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Hydrolytic and enzymatic degradation of copoly(ester amide)s based on L-tartaric and succinic acids

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Abstract

The hydrolytic and enzymatic degradation of a series of crystalline copoly(ester amide)s derived from L-tartaric and succinic acids, 1,6 hexanediamine and 1,6-hexanediol with ester/amide groups ratios 3/97, 10/90, 15/85 and 20/80 was investigated. The hydrolytic degradation study was carried out at 37°C in buffered solution at pH 7.4. Changes taking place in sample weight, molecular weight, chemical constitution and thermal properties of the polymer were evaluated. Degradation proceeded with a notable increment in crystallinity and entailing slight but significant changes in the T_g and T_m temperatures. It was found that copoly(ester amide)s degraded faster than the parent poly(hexamethylene-di-*O*-methyl-L-tartaramide) and that the rate of degradation increased with the content in ester groups. It was also showed that degradation is accompanied by formation of cyclic succinimide units indicative of a scission mechanism based on the occurrence of intramolecular imidation reactions. The enzymatic degradation of these copoly(ester amide)s with papain was comparatively examined for a preliminary evaluation of their potential biodegradability.

Keywords: Poly(ester amide)s; Copoly(ester amide)s hydrolytic degradation; Enzymatic degradation

1. Introduction

Poly(ester amide)s have been the object of extensive investigation during the last few decades. Initially, the main interest was focused on the technical potential of these materials because of their good fiber forming properties [1]. At this aim, ordered poly(ester amide)s having a regular distribution of ester/amide groups were preferentially investigated [2–5] whereas those others with a random constitution were examined only occasionally [6–8]. More recently, a renewed interest has emerged for poly(ester amide)s as promising biodegradable materials [9–11] since they are able to encompass the degradable character of polyesters with the good mechanical properties of polyamides.

Papers published on degradation of poly(ester amide)s by aqueous solutions usually describe the macroscopic changes occurring along the process like viscosity decay, sample weight loss and deterioration of mechanical properties whereas much less attention is paid to evaluate the chemical changes resulting from the hydrolytic reaction. Nevertheless, several works on this subject have recently appeared. Arvanitoyanis et al. [12] studied by NMR the chemical changes occurring during the degradation of a random oligo(ester amide) based on octadecanedioic acid, 1,6-hexanediamine and ϵ-caprolactone. Bueno et al. [13] examined the hydrolytic degradation of a group of poly(ester amide)s derived from carbohydrates. Villuendas et al. [14] reported in detail on the influence that the molecular structure exerts on the susceptibility towards hydrolysis of diverse poly(succinester amide)s and poly(tartarester amide)s.

This paper deals with the degradation undergone by a series of random copoly(ester amide)s made from di-*O*-methyl-L-tartaric acid, succinic acid, 1,6-hexanediamine and 1,6-hexanediol. These polymers are named P6STE$_x$A$_y$, where x and y indicate the percentage content in ester and amide bonds respectively and their representative chemical structure is shown in Fig. 1. According to the method of synthesis applied for the preparation of these copoly(ester amide)s, the ester groups are exclusively succinate units were the amide groups may be both succinamide or tartaramide units. A paper describing in detail the synthesis and characterization of P6STE$_x$A$_y$ and including a preliminary study of their hydrolytic degradation in pH 7.4 aqueous buffer at 37°C has been recently published. [15] The changes taking place in the mechanical properties of these copoly(ester amide)s upon hydrolytic degradation have been also examined [16].

The objective of the present study is to evaluate the effect

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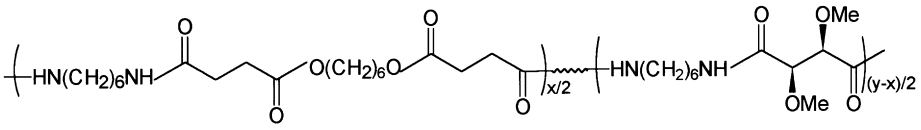

Fig. 1. Chemical structures of copoly(ester amide)s investigated in this work.

that the insertion of ester bonds in the main chain has on the degradability of polytartaramide P6DMLT. The crystal structure and properties of this polyamide have been previously investigated in great detail [17,18] and its degradation has been studied under a wide variety of conditions [16,19,20]. For such purpose a series of P6STE$_x$A$_y$ with contents in ester bonds ranging from 0 to 20% has been subjected to hydrolysis and the chemical changes resulting from degradation examined in detail by IR and NMR spectroscopies. A molecular mechanism is proposed on the basis of the experimental results obtained in this study, the which is supported by recent data published on the degradation of other related poly(ester amide)s derived from tartaric acid or succinic acids and aminoalkanols [14].

2. Experimental

Copoly(ester amide)s investigated in this work were prepared by polycondensation in solution of 2,3-di-*O*-methyl-L-tartaric acid, disuccinyl 1,6-hexanodiol and 1,6-hexanediamine conveniently activated. A detailed account of this synthesis has been published elsewhere [15]. Copolymers containing 3, 10, 15 and 20% of ester groups in addition to the parent polytartaramide P6DMLT were selected for this study in order to correlate composition with degradability. The characteristics of these polymers more relevant to the objectives of this work are summarized in Table 1.

2.1. Measurements

Thin films (100–250 μm) of polymers were prepared by casting from 8% (w/v) chloroform solutions at room temperature on a Petri dish. These films were systematically used to prepare the disks to be used for degradation assays. Infrared spectra were registered on a Perkin–Elmer 2000 FT-IR spectrometer from films casted onto KBr plates. Here ^{1}H and ^{13}C NMR spectroscopy was performed in $CDCl_3$ or $CDCl_3$-TFA-d_1 solutions using a Bruker AMX300 spectrometer operating at 300.13 and 70.48 MHz for ^{1}H and ^{13}C, respectively. Tetramethylsilane was used as internal reference. Compositions were calculated from the integrated area ratios of the proton signals at 2.49 and 4.21 ppm corresponding to the α-methylene units of succinic and tartaric moieties, respectively. Gel permeation chromatography was carried out on a system including pump (Model 510, Water Assoc.), two columns (μ-Styragel, Polymer Lab.) with exclusion limits 10^4 and 10^3 Å, respectively, and refraction index detector (RI 410, Water Assoc.). The chloroform:*o*-chlorophenol (95:5) mixture was used as mobile phase. The sample concentration was 0.25% w/v, the injected volume 50 μl and flow rate 0.5 ml/min. Molecular weights were estimated against polystyrene standards using a Maxima 820 computer program. The thermal behavior of the copoly(ester amide)s was studied at heating rates of 10°C/min by using a differential scanning calorimeter Perkin–Elmer DSC-4 equipped with a cooling accessory and calibrated with indium. Glass transition temperatures (T_g) were determined as the midpoint of the recorded step change in heat capacity and the melting points (T_m) were defined as the temperature of the maximum of the endothermic peaks.

2.2. Degradation experiments

For hydrolytic degradation the films were cut into disks 40–50 mg weight and approximate dimensions of 14 mm diameter and 200–220 μm thick. Degradation assays were carried out at 37 ± 0.1°C in 0.1 M phosphate buffer (pH 7.4) containing 0.03 wt.% sodium azide to prevent bacterial growth. Disks were placed in small bottles containing 30 ml of buffer and samples were periodically removed,

Table 1
Some characterization data of copoly(ester amide)s examined in this work (viscosity, chromathography and compositional data taken from Ref. [16])

Copoly(ester amide)	$[\eta]$ (dl/g)[a]	$\bar{M}_n$[b]	Ester/amide[c]	T_m[d]	T_g[d]	ΔH_m (cal/g)[d]
P6DMLT	2.72	29700	0/100	232	116	10.0
P6STE$_3$A$_{97}$	0.76	17400	3.1/96.9	231	195	8.8
P6STE$_{10}$A$_{90}$	0.75	12600	9.6/90.4	224	84	9.5
P6STE$_{15}$A$_{85}$	1.72	30700	15.2/84.8	203	75	5.0
P6STE$_{20}$A$_{80}$	0.73	11900	19.9/80.1	198	59	4.9

[a] Intrinsic viscosity measured in dichloroacetic acid at 25°C.
[b] Number-average molecular weight measured by GPC.
[c] Ester/amide ratio in the copolymer determined by ^{1}H-NMR.
[d] Measured by DSC on films prepared by casting from chloroform.

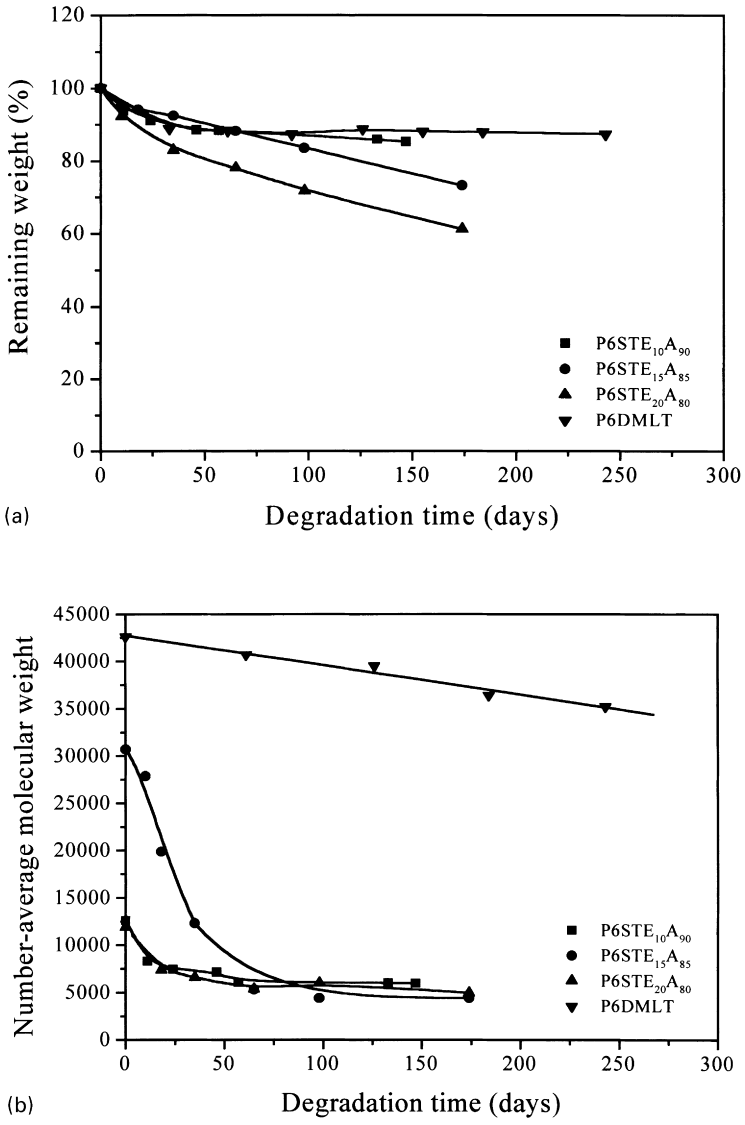

Fig. 2. Changes in sample weight (a), and number average molecular weight (b) during hydrolytic degradation of copoly(ester amide)s in phosphate buffer at pH 7.4 and 37°C.

washed with distilled water, and dried under vacuum to constant weight before analysis. The evolution of degradation was followed by sample weighting, GPC, IR and NMR spectroscopy and DSC.

For enzymatic degradation with papain, disks 15–20 mg weight and approximate dimensions of 12 mm diameter and 100–150 μm thick were cut from films. Papain (30 000 USP-U mg-1, No. 7144) was purchased from Merck and used without further purification. Disks were placed in small bottles containing 10 ml of the enzymatic medium consisting of a pH 8.0 buffered 0.05 M tris(hydroxymethyl)-aminomethane solution containing L-cysteine and papain (10 mg). The reaction solution was incubated for 18 days at 37 ± 0.1°C under gently shaking. Samples were periodically removed, washed with water and dried under vacuum to constant weight. Hydrolytic degradation was also monitored in parallel on replicates exposed to the same solution but in the absence of enzyme. The evolution of the degradation was followed by sample weighting and morphological observation under the optical microscope.

3. Results and discussion

3.1. Hydrolytic degradation

Changes in sample weight and molecular weight taking place upon degradation as a function of incubation time are plotted in Fig. 2 for three P6STE$_x$A$_y$ and the parent polyamide P6DMLT. For all copolymers the loss of mass took place since the very early stages of incubation (Fig. 2a) and continued steadily to end in a complete erosion of the polymer disk. The weight loss rate in the three copoly(ester amide)s was higher than in P6DMLT and such difference

Table 2
Changes in thermal properties of the $P6STE_xA_y$ with degradation

$P6STE_{10}A_{90}$				$P6STE_{15}A_{85}$				$P6STE_{20}A_{80}$			
Degradation days	T_m (°C)	T_g (°C)	ΔH_m (cal/g)	Degradation days	T_m (°C)	T_g (°C)	ΔH_m (cal/g)	Degradation days	T_m (°C)	T_g (°C)	ΔH_m (cal/g)
0	224	84	9.5	0	203	75	5.0	0	198	59	4.9
11	223	81	11.3	10	207	75	10.0	10	202	–	7.8
24	223	76	11.8	18	211	55	8.3	18	205	–	8.1
46	224	78	12.4	35	212	72	9.4	35	203	–	9.1
57	223	75	12.5	65	213	62	9.4	65	207	–	12.4
133	227	72	13.1	98	213	59	11.9	98	208	–	12.2
147	224	71	15.0	174	216	56	14.4	174	211	–	15.3

increased with the content of the polymer in ester groups. The GPC chromatograms revealed a continuous shift of the peaks towards longer elution times and a narrowing of the molecular weight distribution with the advance of degradation. Fig. 2b shows the decay in molecular weight as a function of time. The decrease in molecular weight starts early and continues rapidly during the first two months of incubation, after which a limiting value of about 5000 is reached. The fact that sample erosion in the last degradation stages proceeded with no perceivable changes in molecular weight may be rationalized by assuming the generation of soluble polymer fragments which pass into the solution.

3.2. Changes in thermal properties

Changes in the thermal properties of $P6STE_xA_y$ accompanying their hydrolytic degradation are shown in Table 2 and plotted in Fig. 3 for the particular case of $P6STE_{20}A_{80}$. Such data reveal that all copolymers experiment an important increase in the enthalpy of fusion (ΔH_m) with degradation. Increments of ΔH_m greater than 300% of the initial value could be observed for the case of $P6STE_xA_y$ after 147 days of incubation. The gradual increase of crystallinity with degradation is a well-known phenomenon usually observed in semicrystalline polymers. Degradation first occurs in amorphous domains giving rise to a highly crystalline material which is more resistant to hydrolysis than the initial one. On the other hand, both glass and melting temperatures show a slight but regular variation with time, which is more clearly perceivable for copoly(ester amide)s with higher contents in ester groups. Whereas T_m increases approaching to the melting temperature of polytartaramide P6DMLT, T_g decreases probably due to molecular weight effects.

3.3. Spectroscopic analysis: compositional changes and degradation mechanism

The IR and NMR spectra recorded from disks during the first days of incubation were practically indistinguishable from those obtained from the original polymers. However after 35 days of immersion appreciable differences started to be noticeable revealing significant changes in the composition and the chemical structure of the degraded copolymers.

Fig. 4 shows the 2.4–4.4 ppm region of ^1H NMR spectra of samples of $P6STE_{20}A_{80}$ subjected to increasing degradation times. All significant changes observed in the ^1H NMR spectra are included in this region. It is apparent that the

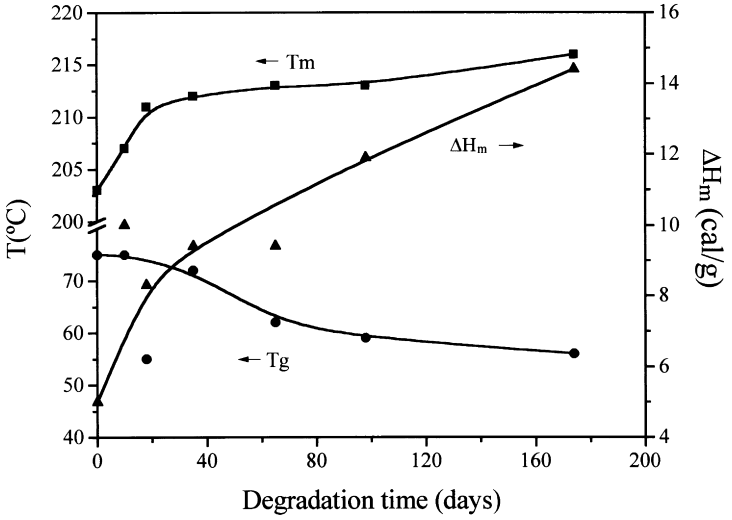

Fig. 3. Variation of T_m, T_g and ΔH_m of $P6STE_{20}A_{80}$ as a function of incubation time.

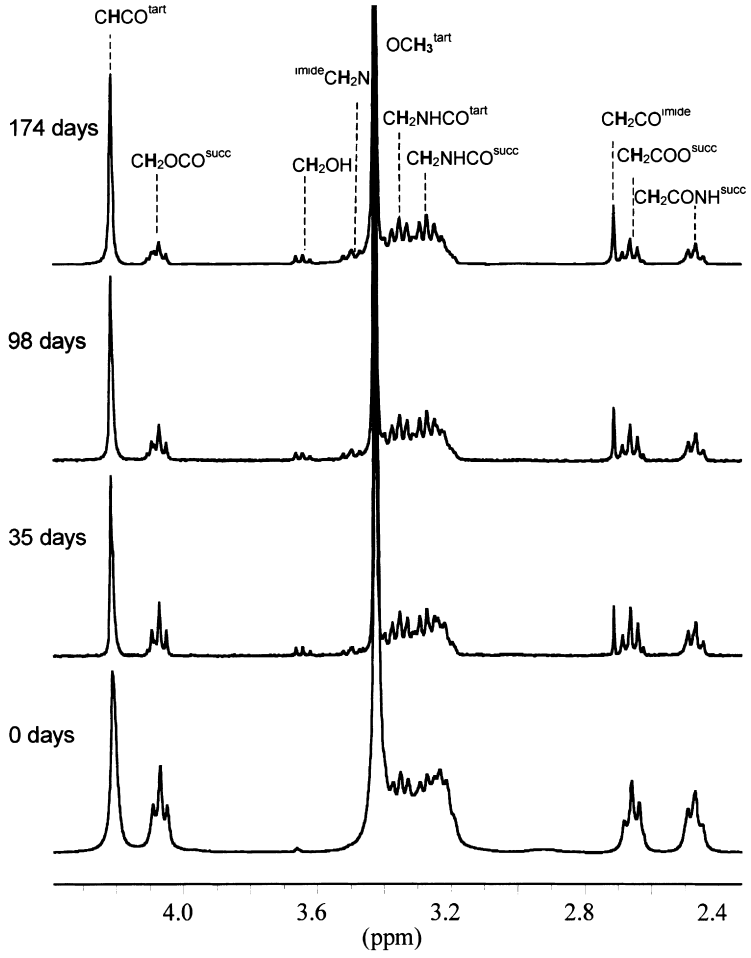

Fig. 4. ^{1}H NMR spectra of P6STE$_{20}$A$_{80}$ at different times of incubation in pH 7.4 phosphate buffer at 37°C.

intensity of the two triplets corresponding to the two methylenes of the succinic unit and the triplet corresponding to the α-methylene of the 1,6-hexanediol unit decreases with time. At the same time a new triplet appeared at 3.64 ppm which is attributable to the methylene adjacent to end hydroxymethyl groups emerging from the hydrolysis of the ester bonds. On the contrary, no changes are detected in the signals arising from the protons contained in the tartaric

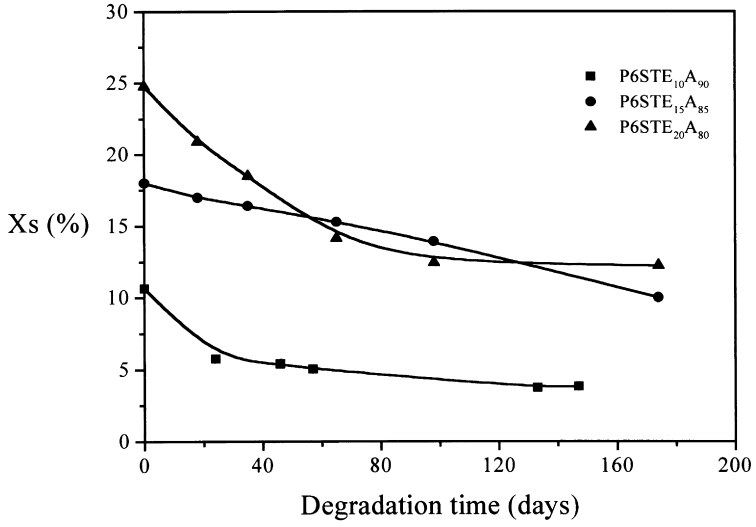

Fig. 5. Variation of the content of P6STE$_x$A$_y$ in succinic units (Xs) with degradation.

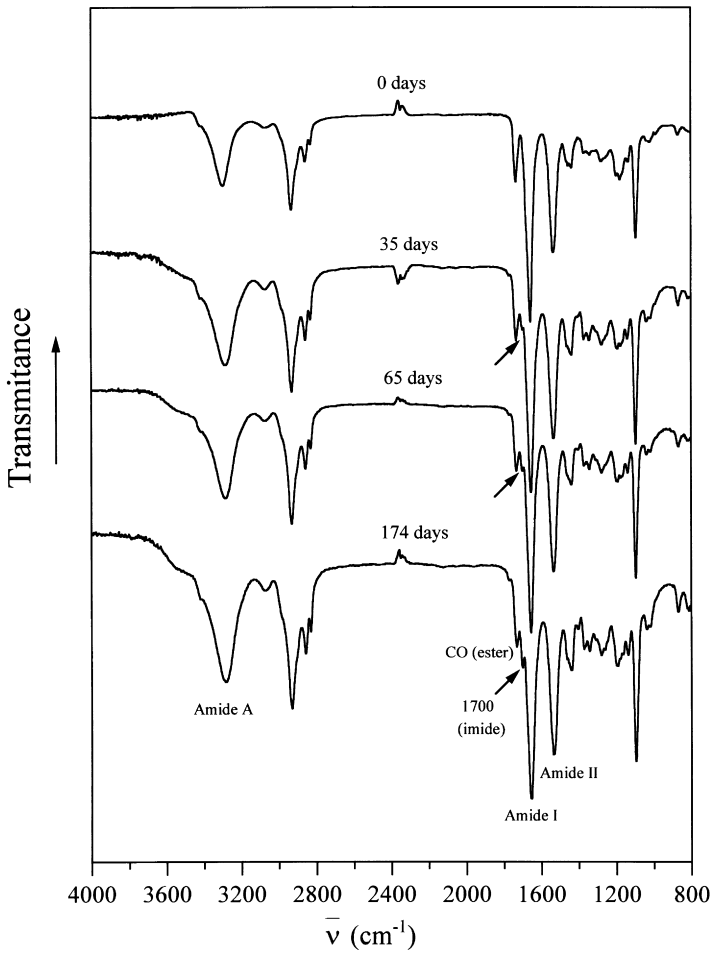

Fig. 6. IR spectra of $P6STE_{20}A_{80}$ at different times of incubation in pH 7.4 phosphate buffer at 37°C.

moieties revealing that neither the methoxy side groups nor the tartaramide bonds are involved in the degradation process. Changes in the composition of $P6STE_xA_y$ with degradation are plotted in Fig. 5 for three different contents in ester groups. In the three cases the content of the remaining sample, in succinic units (Xs), steadily decreases with time reaching a final value more or less constant and consistent with the initial composition of the copoly(ester amide).

Other changes in the chemical structure related with the degradation mechanism were evidenced by spectroscopy. In the IR spectra of degraded polymers a weak peak at 1700 cm^{-1} characteristic of the carbonyl stretching vibration of succinimide ring appears with increasing intensity whereas no other changes were apparent in the spectra (Fig. 6). Such observation is fully consistent with NMR results. In fact two weak signals, a singlet at 2.71 ppm and a triplet at 3.50 ppm attributable to the methylenes in the succinimide ring and to the methylene attached to the succinimide nitrogen are observed with increasing intensity in the ^1H NMR spectra shown in Fig. 4. Furthermore, the ^1H NMR spectra of the residue resulting from the evaporation of the mother solution after 174 days of incubation contains also the singlet at 2.71 ppm characteristic of succinimide units whereas no signals associated to succinamide groups could

Table 3
Chemical shifts of ^1H and ^{13}C NMR of degraded $P6STE_xA_y$

	Succinimide ending fragments $(CH_2CO)_2N–CH_2^{\alpha}–CH_2^{\beta}–CH_2^{\gamma}–CH_2^{\gamma'}–CH_2^{\beta'}–CH_2^{\alpha'}–NHCO–$								Hydroxyl ending fragment $HOCH_2–CH_2^{\alpha}–CH_2^{\beta}–CH_2^{\gamma}–CH_2^{\gamma'}–CH_2^{\beta'}–CH_2^{\alpha'}–OCO–$						
	CO^{imide}	CH_2^{imide}	CH_2^{α}	CH_2^{β}	CH_2^{γ}	$CH_2^{\gamma'}$	$CH_2^{\beta'}$	$CH_2^{\alpha'}$	CH_2^{α}	CH_2^{β}	CH_2^{γ}	$CH_2^{\gamma'}$	$CH_2^{\beta'}$	$CH_2^{\alpha'}$	OCO
^1H NMR	–	2.71	3.50	1.55	1.39	1.39	1.55	3.29	3.64	1.65	1.39	1.39	1.65	4.1	–
^{13}C NMR	177.35	28.18	38.65	27.57	26.24	26.24	29.62, 29.53[a]	39.32, 39.05[a]	62.59	32.55	25.68	25.33	28.49	64.74	173.18

[a] Two peaks corresponding to succinamide or tartaramide units.

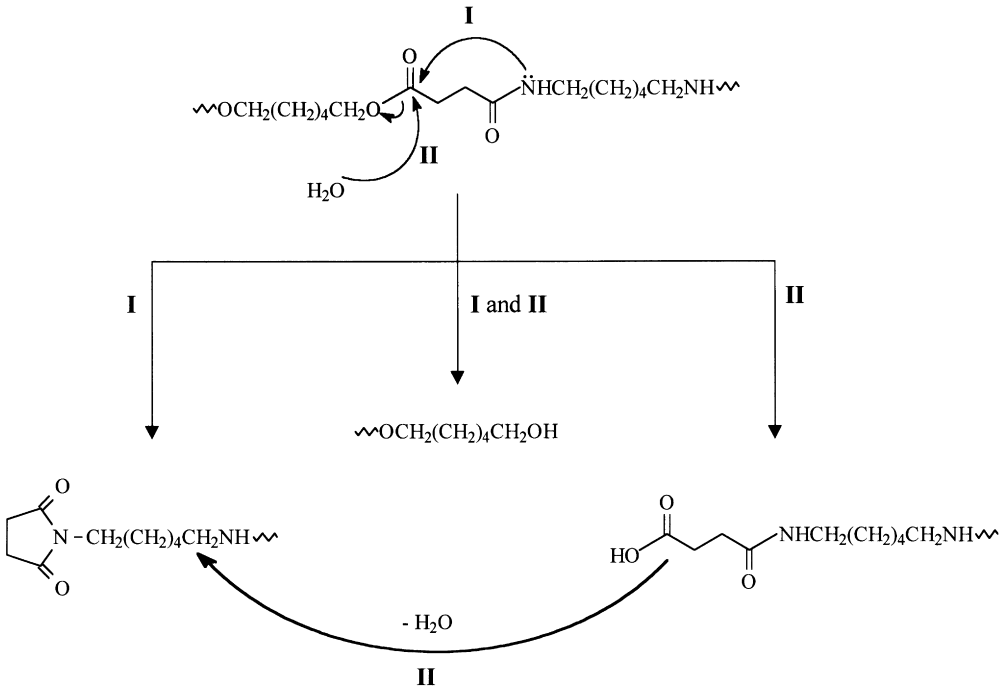

Fig. 7. Possible mechanisms of degradation of copoly(ester amide)s: I. One-step mechanism (amidolysis); II: Two-steps mechanism (hydrolysis followed by imidation).

be detected. These results were confirmed by ^{13}C NMR spectra; peaks at 28.18 and 177.3 ppm attributable to the resonance of the methylene and carbonyl carbons of the succinimide ring respectively were present whereas no traces of carboxylic acid end groups could be observed. Chemical shifts of ^{1}H and ^{13}C NMR spectra of the fragment resulting from the hydrolytic degradation of P6STE$_x$A$_y$ are given in Table 3.

The above results indicate that chain scission must occur preferentially by breaking of the ester linkages as revealed by the disappearance of the succinic units and simultaneous appearance of hydroxymethyl end groups. However since no signs of carboxylic terminal groups were found and succinimide end rings were observed instead, a degradation mechanism implying the occurrence of imidation reactions has to be considered.

By analogy with the mechanism described earlier by us for the hydrolytic degradation of poly(tartarester amide)s [14], scission of P6STE$_x$A$_y$ chains can be thought to occur as a consequence of the intramolecular nucleophilic attack of the amide group onto the neighboring ester linkage leading directly to the imide ring (Fig. 7, pathway I). Accordingly cyclization would be a driving factor for degradation and the remarked degradability of these copoly(ester amide)s will be due not only to the presence of ester groups in the backbone but also to the strategic position of such groups with respect to the amide groups. A second interpretation of the chemical process can be made on the basis of the occurrence of a two-step reaction mechanism (Fig. 7, pathway II). The first step would be the hydrolysis of the ester group and the second one would imply the cyclization of the resulting β-amidocarboxylic acid to form the five-membered succinimide ring. This mechanism is unable however to explain the high degradability displayed by these poly(ester amide)s when compared to other those containing a higher content of ester groups but arranged in such a manner that generation of stable five-membered imide rings is unfeasible [14].

Table 4
Remaining weight after enzymatic degradation with papain

Degradation days	P6STE$_3$A$_{97}$		P6STE$_{10}$A$_{90}$		P6STE$_{20}$A$_{80}$	
	Papain	Control	Papain	Control	Papain	Control
0	100	100	100	100	100	100
6	91.9	90.2	87.1	91.1	79.5	85.0
12	93.0	–	74.3	–	–	–
13	–	–	–	–	21.9	81.3
15	–	89.7	–	87	–	–
18	90.3	–	67.5	–	22.4	77.4

3.4. Enzymatic degradation

The enzymatic degradation of P6STE$_x$A$_y$ with papain was carried out for a period of 18 days. Three copoly(ester amide)s namely P6STE$_3$A$_{97}$, P6STE$_{10}$A$_{90}$ and P6STE$_{20}$A$_{80}$ were subjected to treatment in order to achieve a reliable assessment of the influence of composition on biodegradation. The optical microscopy showed apparent erosion on surface of the degraded disks in all cases. Weight losses taking place upon degradation of the three copoly(ester amide)s with and without the concourse of the enzyme are

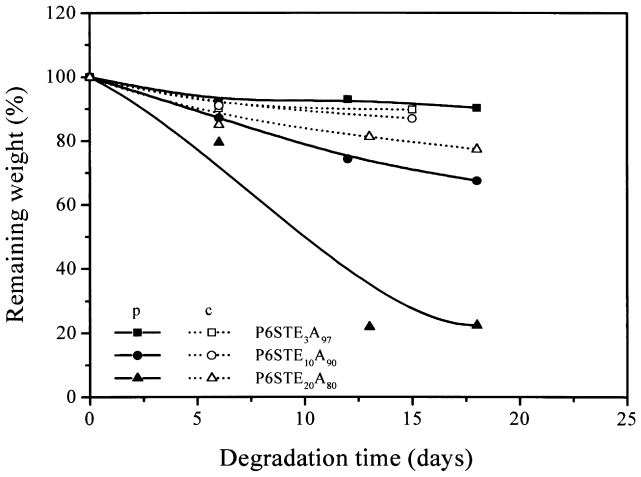

Fig. 8. Weight changes of three representative copoly(ester amide)s during enzymatic degradation with papain.(p: papain, c: control).

collected in Table 4 and data are represented in Fig. 8 for a more vivid comparison. All copolymers show rapid enzymatic degradation with the remaining weight decreasing steadily with time. The highest biodegradation rate was found for $P6STE_{20}A_{80}$ which underwent a weight loss of about 78% but only 20% in the absence of enzyme. On the other hand $P6STE_3A_{97}$ appeared to be scarcely sensitive to the papain action showing a weight loss of less than 10% after incubation whichever the enzyme is present or not. These results indicate therefore that these copoly(ester amide)s are biodegradable and that biodegradability is strongly dependent upon their content in ester groups.

The ^{1}H NMR analysis of copoly(ester amide)s degraded with papain gave results which are worthy to compare with those obtained in the analysis of the same copolymers subjected to degradation in the absence of enzyme. Although the peaks characteristic of imide units were also present in this case, the integrated area ratio of $OC-CH_2$ to $HO-CH_2$ peak areas is significantly lower. This is consistent with a reaction mechanism based on the cleavage of the ester bonds by the action of water, as it should be expected from an enzyme-mediated degradation. The presence of imides would result therefore from amidolysis taking place both before and after ester hydrolysis according to the two mechanisms depicted in Fig. 7.

4. Conclusions

The degradation study carried out in this work revealed that copoly(ester amide)s $P6STE_xA_y$ are much more degradable than their parent polytartaramide P6DMLT and that their degradability increases with the content in ester groups. The process entails a diminution in the molecular weight and a considerable increase in crystallinity. Results obtained in the spectroscopy analysis of degraded products supported a degradation mechanism involving intramolecular amidolysis with subsequent formation of succinimide units. Results obtained in the degradation with papain demonstrated the biodegradability of these polymers and its strong dependence on the content in ester groups.

Acknowledgements

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