Structure–property relationships in the case of the degradation of massive aliphatic poly-(α-hydroxy acids) in aqueous media,

Part 1: *Poly(DL-lactic acid)*

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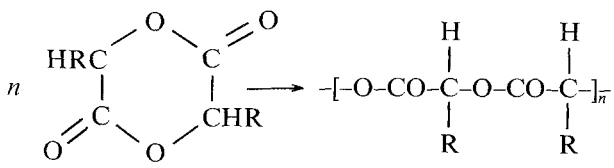
A standard protocol is proposed which has been used to study comparatively the degradation mechanism of bioresorbable poly(α-hydroxy acids) with respect to macromolecular structural characteristics and solid-state morphologies. As a first approach, the hydrolytic degradation of poly(DL-lactic acid) (PLA50) parallelepipedic specimens (15 mm × 10 mm × 2 mm), processed by compression moulding and machining, was investigated in two aqueous media: iso-osmolar saline and pH 7.4 phosphate buffer. Various techniques (namely weighing, size-exclusion chromatography (SEC), potentiometry, cryometry and enzymatic assay) have been applied to these specimens in order to monitor the degradation. Data show conclusively that the degradation of massive PLA50 specimens proceeds more rapidly in the centre than at the surface. This feature has been related to the formation of an outer layer of slowly degrading polymer, which is caused by surface phenomena and entraps degrading macromolecules. Only oligomers can diffuse and dissolve in the surrounding media. Accordingly, the number of carboxylic groups present in the inner part of the degrading specimens becomes larger than at the surface and accelerates ester bond cleavage. The resultant autocatalytic mechanism explains well the fact that partially degraded PLA50 exhibits bimodal SEC chromatograms although this polymer is amorphous.

1. Introduction

Bioresorbable poly(α-hydroxy acids) are polyesters derived from α-hydroxy acids, HO–CHR–COOH, especially glycolic (GA, R = H) and lactic (LA, R = CH_3) acids, the latter being a chiral compound and thus existing under two enantiomeric forms, namely L and D (or *S* and *R* as referred to absolute configurations). These two isomers have similar intrinsic chemical properties but opposite configurational structures.

High molecular weight poly(α-hydroxy acids) can be obtained by ring-opening polymerization of cyclic diesters only [1], according to the equation



(R = H, glycolide; R = CH_3, lactide)

Because of the chirality of lactic repeating units and of the possibility to make GA–LA copolymers, a large number of polymers can be made which are based on LA and GA repeating units. Acronyms are used to identify the composition of polymer chains, which is usually close to that of the feed [2]. A key of these acronyms is given in Table I.

It is now well established that LA–GA polymers are of great interest in the field of human therapy for various reasons which include outstanding biocompatibility, bioresorbability and the fact that wide ranges of physical, thermal, mechanical and biological properties can be covered by varying the chemical and configurational structures in the polyester chains [3]. PGA and LA–GA copolymers with low content of LA units were first introduced and developed as bioresorbable synthetic sutures in the 1960s and 1970s [4]. Homopolymers, copolymers and stereocopolymers of lactic and glycolic acids are currently being investigated in several countries with respect to applications as temporary aids in bone surgery (osteosynthesis and bone reconstruction) and for the design of drug delivery systems [3]. As far as degradation is concerned, it has been shown conclusively that non-toxic products are formed *in vivo* which are easily eliminated from the body [5]. At the moment, degradation of poly(α-hydroxy acids) is increasingly regarded as depending on chemical hydrolysis only, although

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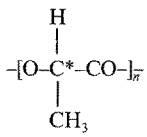
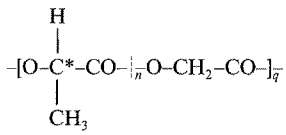
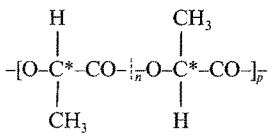
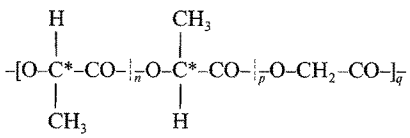
TABLE I Poly(α-hydroxy acids) derived from lactide and/or glycolide

Structure	Acronym
$-[O-CH_2-CO-]_n-$	PGA poly(glycolic acid)
	PLA100 poly(L-lactic acid)
	PLA (100-Y) GA Y ($Y = q/(n + q)$) L-LA–GA copolymers
	PLA X ($X = 100n/(n + p)$) (stereocopolymers)
	PLA X GA Y ($X = 100n/(n + p + q)$) ($Y = 100q/(n + p + q)$) (terpolymers)

enzymatic contribution to *in vivo* degradation is still questioned from time to time [6, 7].

Degradation of aliphatic polyesters has been investigated by many authors, both *in vitro* and *in vivo* [7, 8]. However, data have been generally collected from experiments carried out with compounds of different origins, for devices of different natures, shapes and sizes, and at different implantation sites including different tissues and animals. The difficulties of characterizing LA–GA-containing polyester chains were pointed out for the first time in 1981 when *in vivo* degradation of well-defined LA stereocopolymers was monitored by size-exclusion chromatography (SEC) [2]. One of the striking features was that chromatograms, which were monomodal at the beginning with rather narrow polydispersity, became bimodal as degradation proceeded. Although the number of papers reporting SEC analyses during the degradation of aliphatic polyesters is surprisingly small, several reports of bimodal chromatograms have been done in the case of *in vitro* degradation of various poly(α-hydroxy acids) [9–11]. The bimodal feature was generally related to semicrystallinity and assigned to the difference in degradation rates in amorphous and crystalline microdomains, hydrolysis being faster in amorphous than in crystalline microdomains [9]. This interpretation was acceptable for copolymers and stereocopolymers known as semicrystalline. However, it was unsuitable for totally amorphous devices made of non-stereoregular and non-crystallizable LA-GA copolymers or LA stereocopolymers [11].

In order to understand better the degradation mechanisms of solid-state devices derived from LA–GA polymers with respect to macromolecular structural parameters (gross composition and stereosequence distribution), and to define a trustable method to evaluate and compare their behaviours *in vitro*, we have undertaken, in the past 4 years, detailed investigations of the degradation of poly(α-hydroxy acids) having different chemical and physical characteristics. Our approach was based on two unusual principles as far as the literature is concerned: (1) using well-defined specimens and aqueous ageing media and (2) monitoring the ageing by various techniques applied to specimens with the same history.

In this first paper, we present the protocol that we selected and the results obtained in the particular case of intrinsically amorphous racemic poly(DL-lactic acid) (PLA50), allowed to age in two different aqueous media, namely iso-osmolar saline and pH 7.4 phosphate buffer. The latter was selected because it provides both physiological ionic strength and buffered pH. In contrast, saline was selected to detect the effect of pH changes on ageing, as it provides physiological ionic strength without buffering effect.

2. Experimental

The polymer was obtained by bulk ring-opening polymerization of DL-lactide at 145° C using zinc powder as the initiator with further removal of low molecular weight residual compounds by the dissolution–precipitation method [12]. The solid polymer was first moulded as round plates (75 mm diameter × 2 mm thickness) by compression moulding. Parallelepipedic specimens (15 mm × 10 mm × 2 mm) were machined from the round plates thus obtained.

The specimens were weighed and then placed on the three floors of a glass holder immersed in the aqueous medium contained in a 1 litre vessel. For each degradation time, three specimens were recovered, washed three times with distilled water, weighed and vacuum-dried for 1 week at room temperature before being subjected to the various analyses.

The degraded specimens were first examined visually. Photographs of their surfaces and cross-sections

were taken with a 24 × 36 photo-apparatus adapted for macroscopic view.

Weight loss (WL) and water absorption (WA) were evaluated by weighing. The former was deduced from the relationship $WL\% = 100(W_0 - W_t)/W_0$, where W_0 and W_t are, respectively, the initial weight and the residual weight of the same specimen; the water absorption was deduced from the relationship $WA\% = 100(W_s - W_t)/W_t$, where W_s is the weight of the swollen specimen after wiping the surface with paper.

L-Lactic acid was assayed by using enzymatic titration kits from Boehringer. The absorbance measurements were made at 340 nm with a spectrometer UV/VIS Lambda 15 Perkin–Elmer. The pH of solutions was measured at 25° C, using an Tacussel Ionoprocessor-II. Osmolarity changes were obtained with a Roebling osmolarimeter based on cryoscopic phenomena. SEC chromatograms were obtained with a Waters apparatus equipped with μ-styragel columns. The mobile phase was dioxane and data were expressed with respect to polystyrene standards.

3. Results and discussion

3.1. Standard protocol of investigation

To avoid discrepancies due to uncontrolled sampling or experimentation, we tried to standardize our investigations, To do this, we retained the principle of using specimens fabricated and processed similarly and allowed to age simultaneously at 37° C in aqueous media taken as a model of biological fluids. Distilled water, iso-osmolar saline and pH 7.4 phosphate buffer were selected as suitable to detect the effects of pH and ionic strength by comparison. The specimens were given a parallelepipedic shape with sharp edges, as no biocompatibility evaluation was planned. The dimensions (2 mm × 10 mm × 15 mm) were selected to allow the monitoring of ageing consequences by various techniques including weighing (water absorption and weight loss), SEC (molecular weight and polydispersity), differential scanning calorimetry (DSC) and X-ray scattering (crystallinity), potentiometry and cryometry and enzymatic assay (pH changes and L-lactic acid release), and viscoelasticimetry (mechanical properties).

Data collection was achieved from identified specimens, allowed to age at 37° C in the aqueous media for periods as long as 2 years in order to include the case of long-lasting PLA100, the most stable polymer of the LA–GA series [3]. The principle of data averaging was also selected when necessary to ensure data consistency. When it was acceptable, the same specimen was split into parts to perform measurements with different techniques in order to limit discrepancies related to processing history. Altogether, up to 30 specimens for each polymer were evaluated. Specimens were aged without stirring, since body fluids move slowly in smooth or hard tissues. Accordingly, these specimens were placed in vessels containing three-floor glass holders immersed in the ageing medium, the floors being made of pieces of polyethylene mesh (Fig. 1). Only one vessel was used to accommodate all the specimens of a given polymer. All of the vessels containing the various polymers were accommodated in the same water bath thermostated at 37° C. Several sets of specimens derived from different polymers were thus allowed to age simultaneously.

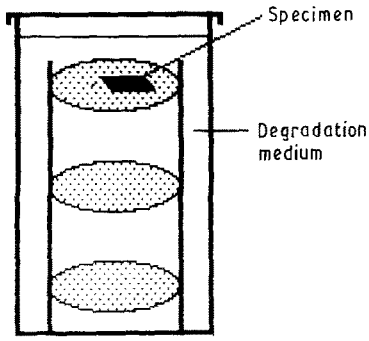


Figure 1 Schematic diagram of the apparatus designed for studying the hydrolytic degradation of poly(α-hydroxy acids) in aqueous media.

3.2. Fate of poly(DL-lactic acid)

The examination of the change of various parameters from a series of similar specimens allowed to age simultaneously has been compiled and the data have been grouped in order to make comparisons easier. For the sake of clarity, the two ageing media selected for the case of PLA50 are discussed separately.

3.3. Ageing in saline

3.3.1. Visual examination

In saline, PLA50 specimens became progressively whitish and exhibited irregular deformations in spite of the absence of mechanical stress. At week 3 the specimens revealed heterogeneous cross-sections after breaking. A whitish outer layer appeared, whereas the inner part was yellowish and transparent. At week 5 the inner part of specimens appeared as a very viscous liquid, whereas the outer layer was still rigid (Fig. 2). At week 12 most of the inner viscous liquid disappeared, whereas the outer layer remained and appeared as a flattened empty shell. No difference was detected between the large surfaces, which had been at the contact of the mould walls, and the small machined surfaces. This finding showed that the formation of the outer layer was not related to the processing history.

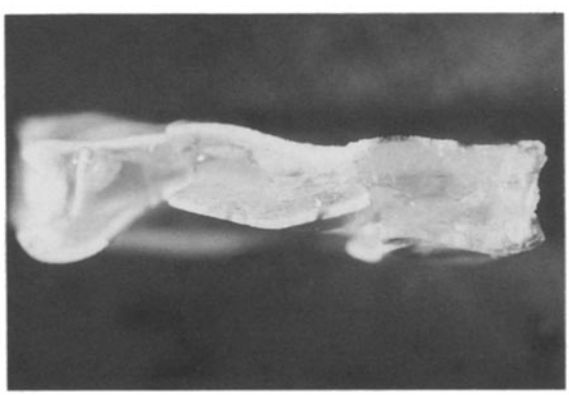


Figure 2 Cross-section of a PLA50 specimen degraded for 5 weeks in saline.

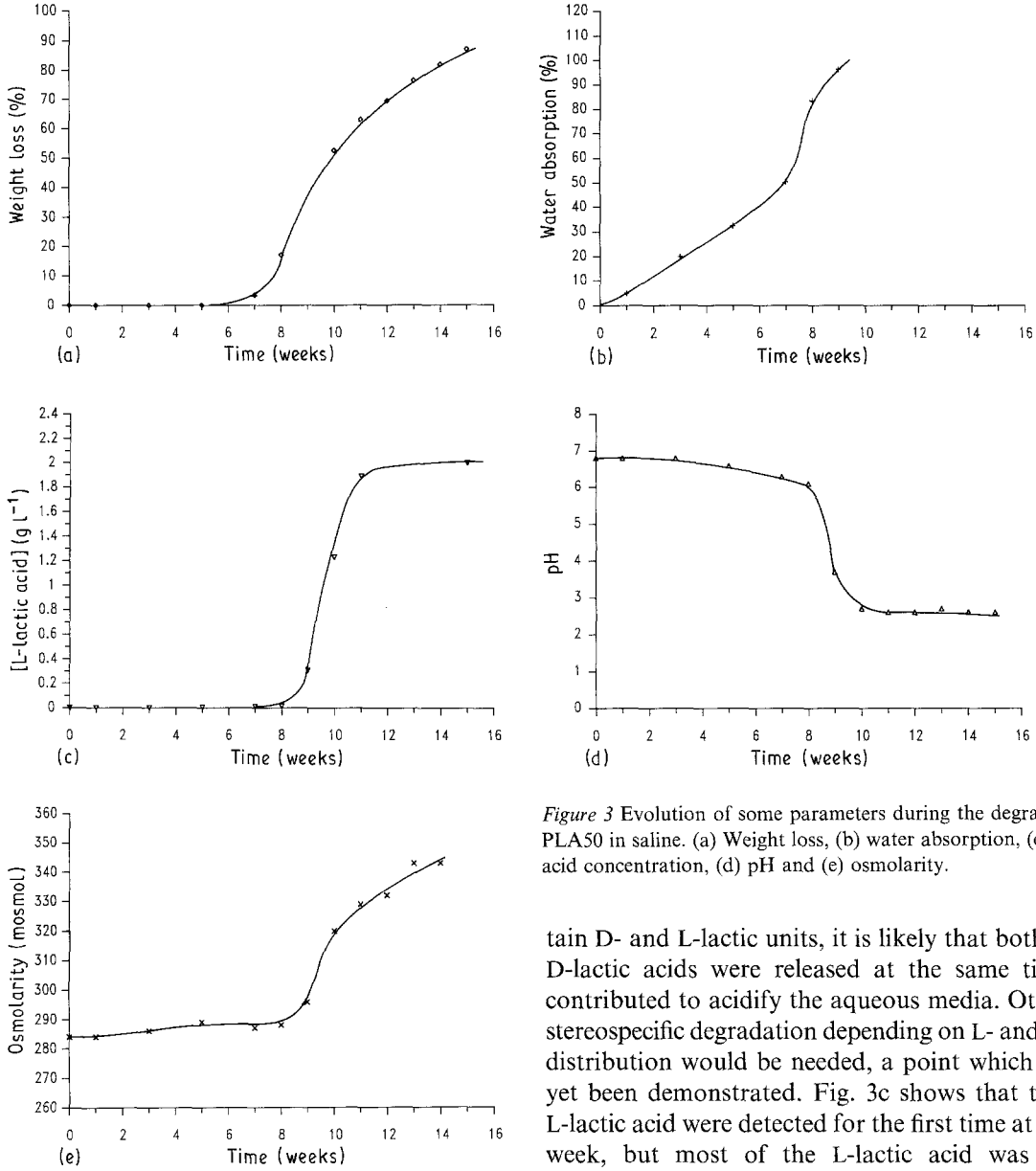


Figure 3 Evolution of some parameters during the degradation of PLA50 in saline. (a) Weight loss, (b) water absorption, (c) L-lactic acid concentration, (d) pH and (e) osmolarity.

3.3.2. *Weight loss (Fig. 3a)*

During the first 5 weeks the weight of specimens remained unchanged in spite of the change of aspect mentioned above. The onset of weight loss was detected at week 7. The increasing weight loss was observed up to week 15 and showed regular increase up to 90% of the initial weight, the whole variation being typically sigmoidal.

3.3.3. *Water absorption (Fig. 3b)*

As the weight loss was known, water absorption was evaluated with respect to the remaining weight. Water absorption was detected just after immersion in saline and increased linearly until the seventh week. Then the rate of water absorption increased significantly to reach 100% at the ninth week. After that, weighing the degraded specimens became problematic because of the fragility of the residual structures.

3.3.4. *Release of L-lactic acid (Fig. 3c)*

Detection of the release of L-lactic acid was achieved by using an enzymatic assay which detected L-lactic acid only. However, as PLA5O polymer chains contain D- and L-lactic units, it is likely that both L- and D-lactic acids were released at the same time and contributed to acidify the aqueous media. Otherwise, stereospecific degradation depending on L- and D-units distribution would be needed, a point which has not yet been demonstrated. Fig. 3c shows that traces of L-lactic acid were detected for the first time at the fifth week, but most of the L-lactic acid was formed between the eighth and the 11th weeks, in agreement with weight loss. Compared with pH and osmolarity changes, this finding shows that water-soluble acidic compounds were released in very low amounts between weeks 3 and 7, whereas fast apparition of lactic acid occurred beyond week 8, as confirmed by osmolarity changes.

3.3.5. *pH variations (Fig. 3d)*

Saline being unbuffered, its pH was considered a good parameter to monitor the release of acidic compounds from the whole series of specimens. For the first 5 weeks the pH remained constant, whereas in the period from week 8 to week 10 a dramatic drop to 2.6 was observed.

3.3.6. *Osmolarity changes (Fig. 3e)*

Osmolarity changes were used to monitor the release of both non-ionic and ionic species, regardless of the molecular weight. Initially fixed at 285 mosmol (the physiological value), osmolarity remained constant until the seventh week and then increased rapidly in the period from week 8 to week 12. It was not possible to correlate the release of water-soluble acid products with osmolarity changes and with weight loss, because

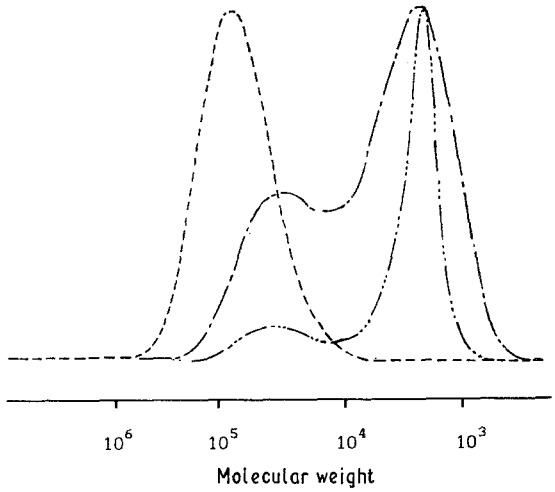


Figure 4 SEC chromatograms of PLA50 after (– – –) 0, (– · –) 7, (– · · · –) 15 weeks in saline.

specimens were withdrawn from the vessel during the experimentation, making correlation between weight and concentration problematic. However, it can be said qualitatively that chemical species were released early during ageing, since a slight increase of osmolarity was detected in the period from week 3 to week 7 before the dramatic increase observed from week 8 to week 13. A similar release of soluble oligomers and the existence of an equilibrium with lactic acid were discussed recently in a convincing manner [13].

3.3.7. Loss of impact strength

The loss of impact strength appeared much earlier than the loss of weight, as already pointed out by many authors. Only relative data were obtained, as the shape of the specimens was not preserved. Nevertheless, we were able to conclude that the impact strength increased slightly during the first week. This increase was probably related to the plasticizing effect of the absorbed water. After 3 weeks swelling and deformation were too important to allow significant evaluations in spite of the retention of some rigidity after drying. Beyond week 5 the specimens became more fragile.

3.3.8. Molecular weight changes

The rather complex mechanism of macromolecules degradation precluded the possibility of considering solely the variations of the average molecular weight values. It is essential to discuss the whole SEC chromatograms against time (Fig. 4). At time 0, PLA50 specimens exhibited monomodal SEC chromatograms with no contribution in the range of elution volumes corresponding to low molecular weight compounds. The average molecular weight at the SEC peak corresponded to 65 000 with respect to polystyrene standards. Polydispersity was rather narrow with $I = 1.6$. After 7 weeks the SEC chomatograms of partially degraded specimens appeared bimodal, with two peaks corresponding to intermediate (22 000) and low molecular weight (1500) values, respectively. At week 15, i.e. when specimens were reduced to hollow forms, the SEC chromatogram was still bimodal but with predominance of a narrow low molecular weight peak. Surprisingly, the SEC analysis of the outer and inner parts of partially degraded specimens revealed that degradation was dramatically larger in the inner part than at the surface. The differentiation between surface and centre appeared during the third week (Fig. 5a). However, at this time SEC curves of both parts were still monomodal. At week 7 monomodal SEC pattern was found for the inside matter, whereas the outer led to a bimodal one (Fig. 5b). It is likely that the presence of the peak corresponding to low molecular weight compounds in the SEC pattern of the outer layer was related to pollution by the inner matter, the separation of the crust from the viscous core being physically difficult.

3.4. Ageing in phosphate buffer

The degradation in phosphate buffer appeared to be very analogous to that in saline. Visual examination revealed similar features: swelling and heterogeneity of the cross-sections after breaking of partially degraded specimens (Fig. 6). Contrary to saline, the buffered aqueous media did not cause any flattening of the hollow specimens, which retained the swollen shape formed during the first 3 weeks (Fig. 7).

Fig. 8 presents the variations of weight loss, water absorption, pH and osmolarity with respect to degradation time. The pH remained rather stable during the experimentation and decreased slowly to 6.7 at the end. The other parameters led to variations similar to those observed in saline. Therefore, the pH

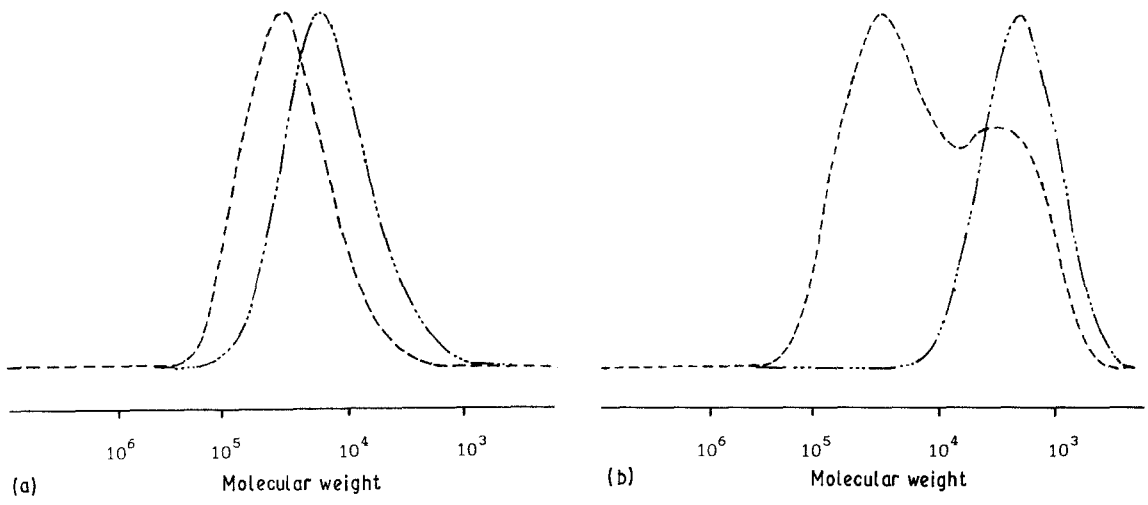


Figure 5 SEC chromatograms of PLA50 after (a) 3 and (b) 7 weeks in saline. (– – –) Surface and (—· · ·—) centre.

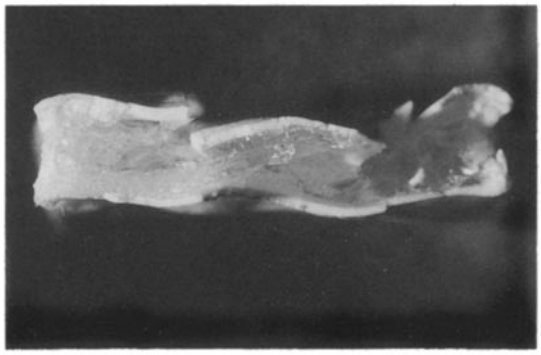


Figure 6 Cross-section of a PLA50 specimen degraded for 5 weeks in phosphate buffer.

Figure 7 Empty shell of a hollow PLA50 specimen as it appeared after 12 weeks in phosphate buffer.

difference between the two ageing media did not affect the degradation significantly.

Fig. 9 shows SEC chromatograms at different ageing times. Bimodal profiles were also found in the period from week 7 to week 12. At week 12, however, the profile of the hollow specimens was back to monomodal, in contrast with what was found in saline. At week 14 the SEC pattern was again bimodal, probably because of advanced degradation of the crust.

From the various data reported above, it can be concluded that degradation of PLA50 started immediately after immersion of the specimens in the aqueous media and occurred preferentially in the bulk, as already mentioned in the literature [14]. However, it is now clearly shown that under the selected conditions degradation does not proceed homogeneously. Similar findings have been suspected for implants as well as for microspheres [13, 15]. However, it had never been demonstrated that degradation was faster in the centre than at the surface. Whenever the observation of heterogeneous degradation was reported, people called for differences of degradation rates in amorphous and crystalline microdomains to account for this particularity, even for polymers or copolymers known to be intrinsically amorphous [13, 15].

Actually, the degradation of PLA50 is much more complex than was so far suspected. Our present understanding of the phenomenon is schematized in Fig. 10 and separated in five steps. The specimens are initially homogeneous (step 1). Once placed in an aqueous medium, they absorb water and hydrolytic cleavage of the ester bonds starts as shown by the decrease of molecular weight (step 2). It can reasonably be thought that, at the very beginning, degradation is faster at the surface than in the core, according to the gradient of absorbed water. Degradation products are formed at the surface as well as in the inner part, but those localized near the surface can dissolve more easily in the medium than those located inside the specimens. The concentration of carboxylic endgroups increases in the centre and thus catalyzes ester degradation, resulting in a surface–centre segregation (step 3). The weight loss observed for individual specimens (Figs 3a and 8a) showed that almost no soluble products were released before week 7. However, the slight decrease of pH in saline observed before week 7 and the increase of osmolarity suggested that acidic compounds did diffuse in rather small amounts in the aqueous medium. Both features are in good agreement with our hypothesis. The membrane-like layer formed at the surface actually separates two phases of different and changing compositions and chemical potentials. Therefore, it can be the source of osmotic forces, and thus of swelling and blistering. With advanced degradation, the segregation is amplified and the inner part becomes a very viscous liquid consisting essentially of oligomers (molecular weight ≈ 1500). In the meantime the outer layer becomes thinner as the degradation propagates toward the surface. When the surface becomes permeable for internal oligomers, a weight loss occurs. The specimens hollow out gradually to give a crust (step 4). During this period all of the characteristics of the medium are subjected to a dramatic evolution. Finally, the crust degrades at a reduced rate (step 5), whereas the pH, osmolarity and L-lactic acid concentration of the medium no longer change significantly. It is noteworthy that no major differences were observed between the degradations in the two isoosmolar media, in spite of the pH difference.

Although theoretical derivations are difficult because of the time dependence of most of the material characteristics (diffusion coefficient, hydrophilic–hydrophobic balance, dimensions and forms eventually), it can reasonably be thought that the thickness of the outer layer should depend on the relative rates of diffusion and degradation. Accordingly, degradation of massive PLA50 should be faster than that of small devices such as films or microspheres, because the surface–centre segregation is favoured in massive samples. So far, no significant data are available to check this point.

The formation of a degradation-resistant crust could have been assigned to morphological heterogeneities related to processing and faster cooling at the contact of the mould. Actually, the formation of a crust was observed both for large surfaces, which were in contact with the mould, and for small surfaces, which were machined. Therefore, it can be concluded that the segregation between surface and core reflected the particular degradation mechanism proposed in Fig. 10. Last, but not least, the difference of molecular weights between surface and core explains well the finding of bimodal SEC chromato-

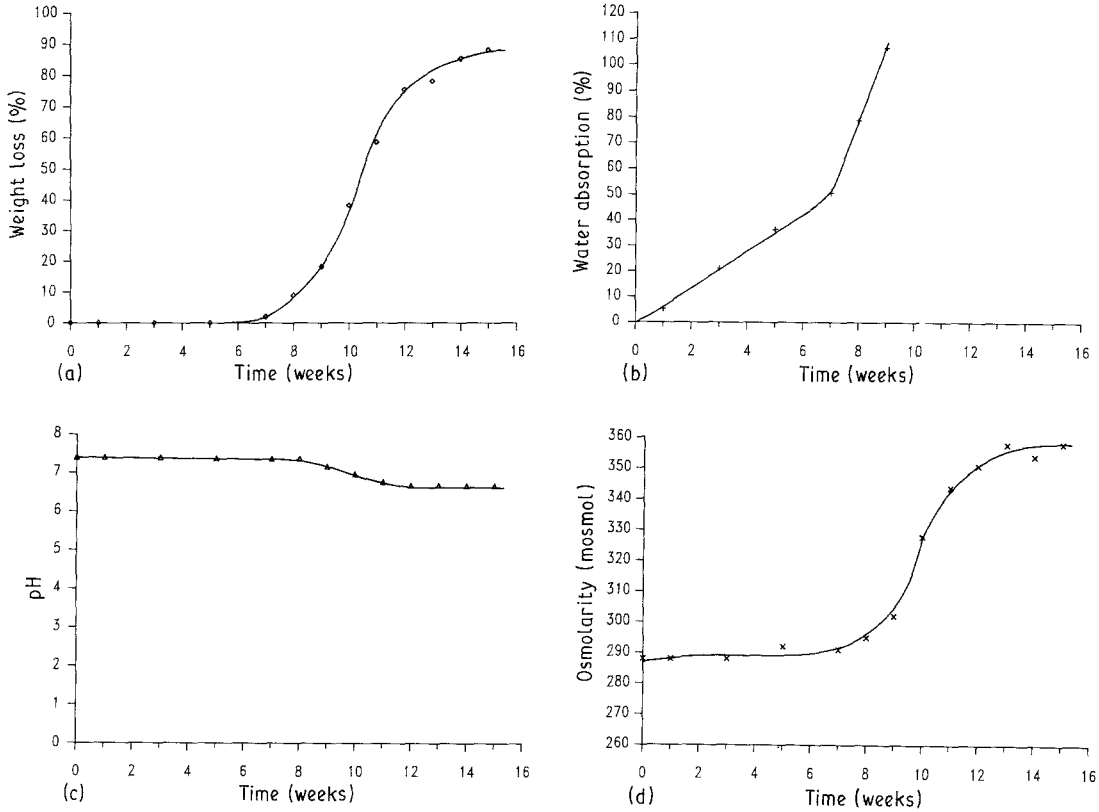


Figure 8 Evolution of some parameters during the degradation of PLA50 in phosphate buffer. (a) Weight loss, (b) water absorption, (c) pH and (d) osmolarity.

grams for partially degraded intrinsically amorphous samples.

The inner autocatalytic effect should be a general behaviour for the degradation of aliphatic polyesters. However, the fate of the specimens is very strongly dependent on the chemical and configurational structures of the polymer chains and on the morphology of the macroscopic devices. These conclusions are of great interest for the understanding of the behaviour of large devices to be used in bone surgery or of tiny particles to be used for drug delivery. According to our findings, an entrapped basic drug should affect the autocatalytic effect very strongly if acid–base reactions

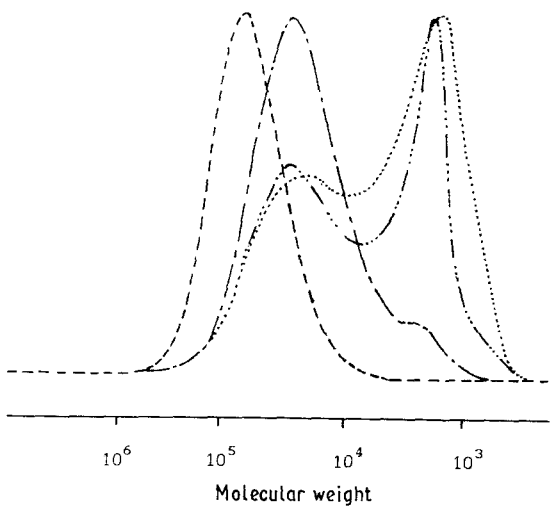


Figure 9 SEC chromatograms of PLA50 after (– – –) 0, (· · · ·) 8, (—·—) 12 and (—···—) 14 weeks in phosphate buffer.

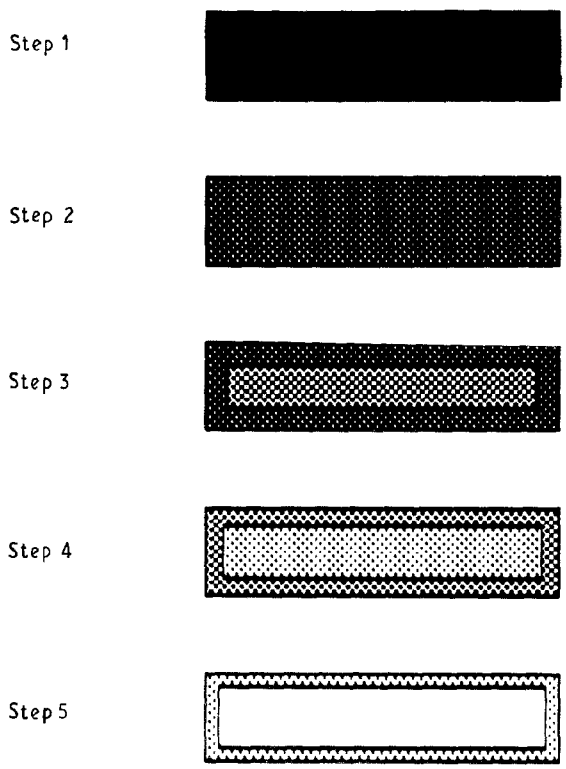


Figure 10 Schematic representation of different steps of the degradation of PLA50 specimens in an aqueous medium. Step 1: initial specimen at time 0. Step 2: water absorption, beginning of ester bond cleavage and molecular weight decrease. Step 3: differentiation between surface and centre with dramatic decrease of molecular weight in the inner part of the specimen. Step 4: diffusion of oligomers through the thinning surface layer when molecular weight is low enough to allow solubilization in the medium. Step 5: shell remaining after the release of oligomers and slow degradation of the shell.

can occur. This statement is supported by recent data in the field of drug delivery systems involving basic drugs [16].

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