieux) added with gentamicine at 25°C for 1 week. A conidia suspension was prepared and poured into a glass-stoppered Erlenmeyer flask containing 45 ml of sterile water and 10–15 glass beads. The flask was shaken vigorously to liberate the conidia and the suspension was filtered in order to remove mycelial fragments. The filtered spore suspension was centrifuged at 2500 rpm for 20 min, the supernatant discarded, and the sediment resuspended in 50 ml of sterile water and centrifuged again. This process was repeated three times. The final washed residue was diluted in 2% Sabouraud dextrose broth (BioMérieux) and gentamicine, and the concentration of inocula determined as indicated by the International Standard ISO 7954. An inoculum concentration of 3.7×10^5 CFU ml^{-1} was measured after 3 days of incubation.

Discs with a diameter of 6 mm and a thickness ranging from 70 to 120 μm were used for biodegradation assays. Three replicates were used for each polyamide. The discs were sterilized by exposing them to UV radiation for 60 min (30 min per each side). Then they were individually incubated for 21 days at 25°C in Petri dishes with 8 ml of inoculant *A. niger* (pH 5.6). A fourth disc was used as a blank. After incubation, the discs were rinsed with sterile physiologic serum and examined by optical and scanning electron microscopy. To evaluate the extent of biodegradation, the density of hyphae invading the discs was quantified by means of optical microscopy. Four grades of invasion ranging from 0 to 3 were established as a function of the number of hyphae observed inside the disc. Grade 0 indicated absence of invasion; grade 1 was considered to correspond to a low attack with a maximum of 20 hyphae counted at the same focal plane; grade 2 indicated an invasion of moderate intensity with the number of penetrating hyphae

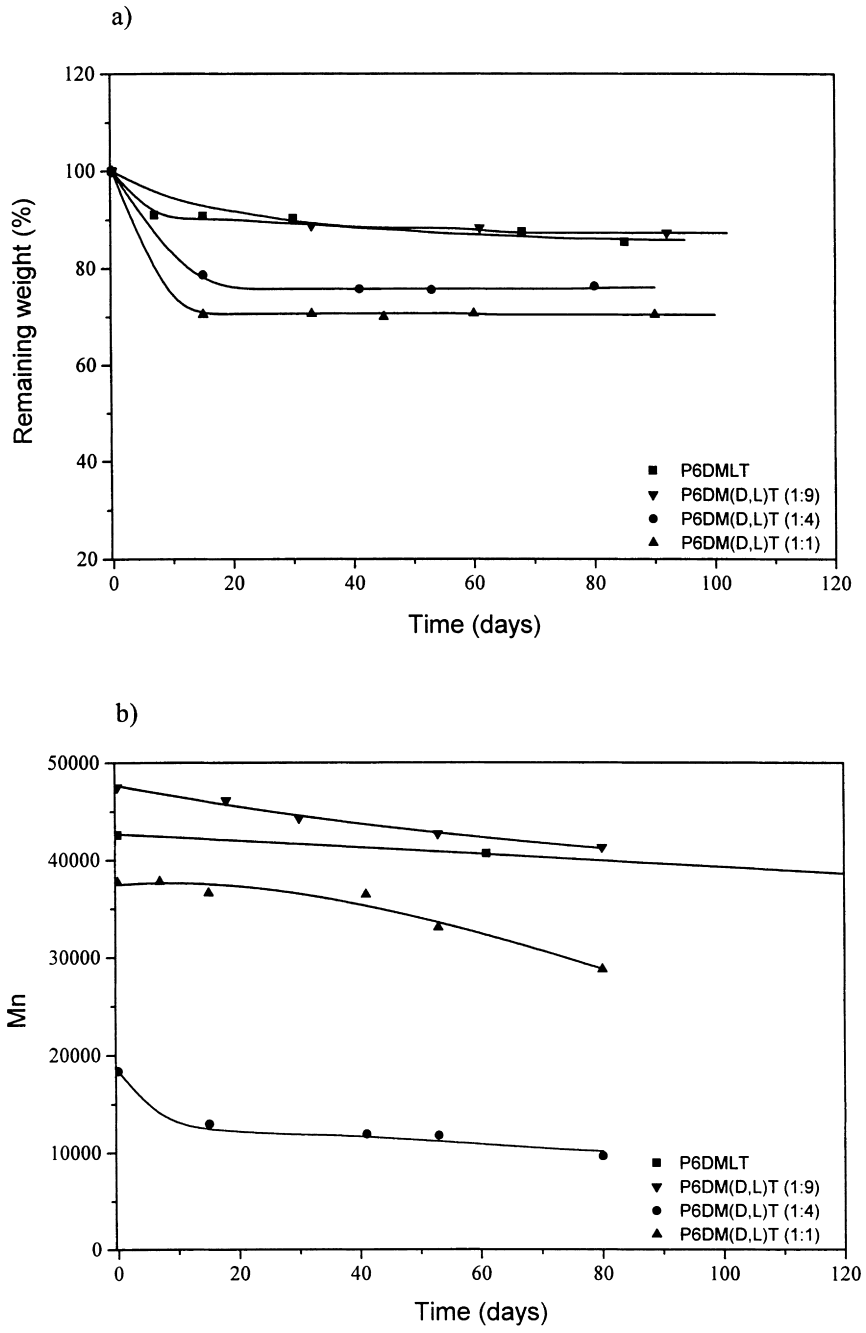

Fig. 3. (a) Weight loss; (b) and number average molecular weight decay as a function of the incubation time.

per focal plane ranging from 21 to 40; and grade 3 expressed a high degree of penetration with more than 40 hyphae per focal plane.

2.3. Optical and scanning electron microscopy

In order to attain a reliable observation of the polymers, before and after degradation, as well as of the invading mycelia, discs before and after incubation with the fungus were freeze-processed for each examined polyamide. For this, a piece of the disc was embedded in TBS and frozen at −50°C. Sections of approximately 10 μm were cut with a Leica cryostat CM3050 at a chamber temperature of −20°C. Glass slides were conditioned by dipping them in an aqueous solution of gelatine (2%) and $CrK(SO_4)_2 \cdot 12H_2O$. Sections were adhered to the slides and observed under an optical microscope, Nikon Optiphot, in a 100–200 range of magnification.

A selection of the incubated specimens was also examined by scanning electron microscopy. For this purpose,

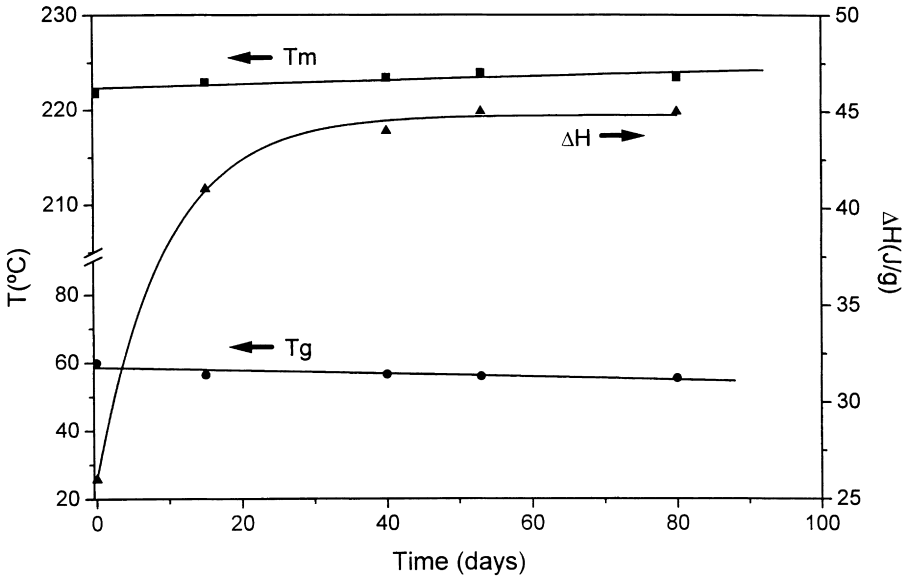

Fig. 4. Variation of T_m, T_g and ΔH_m as a function of incubation time for polyamide P6DM(D,L)T(1:4).

discs were quenched in liquid nitrogen and broken into small pieces. Free-fractured surfaces were sputtered with gold and visualized on a Jeol JSM 6400 instrument.

3. Results and discussion

3.1. Hygroscopicity

Water adsorption displayed by polyamides P6DM(D,L)T upon exposure to 100% relative humidity atmosphere is shown in Fig. 2. In all cases, the amount of sorbed water increased exponentially at the beginning to attain a constant value which increases from 8 to 25% as the D:L ratio of the polyamide increases from 0 to 1. As expected, the behavior displayed by P6DMDT (not shown) was essentially the same as that found for P6DMLT. At first sight it could be thought that differences in water uptake were due to differences in the degree of crystallinity that could be expected for stereocopolymers varying in the enantiomeric composition. However, it has been shown in previous work that crystallinity of P6DM(D,L)Ts is hardly affected by the D:L ratio, and that the same crystal structure is shared by the whole series including both the homopolyamide P6DMLT and the racemic polymer P6DM(D,L)T(1:1) [13]. In contrast, the influence of chain ends on hygroscopicity should be disregarded since we are dealing with polymers with polymerization degrees in the range 100–250. It can be concluded therefore that differences in the packing of the polymer in the amorphous phase rather than in crystallinity should be responsible for the observed differences. A more loose structure seems to be adopted by the polymer filling the non-crystallized fraction as the system becomes more optically compensated. This is in full agreement with T_gs determined by DSC which go down from 106 to 68°C as the D:L ratio increases from 0 to 1 (see Table 1).

3.2. Hydrolytic degradation

Hydrolytic degradation results are fully consistent with the foregoing observations. Since exactly the same behavior was found for both optically pure enantiomorphs, results are illustrated only for P6DMLT. Variations taking place in both specimen weight and molecular weight of the polymer as a function of incubation time are shown in Fig. 3. A higher susceptibility to the water attack is displayed by polyamides P6DM(D,L)T as the content in the D-isomer increases. This is best reflected in the remaining weight vs. time curves plotted in Fig. 3a which show weight losses ranging from 10% for P6DMLT to 30% for the racemic P6DM(D,L)T(1:1). As it can be seen in the plot shown in Fig. 3b, the number average molecular weight is reduced to 20–30% of the initial value for the stereocopolymers, whereas such reduction turns out to be fewer than 10% for homopolyamide P6DMLT. A simple calculation reveals that only a few backbone bonds per chain are broken at the end of the treatment.

Both water adsorption and hydrolytic degradation results suggest the presence of a relative accessible amorphous phase that is responsible for the action of water. Furthermore, the fact that neither effect increases continuously with time indicates that the action of water must be confined to a certain fraction of the sample. In order to verify this point, a series of P6DM(D,L)T(1:4) samples degraded at different times were analyzed by DSC. Results obtained from such measurements are plotted in Fig. 4. No significant variations in either melting point or glass temperature were found to take place upon degradation. On the contrary, a steady increase in the fusion heat was observed to occur with the

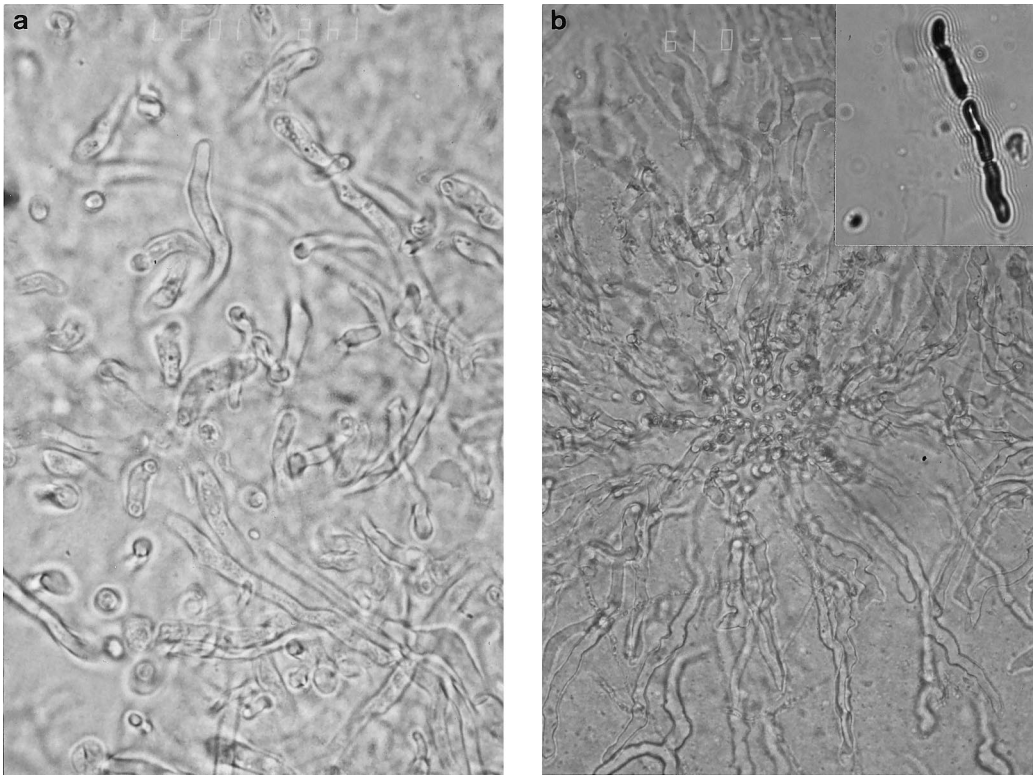

Fig. 5. Optical micrographs of *A. niger* growths in (a) P6DM(D,L)T(1:1) (×1250); and (b) P6DM(D,L)T(1:4) (×500); Inset: section of the disc showing internal hyphae (×1250).

advancement of the degradation. These results are consistent with a selective hydrolysis confined to the amorphous phase and that does not affect the polymer-chain-forming part of crystallites.

3.3. Fungal degradation

This test was carried out on the two optically pure polyamides and on the stereocopolyamides with D:L ratios 1:4 and 1:1. An unaided visual observation of the different dishes containing the polymer discs incubated with *A. niger* revealed extensive growth of the fungus with massive generation of pigmented conidia. No contamination on the controls was detected. The pH value of the medium was measured after incubation and found to range from 3.5 to 4.0. Observation of the discs under the optical microscope

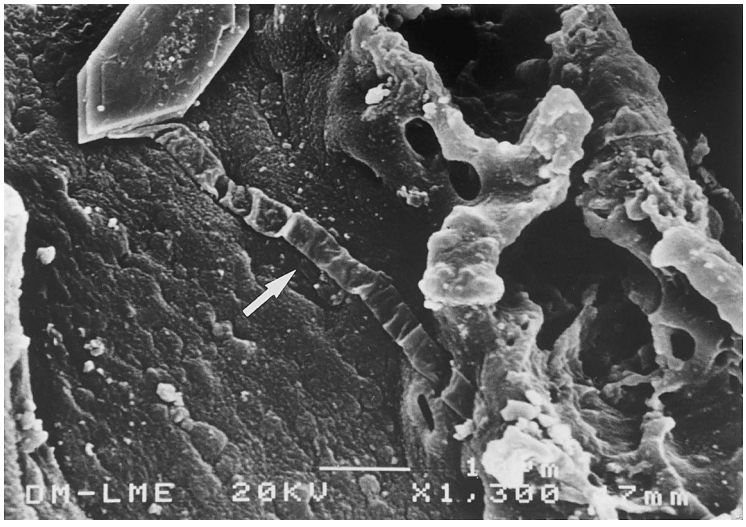

Fig. 6. Scanning electron micrograph of P6DM(D,L)T(1:1) invaded by *A. niger*. Collapsed pervading hyphae as that shown by an arrow on the micrograph are detected throughout the bulk of the specimen (×1800).

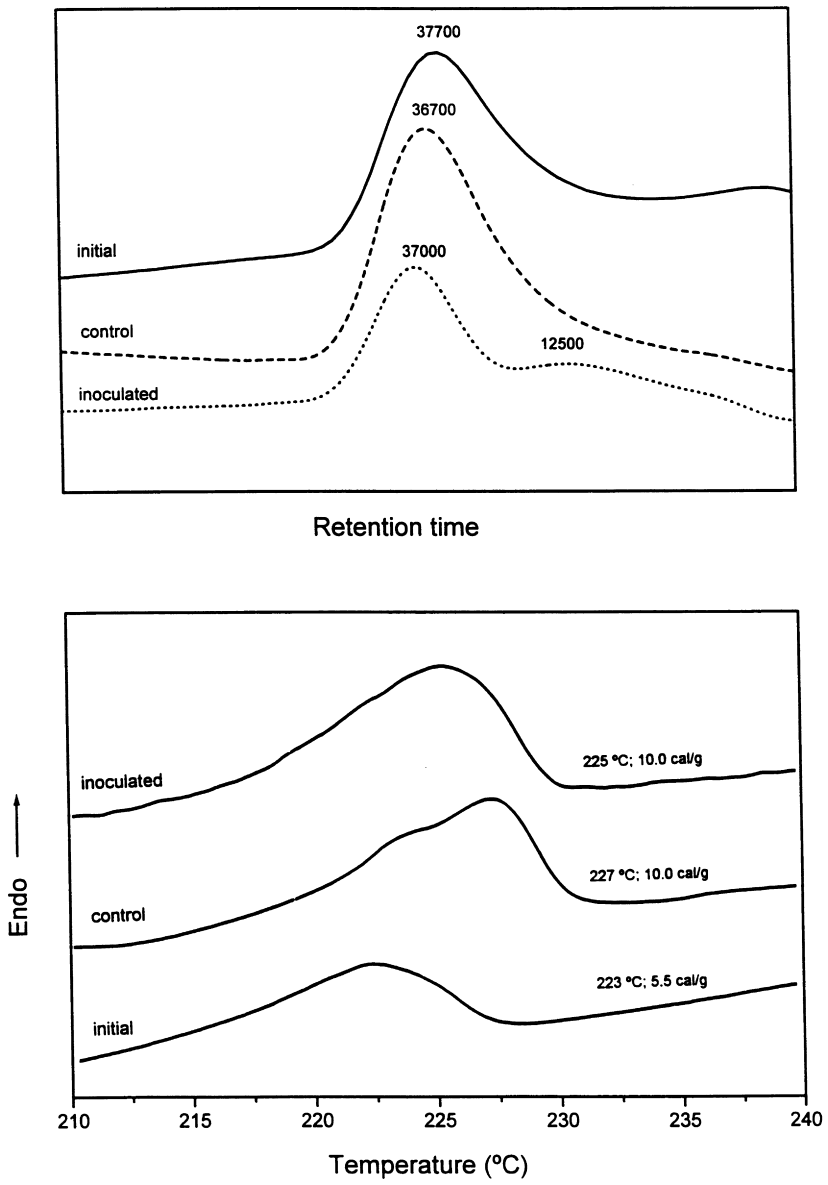

Fig. 7. (a) Compared GPC elution profiles; and (b) DSC traces of P6DM(D,L)T (1:1) before and after 1 month ageing on the inoculated and the inoculate-free (control) culture medium.

showed that conidia adhesion was restricted to stereocopolyamides. Surprisingly, no invasion at all was observed in discs made from either of the two optically pure enantiomorphs, i.e. P6DMDT and P6DMLT. The appearance of P6DM(D,L)T(1:1) and P6DM(D,L)T(1:4) discs drawn from the culture medium after 21 days of incubation is shown in the micrographs included in Fig. 5a. On micrograph 5a, axial views of hyphae penetrating inside the disc are frequently seen. A high density colony is observed at the center of the field of micrograph 5b with peripheral branching extended throughout the whole matrix of the polymer. Colonising hyphae are estimated to occupy between 80 and 100% of the surface of each disc with a density of grade 3 in the two analyzed stereocopolyamides. Optical microscopy inspection of sections cut from the discs revealed that the material has swollen by approximately 20% upon contact with the culture medium and that frequent cracking has evolved on the inner side of the samples. The optical micrograph recorded from a section of P6DM(D,L)T(1:4) invaded by *A. niger* is depicted in the inset of Fig. 5b. The grown fungic mycelium shown at the middle of the image is longitudinally viewed, whereas other hyphae oriented normal to the cutting plane are seen in the surroundings.

Invasion of the polymer bulk by mycelium was also made evident by scanning electron microscopy of free-fractured surfaces of discs that were broken after quenching. The presence of a hyphae that has penetrated through the matrix of a P6DM(D,L)T(1:1) is shown in Fig. 6. The ribbon-like appearance of the hyphae is due to the fact that no specific precautions to avoid collapse of the biological structures

were taken in the preparation of the sample. On the contrary, the micrograph vividly illustrates that an extensive alteration of the material has occurred upon degradation.

A point deserving special attention is to know whether colonization of the polyamide by *A. niger* entails the molecular degradation of the polymer in addition to mechanical erosion. This is not an easy task since chemical hydrolysis takes place simultaneously and removal of the biomass from the polymer is not straightforward. Nevertheless, GPC and DSC measurements were made on P6DM(D,L)T (1:1) samples before and after incubation in order to see changes taking place in molecular weight and crystallinity, and results are shown in Fig. 7. The GPC profile given by the colonized sample shows a bimodal distribution of molecular weights characteristic of a partially degraded material, whereas no differences were observed between the curves obtained for an initial polymer sample and for a control sample aged in the inoculate-free culture medium. In contrast, the DSC traces reveal a similar increase in crystallinity for both the control and the fungi cultivated samples when compared to the pristine material. The increase in crystallinity observed in the aged sample in absence of fungi is based on the results obtained in the chemical hydrolysis experiments described above (Fig. 4). This level of crystallinity does not increase further since no mass loss seems to occur upon fungal degradation.

The fact that fungal invasion is restricted to stereocopolymers containing considerable amounts of the two tartaric acid enantiomers, whereas no attack occurs on optically pure polyamides of either sign leads us to discard a stereospecific action of the microorganism. If hydrolytic degradation and water sorption results are taken into account, it can be reasonably concluded that the sensitivity of the material to fungal attack is determined by the accessibility of the amorphous phase to water. The mechanism may be envisioned as the one taking place by growth and penetration of hyphae in water-enriched zones with concomitant chemical degradation of the polymer. Hydrolytic degradation to leachable oligomers most likely takes place simultaneously giving rise to pores which facilitate fungal invasion inside the polymer mass.

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References

[1] Thiem J, Bachmann F. Trends Polym Sci 1994;2:425.
[2] Gonsalves KE, Mungara PM. Trends Polym Sci 1996;4:25.
[3] Molina I, Bueno M, Galbis JA. J Polym Sci, Chem Ed 1995;28:3766.
[4] Kiely DE, Chen L, Lin TH. J Am Chem Soc 1994;116:571.
[5] Bou JJ, Rodríguez-Galán A, Muñoz-Guerra S. Encyclopedia of polymeric materials, vol. 1. Boca Raton, FL: CRC Press, 1996. 561p.
[6] Rodríguez-Galán A, Bou JJ, Muñoz-Guerra S. J Polym Sci, Chem Ed 1992;30:713.
[7] Bou JJ, Rodríguez-Galán A, Muñoz-Guerra S. Macromolecules 1993;26:5664.
[8] Bou JJ, Iribarren I, Muñoz-Guerra S. Macromolecules 1996;27:5263.
[9] Bou JJ, Muñoz-Guerra S. Polymer 1995;36:181.
[10] Iribarren I, Alemán C, Bou JJ, Muñoz-Guerra S. Macromolecules 1996;29:4397.
[11] Ruíz-Donaire P, Bou JJ, Muñoz-Guerra S, Rodríguez-Galán A. J Appl Polym Sci 1995;58:41.
[12] Huang SJ, Ho LH, Huang MT, Koenig MF, Cameron JA. In: Doi Y, Fukuda K, editors. Biodegradable plastics and polymers. Amsterdam: Elsevier, 1994. p. 3.
[13] Regaño C, Martínez de Ilarduya A, Iribarren I, Rodríguez-Galán A, Galbis JA, Muñoz-Guerra S. Macromolecules 1996;29:8404.
[14] Iribarren I, Alemán C, Regaño C, Martínez de Ilarduya A, Bou JJ, Muñoz-Guerra S. Macromolecules 1996;29:8449.
[15] Saotome Y, Miyazawa T, Endo T. Chem Lett 1991;1:21.
[16] Doi Y, Kumagai Y, Tanashashi N, Mukai K. In: Vert M, Feijen J, Albertsonn A, Scott G, Chiellini E, editors. Biodegradable polymers and plastics. Cambridge: Royal Society of Chemistry, 1992. p. 139.
[17] Vert M, Torres A, Li SM, Roussos S, Garreau H. In: Doi Y, Fukuda K, editors. Biodegradable plastics and polymers. Amsterdam: Elsevier, 1994. p. 11.
[18] Torres A, Li SM, Roussos S, Vert M. J Appl Polym Sci 1996;62:2295.
[19] Timmis MR, Lenz RW, Hocking PJ, Marchessault RH, Fuller RC. Macromol Chem Phys 1996;197:1193.
[20] Okada M, Okada Y, Tao A, Aoki K. J Appl Polym Sci 1996;62:2257.
[21] DiBenedetto LJ, Huang SJ, Cameron JA. Proceedings of the VI international conference on polymers in medicine and surgery. London: The Plastic and Rubber Institute, 1989. p. 404.
[22] Bailey WJ, Okamoto Y, Kuo W-C, Narita T. In: Sharpley JM, Kaplan AM, editors. Proceedings of the third international biodegradable symposium. Barking, NY: Applied Science, 1976. p. 765.