Communications to the Editor

Degradation of Nonalternating Poly(ester–amides)

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Introduction. Aliphatic polyesters are an important class of biodegradable and hydrolyzable synthetic polymers.[1-5] A series of α-hydroxypolyesters, such as poly(glycolic acid) (PGA), poly(lactic acid) (PLA), and their copolymers, have been successfully used as bioabsorbable sutures and surgical implants.[1,3] Other types of polyesters,[4,5] such as poly(caprolactone), poly(β-propiolactone), and poly(butyrolactone), can also be potentially used as biodegradable polymers in drug delivery and agricultural uses.

On the other hand, commercial aliphatic polyamides (nylon 6 and nylon 66) are nonbiodegradable. The large concentration of hydrogen bonds and the high regularity of the polyamide structure are the possible reasons for the inertness of these nylons to biodegradation. On the basis of the above facts, poly(ester–amides) (PEAs) composed of esters and amides could potentially be a class of biodegradable polymers which would have better physical properties than polyesters.[6-11]

Recently, aliphatic poly(ester–amides) containing α-hydroxy or α-amino acid moieties have been studied for their potential biomedical applications. Helder and coworkers[6] studied the in vitro degradation of nonalternating glycine/DL-lactic acid copolymers. Barrows and coworkers[7,8] also synthesized a series of alternating poly(ester–amides) by using a two-step polycondensation reaction from glycolic acid, diamine, and diacoyl chloride and investigated the in vivo degradation of the copolymers for the purpose of developing new surgical implants. However, very few studies on the degradation of poly(ester–amides) have been reported. Tokiwa et al.[9,10] showed that poly(ester–amides), made by amide–ester interchange reactions of nylons and polycaprolactone, were degraded by lipase. To develop degradable materials for general applications, two types of nonalternating PEAs were synthesized by anionic[11] and interfacial[12] polymerizations. The hydrolysis (in buffer solution) and biodegradation (under attack of fungi) of these polymers were studied.

Experimental Section. Nylon 6 was obtained from Polysciences Inc. and low-density polyethylene (LDPE) from Union Carbide (No. 6201). 1,6-Hexanediamine was purified by vacuum sublimation. ε-Caprolactone, 1,6-hexanediol, and adipoyl chloride were distilled at reduced pressure. ε-Caprolactam was recrystallized from reagent-grade acetone three times and dried in vacuum. Alcohol-free chloroform was prepared by washing with distilled water three times, followed by drying with anhydrous $CaCl_2$ and distillation. Dry methanol was prepared from absolute-grade methanol by drying with potassium methoxide followed by distillation. The catalyst, sodiocaprolactam, was made by reacting ε-caprolactam with sodium methoxide in anhydrous methanol under argon. Excess methanol was distilled off and the residue heated under reduced pressure at 120–130 °C for 2 h to remove any remaining methanol and lactam.

The intrinsic viscosities of the polymers were measured in a 0.5 g/dL solution of 90% formic acid at 25 °C. The infrared spectra of the polymer films, cast from methanol or formic acid solutions, were recorded on a Nicolet 60SX FTIR spectrometer. ^1H NMR spectra were obtained on an IBM AF-270 NMR spectrometer (270 MHz), where CF_3COOD was used as a solvent. Thermal analysis was made using a Perkin-Elmer DSC-7 in N_2 at a heating rate of 20 °C/min. Molecular weights and molecular weight distributions were measured by using a Waters 150-C ALC/GPC equipped with μ-Styragel HT columns of 10^4-, 10^3-, and 10^3-Å pore sizes at a flow rate of 1 mL/min. *m*-Cresol and DMAC (dimethylacetamide) were used as solvents at 100 and 70 °C, respectively. Polystyrene narrow MW standards were used for calibration.

The polymer films for the biodegradation tests were prepared by molding at 10–20 °C beyond their melting points. The samples used for the tensile test were molded with a thickness of 0.7 mm and cut into dumbbell shapes with a width of 0.315 mm in the middle uniform portion. Tensile tests were made using an Instron Model 1101 at a speed of 1 in./min.

Poly(ester–amides) (PEA-I) were made by the anionic ring-opening copolymerization of ε-caprolactam and ε-caprolactone at 100–160 °C with sodiocaprolactam as a catalyst.[11a]

Interfacial poly(ester–amides) (PEA-II) were made by using a method similar to Castaldo et al.[12] A typical procedure is described as follows: 1,6-Hexanediol, 4.73 g (40 mmol), was added under argon to adipoyl chloride, 11.63 mL (80 mmol). While stirring, the mixture was heated to 80 °C and kept at this temperature for 1.5 h until the hydrogen chloride evolution ceased and then dissolved in 150 mL of chloroform. Under vigorous stirring, the solution was poured into a blender containing a solution of 1,6-hexanediamine, 4.65 g (40 mmol), and sodium hydroxide, 3.2 g (80 mmol), in 350 mL of water. The precipitated random copolymer RA50 (containing 50% amide and 50% ester) was washed with many portions of water and methanol and finally dried at 80 °C in vacuum overnight (yield: 13.70 g, 75.3%).

The hydrolysis of the copolymers was performed according to the following procedure: a piece of polymer film (0.7 mm thick, 100 mg) was placed in a vial containing 10 mL of a buffer solution (pH 7.4) with 0.03% sodium azide to inhibit bacterial growth.[6] The vial was maintained at a certain temperature (20, 37, or 55 °C), and the buffer solution was refreshed once the change in the pH was larger than 1.0.

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Table I
Compositions, Intrinsic Viscosities, and DSC Data of Copolymer Series I (PEA-I)

	sample[a]							
	PCL	AE25	AE45	AE55	AE70	AE80	AE90	nylon 6
amide content, %								
feed	0	25	45	55	70	80	90	100
found by NMR	0	18.0	31.8	44.3	56.7	70.0	87.7	100
$[\eta]$, dL/g		0.18	0.26	0.35	0.34	0.36	0.74	
T_m(DSC), °C	63.2	56.6	46.7	89.0	115.7	140.8	205.4	222.3
ΔH, J/g	100.1	82.1	27.3	24.9		36.4	43.6	50.0

[a] AE90 represents a lactam/lactone copolymer with a 90% lactam feed ratio.

Table II
Compositions, Intrinsic Viscosities, and DSC Data of Copolymer Series II (PEA-II)

	sample[a]		
	RA25	RA40	RA50
$[\eta]$, dL/g	1.09	1.30	0.57
diol %			
feed ratio	25	40	50
found by NMR[b]	42	46	66
DSC data			
ester transition			
T, °C	c		52.01
ΔH, J/g			19.20
amide transition			
T, °C	255.68	252.21	247.02
ΔH, J/g	41.34	15.64	12.88

[a] RA25 represents a random copolymer with adipoyl chloride with 25% diol. [b] Diol percents were calculated from the peak integration ratios of methylene groups next to –COO– and –CONH– groups. [c] The peak was too weak to be calculated.

Table III
Tensile Properties of PEA-I and PEA-II (25 °C)

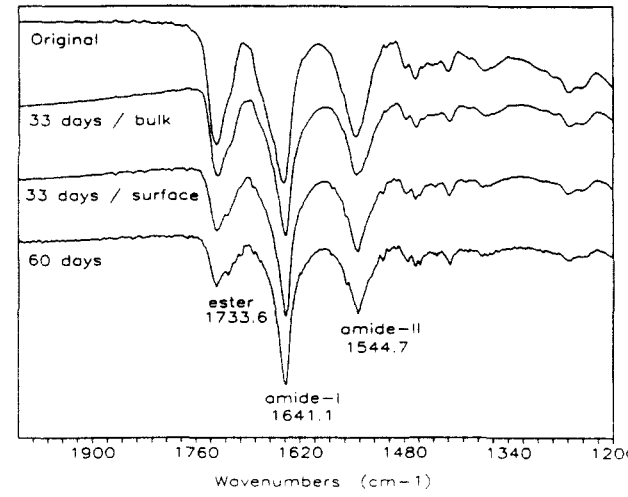
	sample						
	nylon 6	RA40	RA50	AE90	AE70	AE55	LDPE
amide % (NMR)	100	54.0	34.0	87.7	56.7	44.3	
strength, 10^3 psi							
at yield	10.0	3.26	2.09	7.28	2.60	1.60	1.45
at break	10.1	4.44	2.18	8.18	1.32	1.37	2.39
elongation, %	278	343	89	320	60	609	554

Biodegradation tests were conducted as follows: a piece of polymer film was placed on top of a layer of basal mineral salts (BMS) agar[13] and overlayed with a thin film of molten, tempered (50 °C) Sabouraud's dextrose agar. After the agar had hardened, the area over the sample was inoculated with a suspension of spores of either *Fusarium moniliforme* or *Aspergillus niger*, and the plates were incubated at 20 °C.

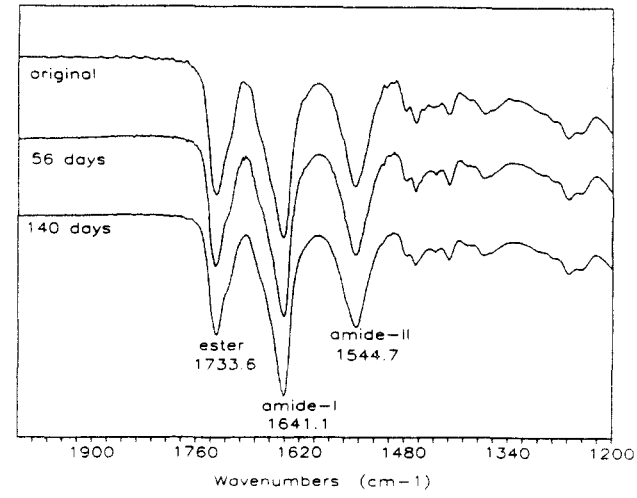
At various times, the samples were removed from buffer solutions or agar media, washed with distilled water, and dried in vacuum for 2 days. These samples were then examined by FTIR and GPC.

Results and Discussion. The compositions, intrinsic viscosities, and DSC data of PEA-I copolymers are shown in Table I. These types of PEAs possess a random microstructure, with chains composed of $-NH(CH_2)_5CO-$ (lactam) and $-O(CH_2)_5CO-$ (lactone) groups.[11] Each of the copolymers displayed a single melting point on DSC analysis. As seen in Table I, the thermal transitions of the copolymers showed a eutectic minimum at an amide content of about 45%. The crystallinities of the copolymers with 45–80% amide contents were significantly reduced as compared to those of nylon 6 and polycaprolactone, a trend which can be indicated from their lower ΔH values as observed in Table I.

Similarly, the compositions, η (intrinsic viscosity), and DSC data of PEA-II copolymers are shown in Table II.



a



b

Figure 1. Infrared spectra of AE70 (PEA-I) before and after degradation: (a) incubated with *Fusarium moniliforme*; (b) hydrolyzed in pH 7.4 buffer solution at 55 °C.

The intrinsic viscosities of these copolymers are moderately high. It can be observed that copolymers with a higher amide content (RA25 and RA40) possess only polyamide melt transitions, while a copolymer with a higher ester content (RA50) shows both polyamide and polyester transitions. These results are similar to those of Castaldo et al.[12] for analogous poly(ester–amides). It is interesting to note that PEA-II copolymers possess polyamide melt transitions, while the melt transitions of PEA-I (see Table I) and alternating poly(ester–amides)[7,12] fall between those of the corresponding polyamide and polyester.

The tensile properties of the PEA-I copolymers listed in Table III indicate that the mechanical strength of the copolymer increases with increasing lactam content. This suggests that the ester component in the copolymer acts

Table IV
GPC Data of AE70 Degraded by Fungi

fungus[a]	time, days	sample[b]	in *m*-cresol M_w	in *m*-cresol M_n	in *m*-cresol M_w/M_n	in DMAC M_w	in DMAC M_n	in DMAC M_w/M_n
	0		50 977	14 394	3.54	24 327	12 408	1.961
F	33	surface	44 696	13 976	3.21			
		bulk	42 440	14 347	2.96			
F	60	bulk	34 444	4 794	7.18	20 279	3 970	5.108
A	60	surface	43 714	10 657	4.10			
		bulk	44 804	12 354	3.63	24 338	12 907	1.886

[a] F is *Fusarium moniliforme*; A is *Aspergillus niger*. [b] Surface area means 0.2 mm in depth.

Table V
RA40 Degraded by *Fusarium moniliforme* and Hydrolysis

condition	time, days	M_w	M_n	M_w/M_n	wt loss, %
	0	130 192	17 321	7.517	0
F. moniliforme, 20 °C	50	59 892	9 924	6.035	25 ■ 5
pH 7.4, 37 °C	36	104 981	13 060	8.039	1.50
(hydrolysis)	165	60 578	9 257	6.544	7.25

Table VI
Weight Loss and GPC Data of AE70 before and after Hydrolysis

T, °C	time, days	wt loss, %	M_w	M_n	M_w/M_n
	0	0	24 327	12 408	1.961
20	164	1.91	24 053	12 537	1.918
	232	1.93	23 065	11 860	1.945
37	17	0.85	23 143	12 468	1.856
	165	2.40	20 939	11 939	1.754
55	22	1.20	20 125	10 817	1.860
	76	3.70	18 595	10 175	1.827
	140	10.2	11 561	6 847	1.688

as plasticizer reducing the strength of the copolymer.[11] AE55 showed almost the same yield tensile strength as LDPE. The others (AE70 and AE90) were stronger than LDPE but weaker than nylon 6. All showed reasonable elongation at break. A similar trend was observed for the mechanical properties of PEA-II, as seen in Table III.

Typical film samples, AE70 (0.7 mm thick) of PEA-I and RA40 (0.2 mm thick) of PEA-II, were subjected to biodegradation testing in solid agar media, in which *F. moniliforme* and *A. niger* were used as microorganisms. GPC analysis showed that both AE70 and RA40 were degraded by the attack of *F. moniliforme*, as indicated by the decrease of molecular weight in Tables IV and V. As seen from Table IV, AE70 can also be degraded by *A. niger*. However, *F. moniliforme* degraded this copolymer more efficiently than *A. niger*.

In the case of AE70 attacked by *F. moniliforme*, surface erosion was observed after 33 days of incubation at 20 °C, while the weight loss was negligible. After 60 days, a weak, porous sample was recovered and significant weight loss (30 ± 5%) was observed. GPC analysis in both *m*-cresol and DMAC showed a significant decrease of molecular weight after 60 days of incubation, as seen in Table IV. This indicated that the degradation had occurred not only on the surface but also in the bulk region after 60 days. For *A. niger*, only slight erosion occurred on the surface after incubation for 60 days; however, the bulk remained unaffected.

For differentiating between the abiotic and biotic degradation of the poly(ester–amides), hydrolysis studies of PEA-I and PEA-II were conducted in pH 7.4 buffer solutions at various temperatures. As observed in Table VI, no significant weight loss or molecular weight decrease was observed for AE70 (PEA-I) subjected to hydrolysis for 6–8 months at 20 and 37 °C. For hydrolysis at 55 °C, significant weight loss and an obvious decrease of molecular weight were only observed after 76 days (2.5 months). All the samples retained their original shapes and smooth surfaces, except for the sample hydrolyzed for 140 days at 55 °C on which cracks were observed.

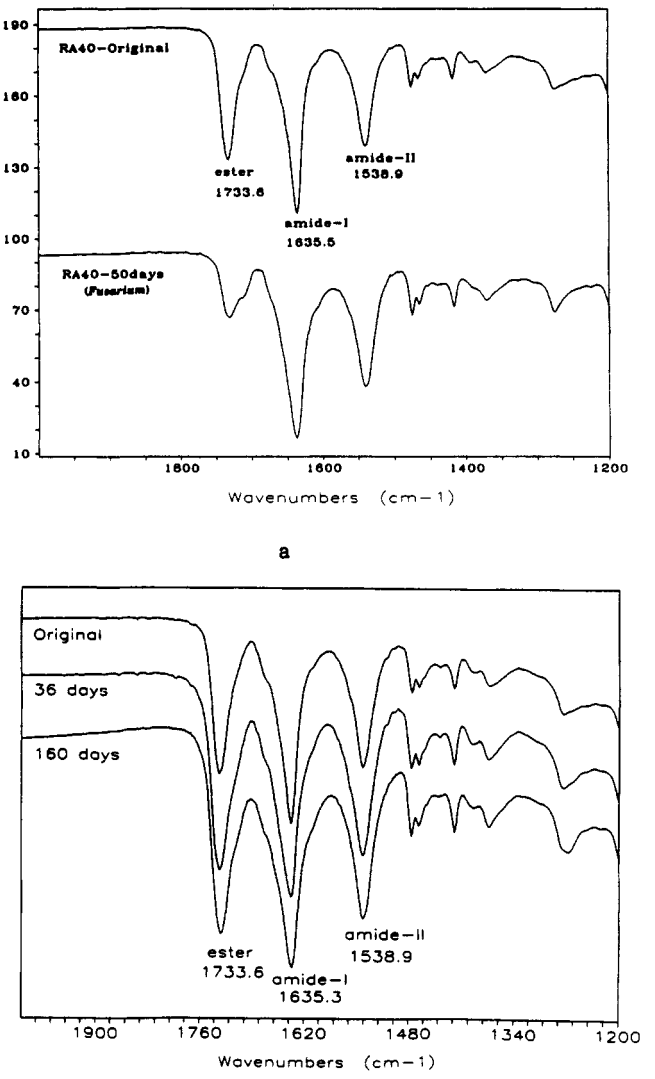


Figure 2. Infrared spectra of RA40 before and after degradation: (a) incubated with *Fusarium moniliforme*; (b) hydrolyzed in pH 7.4 buffer solution at 37 °C.

Similarly, the interfacially polymerized poly(ester–amide) RA40 (PEA-II) was degraded by *F. moniliforme*. The weight loss and GPC data of RA40 after biodegradation, compared with that after hydrolysis in pH 7.4 buffer solution at 37 °C, are shown in Table V. It can be clearly seen that the hydrolysis of RA40 is much slower than its degradation by *F. moniliforme*.

On the basis of the above results of hydrolysis studies, it appears that no appreciable degradation occurs under abiotic conditions for a considerable period at 20 °C. To

investigate the mechanisms of degradation of these poly(ester–amides), the samples were analyzed by FTIR spectroscopy for elucidating any structural changes after degradation. The infrared spectra of AE70 and RA40 after biodegradation compared with the spectra of the original samples, are shown in Figures 1a and 2a, respectively. On observing the carbonyl absorption region for RA40, it appears that after incubation with *F. moniliforme* for 50 days there is a significant decrease in the ester absorption intensity. This is further corroborated by a decrease in the molecular weight as M_w/M_n changed from 130 192/17 321 to 59 892/9924 (Table V). In the case of AE70 exposed to *F. moniliforme*, a corresponding significant decrease of the ester absorption intensity was observed after 60 days (Table IV), though the ongoing surface degradation could be discerned after 33 days. However, in all cases, the amide groups remained virtually unchanged, for there were no significant decreases in the intensities of both the amide-I and -II bands. The infrared spectra of AE70 and RA40, after hydrolysis in pH 7.4 at 55 and 37 °C, are shown in Figures 1b and 2b, respectively. No appreciable changes of peak shapes and ester peak heights were observed for both AE70 and RA40 after hydrolysis, although GPC measurements (Tables V and VI) indicated some extent of decrease in the molecular weights.

Thus, it can be concluded that, in the biodegradation of poly(ester–amides) by the attack of *F. moniliforme*, the breakdown of the ester bonds occurs, followed possibly by the utilization of the products by the microorganisms. However, the mechanism might be extremely complex, involving a series of enzymatic and nonenzymatic reactions. Further studies are underway to elucidate the degradation pathways.

Conclusions. Two types of poly(ester–amides) were synthesized by convenient methods for degradation studies. Both are degradable under attack of *F. moniliforme*. These copolymers are also degradable by hydrolysis in pH 7.4 buffer solutions, however, at much slower rates. Furthermore, under the biotic conditions of this work, the degradation of the poly(ester–amides) mainly involves the breakdown of the ester bonds.

References and Notes

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