Hydrolysis and Erosion Studies of Autocatalyzed Poly(ortho esters) Containing Lactoyl−Lactyl Acid Dimers

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ABSTRACT: Poly(ortho esters) based on 3,9-diethylidene-2,4,8,10-tetraoxaspiro[5.5]undecane, 1,10-decanediol, and 1,10-decanediol dilactate were prepared and their hydrolysis in phosphate buffer at 37 °C and pH 7.4 was determined as a function of time. Specifically, kinetics of polymer weight loss, polymer molecular weight changes, release of lactic acid and release of propionic acid were determined. A significant decrease in molecular weight occurred within about 20 days. Weight loss and evolution of hydrolysis product occurred after an induction period that decreased with increasing content of the lactoyl−lactyl segment in the polymer. After the induction period, a concomitant rate of weight loss and release of hydrolysis products was noted. The rate increased with increasing content of the lactoyl−lactide segment. After a certain time period, size-exclusion chromatography revealed the formation of a peak at an apparent molecular mass of about 550 Da. The compounds under the peak were analyzed by electrospray ionization/mass spectrometry (ESI/MS) and found to consist of a multiplicity of lower molecular weight polymer hydrolysis fragments. On the basis of expected hydrolysis products, it was possible to assign a structure to most molecular weight peaks in the ESI/MS spectrum. The data were found to be consistent with a hydrolysis that takes place predominantly in the surface layers of the solid polymer.

Introduction

Drug release from bioerodible drug delivery devices can best be controlled if drug release is determined primarily by the kinetics of polymer erosion rather than drug diffusion, and desired drug release kinetics can best be achieved if the erosion process is well understood. To gain such an understanding, it is necessary not only to follow polymer weight loss and changes in molecular weight as a function of time but also to determine kinetics of release of polymer hydrolysis products.

Polymer erosion has traditionally been discussed in terms of two extreme mechanisms, surface erosion and bulk erosion.[1] Surface erosion, also known as heterogeneous erosion, is defined as a process where erosion is confined to the outer surface of a solid device. Clearly, to achieve surface erosion, whatever hydrolysis is responsible for the erosion process cannot extend into the bulk of the material, so that surface erosion requires a fast hydrolysis reaction and no water penetration into the bulk material. The only exception to this requirement is erosion that occurs by a slow chemical hydrolysis and a fast enzymatic hydrolysis. Such a process has been described for flexible polyesters where hydrolysis caused by esterases produced true surface erosion because enzymes are unable to penetrate the bulk material and can only act on ester linkages at the polymer surface.[2] Bulk erosion, also known as homogeneous erosion, is a process where polymer hydrolysis occurs at more or less uniform rates throughout the bulk of the material. Although in the literature there has been considerable discussion of surface erosion versus bulk erosion, little convincing evidence for surface erosion has been presented.

Poly(ortho esters) are one class of bioerodible polymers that have been described as surface eroding systems,[3] but only circumstantial evidence to substantiate such claims has been presented.[4] These polymers are stable at alkaline pH and hydrolyze at increasingly rapid rates as the pH is lowered. Because hydrophobic poly(ortho esters) erode at very slow rates at body pH, many applications require an increased hydrolysis rate, which can be achieved by providing an acidic environment within the polymer matrix. Early work used acidic excipients such as anhydrides,[4,5] various aromatic and aliphatic acids,[6] and sebacic acid[7] to increase hydrolysis

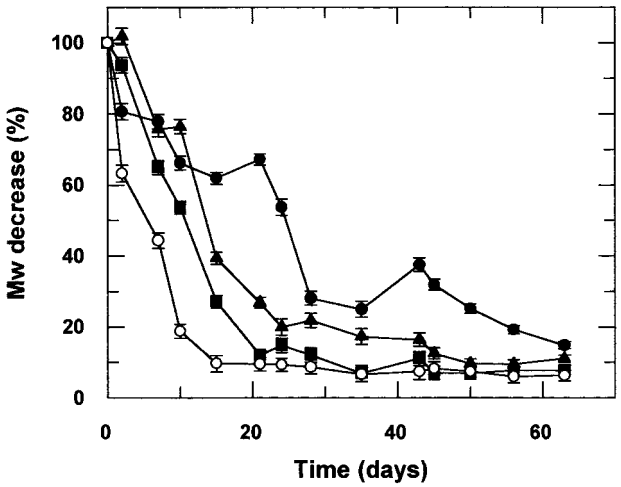

Figure 1. Decreases of weight-average molecular weights for various autocatalyzed POE_xLA_y during in vitro degradation in 0.13 M sodium phosphate buffer, pH 7.4, 37 °C, ($n = 3 \pm$ sdm): (●) POE_{100}, (▲) $POE_{95}LA_5$, (■) $POE_{90}LA_{10}$, and (○) $POE_{70}LA_{30}$.

Table 1. Weight and Number Average Molecular Weights

	POE_{100}	$POE_{95}LA_5$	$POE_{90}LA_{10}$	$POE_{70}LA_{30}$
M_w	36 000	60 100	52 500	30 000
M_n	22 100	38 200	30 800	18 800
M_w/M_n	1.63	1.57	1.70	1.60

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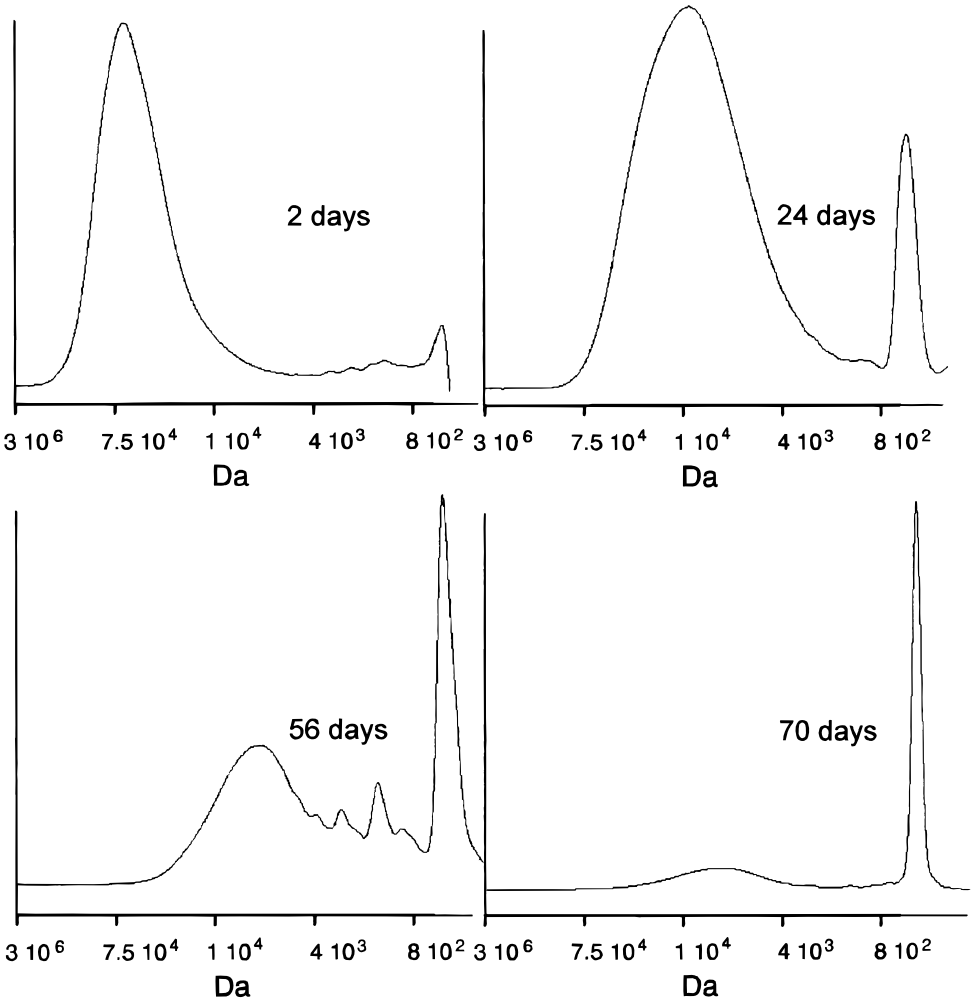
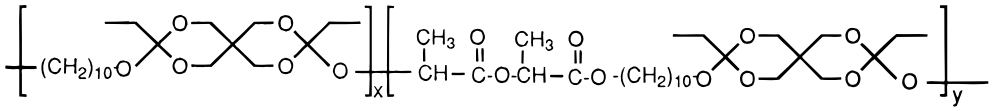


Figure 2. Size-exclusion chromatograms of $POE_{95}LA_5$ at 2, 24, 56, and 70 days, in 0.13 M sodium phosphate buffer, pH 7.4, at 37 °C.

Scheme 1



rates. However, when acidic excipients are used to accelerate hydrolysis, water intrusion into the inner core of the matrix leads to bulk erosion. Further, due to diffusion of the acidic excipient from the matrix, incomplete polymer erosion was noted because excipient-depleted devices were eventually produced.

We have recently reported the preparation of autocatalyzed poly(ortho esters) containing lactoyl−lactyl acid dimer segments in the polymer backbone and have shown that erosion rates of this polymer could be controlled within very wide limits by varying the concentration of the lactoyl−lactic acid dimer segment in the polymer.[8,9] In this polymer system, hydrolysis releases lactic acid, which then acts as a catalyst to accelerate hydrolysis of the ortho ester linkages in the polymer.

The present study describes erosion studies of poly(ortho esters) based on the diketene acetal 3,9-diethylidene-2,4,8,10-tetraoxaspiro[5.5]undecane, 1,10-decanediol, and 1,10-decanediol dilactate having the structures shown in Scheme 1. To gain a better understanding of the erosion mechanism, an in vitro hydrolysis study of this polymer in PBS buffer at pH 7.4 and 37 °C was carried out, where kinetics of weight loss were determined gravimetrically, changes in molecular weight were determined by size-exclusion chromatography, kinetics of release of lactic acid were determined by an enzymatic assay, and kinetics of release of propionic acid were determined by HPLC.

Experimental Section

Materials. Autocatalyzed poly(ortho esters), POE_xLA_y, where LA stands for lactide and x and y for the molar ratios of ortho ester and lactic acid units, were synthesized by an acid-catalyzed condensation of 3,9-diethylidene-2,4,8,10-tetraoxaspiro[5.5]undecane with 1,10-decanediol and 1,10-decanediol dilactate as previously described.[10] Low molecular weight oligomers, unreacted monomers, and catalyst were removed by a dissolution−precipitation method with tetrahydrofuran (THF) and methanol as solvent and nonsolvent, respectively. The precipitated polymer was dried under vacuum at 40 °C for 48 h. The following four poly(ortho esters) were prepared: POE_{100}, $POE_{95}LA_5$, $POE_{90}LA_{10}$, and $POE_{70}LA_{30}$. Integration of the polymer ^{1}H NMR spectra provides a relative estimate of the chemical composition.[10] The measured y values were found to be 7.5, 11.3, and 26.7, which are in reasonable agreement with the calculated values of 5, 10, and 30.

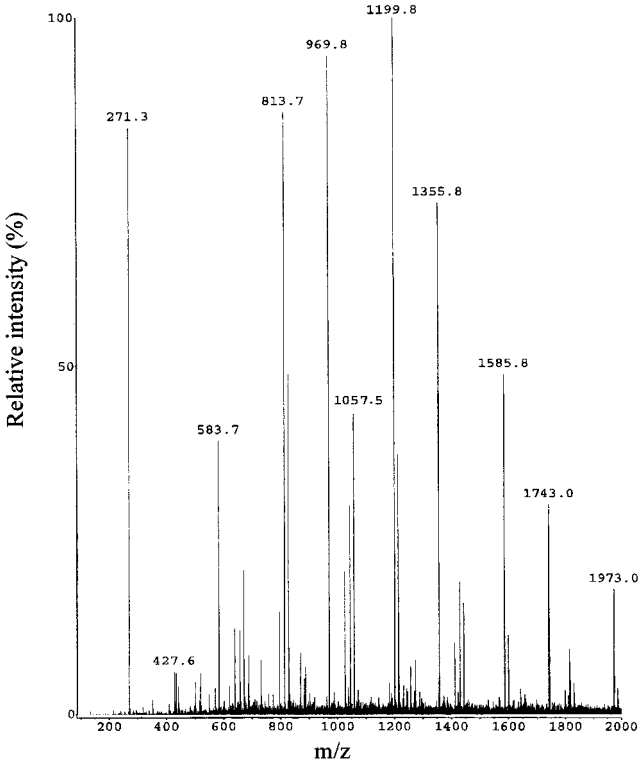


Figure 3. Electrospray/mass spectrometry spectrum for a $POE_{95}LA_5$ polymer residue after 77 days in 0.13 M phosphate buffer, pH 7.4, at 37 °C.

Sodium dihydrogen orthophosphate (NaH_2PO_4), disodium hydrogen phosphate 2-hydrate ($Na_2HPO_4 \cdot 2H_2O$), triethylamine, and orthophosphoric acid were purchased from Fluka Chemie AG, Buchs, Switzerland. Propionic acid and acetonitrile (HPLC-grade) were purchased from Sigma−Aldrich, Gillingham, England. THF (HPLC-grade) was purchased from Romil Chemical, Leicestershire, England. The lactate reagent kit was purchased from Sigma Diagnostics, St. Louis, MO.

Methods. For erosion studies, 50 mg pieces of polymer cut from precipitated material were added to 5 mL of 0.13 M PBS at pH 7.4 and slowly agitated at 100 rpm at 37 °C in a horizontal shaker (GFL, Burgwedel, Germany). At predetermined times, polymers were collected, rinsed with distilled water to remove buffer salts, and freeze-dried to constant weight prior to determination of mass loss and average molecular weight. Mass loss (ML %) was evaluated by gravimetric analysis and calculated from:

$$\text{ML \%} = 100\,(W_0 - W_t)/W_0$$

where W_0 and W_t are the initial weight and the residual weight of the dry polymer at time t.

To determine molecular weight, samples were dissolved in THF containing 100 ppm of triethylamine, and their weight- and number-average molecular weights were determined by analytical high-pressure size-exclusion chromatography (HPSEC). A Waters 600 E system with four Styrogel HR columns (10^5, 10^4, 10^3, and 500 Å) connected in that order in series as the stationary phase, an autosampler Waters 717 plus, and a refractometer Waters 410 was used. Stabilized THF was used as the mobile phase and calibration was carried out with polystyrene standards (Tosoh Corporation, Tokyo, Japan) covering the 500−96 400 Da range.

Propionic acid release was determined with an isocratic HPLC system with LC module I Plus (Waters, Milford, MA), a pump (Waters 600E power line controller), an autoinjector (Waters 715 Ultra WISP), a UV detector (Waters tunable

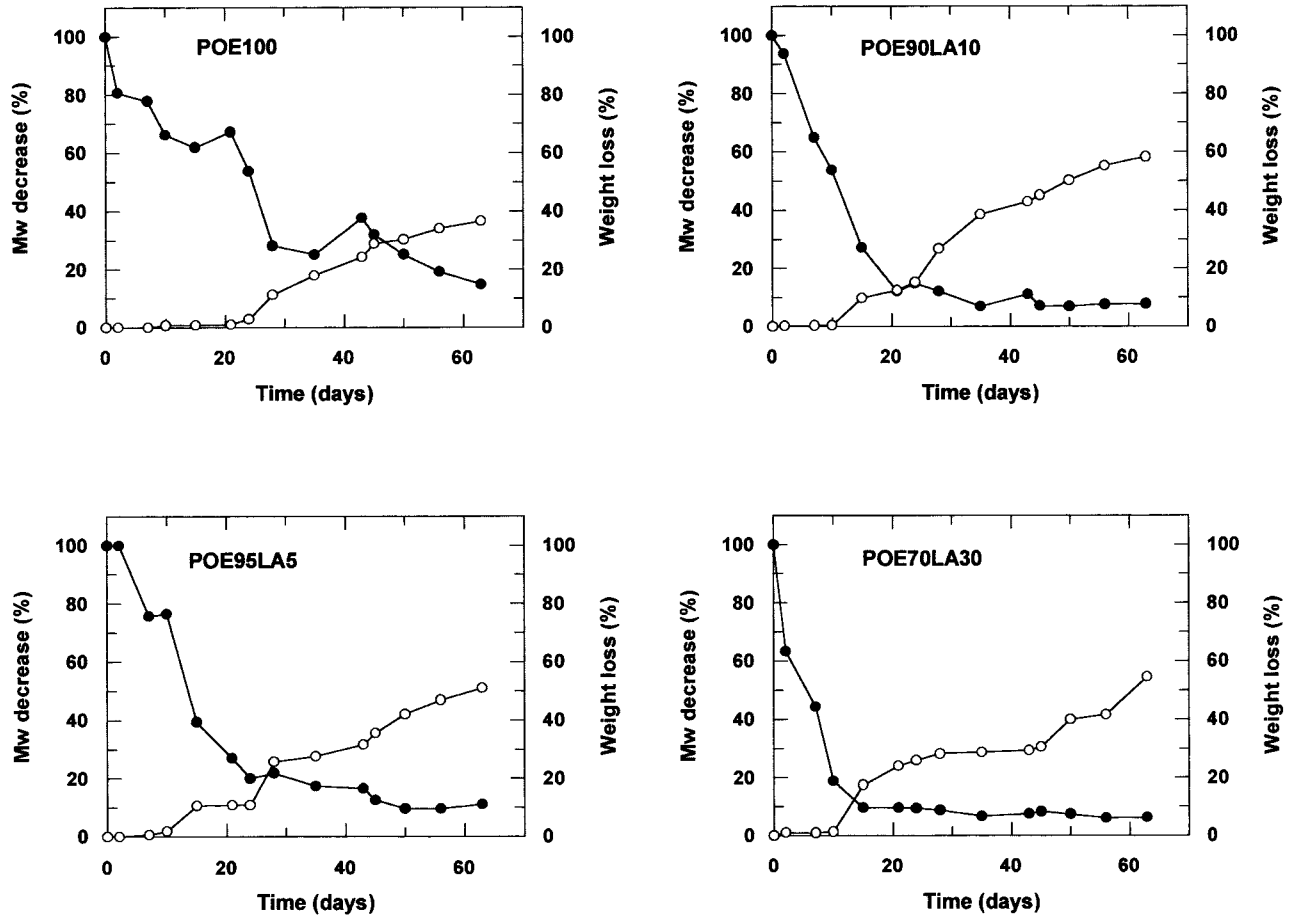
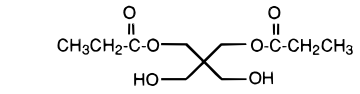
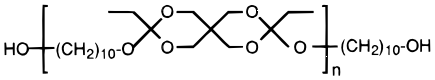


Figure 4. Relationship between the decrease in weight-average molecular weight (●) and weight loss (○) for a range of autocatalyzed POE_xLA_y during in vitro degradation in 0.13 M sodium phosphate buffer, pH 7.4, at 37 °C.

Table 2. Structures and Molecular Weight Assignments



I



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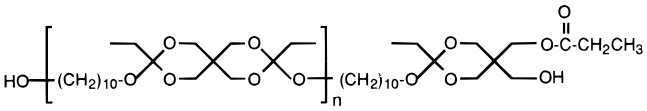

III

structure	peak	peak − Na[a]
I	271	248
II $n = 1$	584	561
II $n = 2$	970	947
II $n = 3$	1356	1333
II $n = 4$	1743	1720
III $n = 1$	814	791
III $n = 2$	1200	1177
III $n = 3$	1586	1563
III $n = 4$	1973	1950

[a] Actual molecular weights are peak molecular weights minus the atomic weight of sodium contaminant.

absorbance detector), and an integrator (Millenium software, Waters). The columns were Nucleosil 100-5 C 18, (Macherey-Nagel Gmbh & Co., Düren, Germany), 5 μm particle size, 250 mm long, and 4 mm inner diameter. The mobile phase was a mixture of 0.01 M phosphate buffer and acetonitrile (80/20 v/v) containing 0.036 M triethylamine and orthophosphoric acid to give a pH of 2.3. This phase was filtered through a 0.45 μm membrane filter (Millipore) and degassed with helium prior to use. The column was preconditioned with the mobile phase until a stable baseline was obtained. A flow rate of 1 mL min^{-1} was used with a run time of 10 min and the eluent was monitored at 208 nm. In each case, 20 μL samples were injected. Seven standard solutions were prepared in duplicate, containing propionic acid in a concentration range from 1.024 to 5.120 mg/mL in phosphate buffer (0.13 M, pH 7.4), and a calibration curve was constructed. The typical retention time of propionic acid was 4.45 min.

Lactic acid was detected by an enzymatic method relying on the quantitative oxidation of lactate to pyruvate in the presence of lactate reagent composed of lactate oxidase, peroxidase, and a chromogen precursor. Since DL-lactide was used in the synthesis and only L-lactic acid is detected enzymatically, DL-lactic acid was used in preparing calibration standards. In this assay, L-lactic acid is converted to pyruvic acid and hydrogen peroxide (H_2O_2) by lactate oxidase. In the presence of the H_2O_2 formed, peroxidase catalyzes the oxidative condensation of the chromogen precursors to produce a colored dye with an absorption maximum at 540 nm. The increase in absorbance at 540 nm is directly proportional to lactic acid concentration in the samples (standards and polymer degradation media). Lactate reagent was reconstituted with 10 mL of deionized water. A 10 μL aliquot of DL-lactic acid standard solution in phosphate buffer (0.13M, pH 7.4) was added to 1 mL of lactate reagent solution and incubated for 10 min. The absorbance was then read at 540 nm. Good linearity was obtained for lactic acid concentration ranging from 10 to 1200 μg/mL.

Electrospray Ionization/Mass Spectrometry. ESI/MS was performed on a Finnigan MAT SSQ7000 quadrupole mass spectrometer. Samples of hydrolysis product were dissolved in methanol and infused into the ESI source at 3 mL/min by a syringe infusion pump (Harvard Apparatus). An optimum voltage of 4500 V was maintained on the ESI/MS electrode in order to form the multiprotonated ions of the product. Mass was scanned from 100 to 2000 mass/charge units in 1 min, and 200 scans were averaged to obtain the mass spectra.

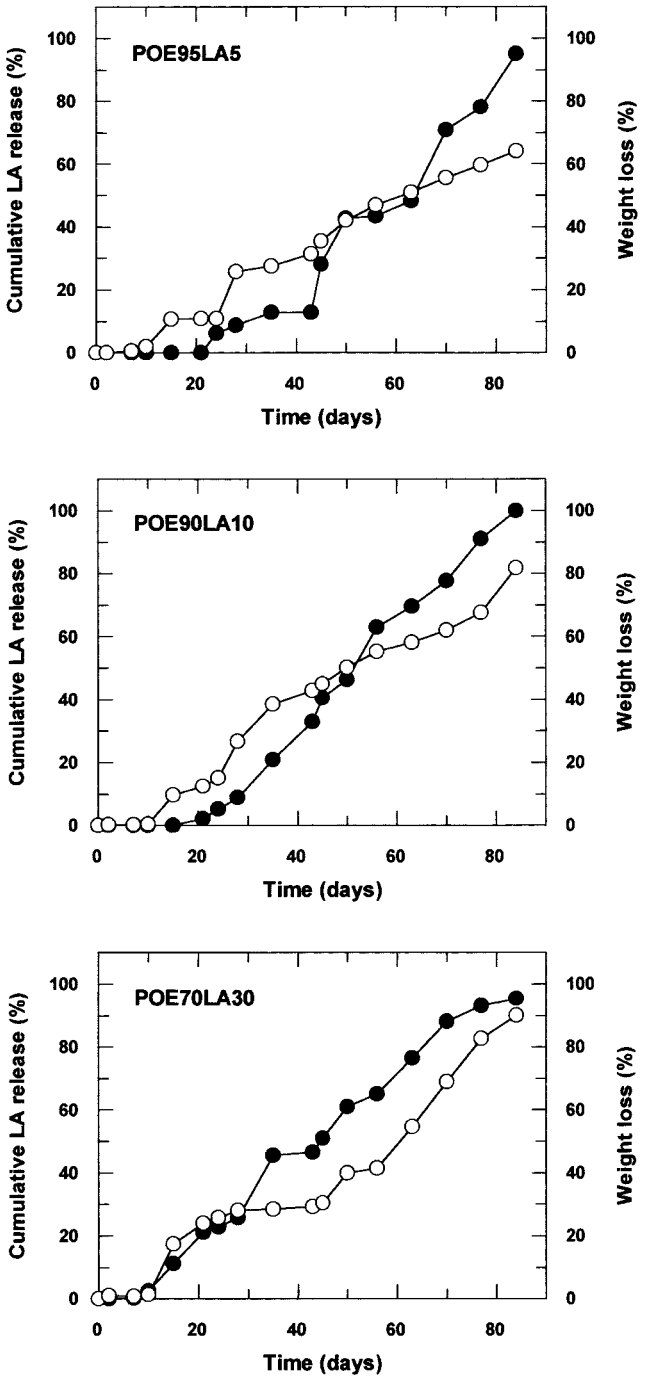

Figure 5. Relationship between lactic acid release (●) and weight loss (○) for a range of autocatalyzed POE_xLA_y during in vitro degradation in 0.13 M sodium phosphate buffer, pH 7.4, at 37 °C.

Results

Molecular Weight Changes. Molecular weights and polydispersity of the four polymers investigated are shown in Table 1. Changes in weight-average molecular weight are shown in Figure 1. All four polymers showed an initial rapid decrease in molecular weight followed by a slower decrease in a second phase. For POE_{100} and

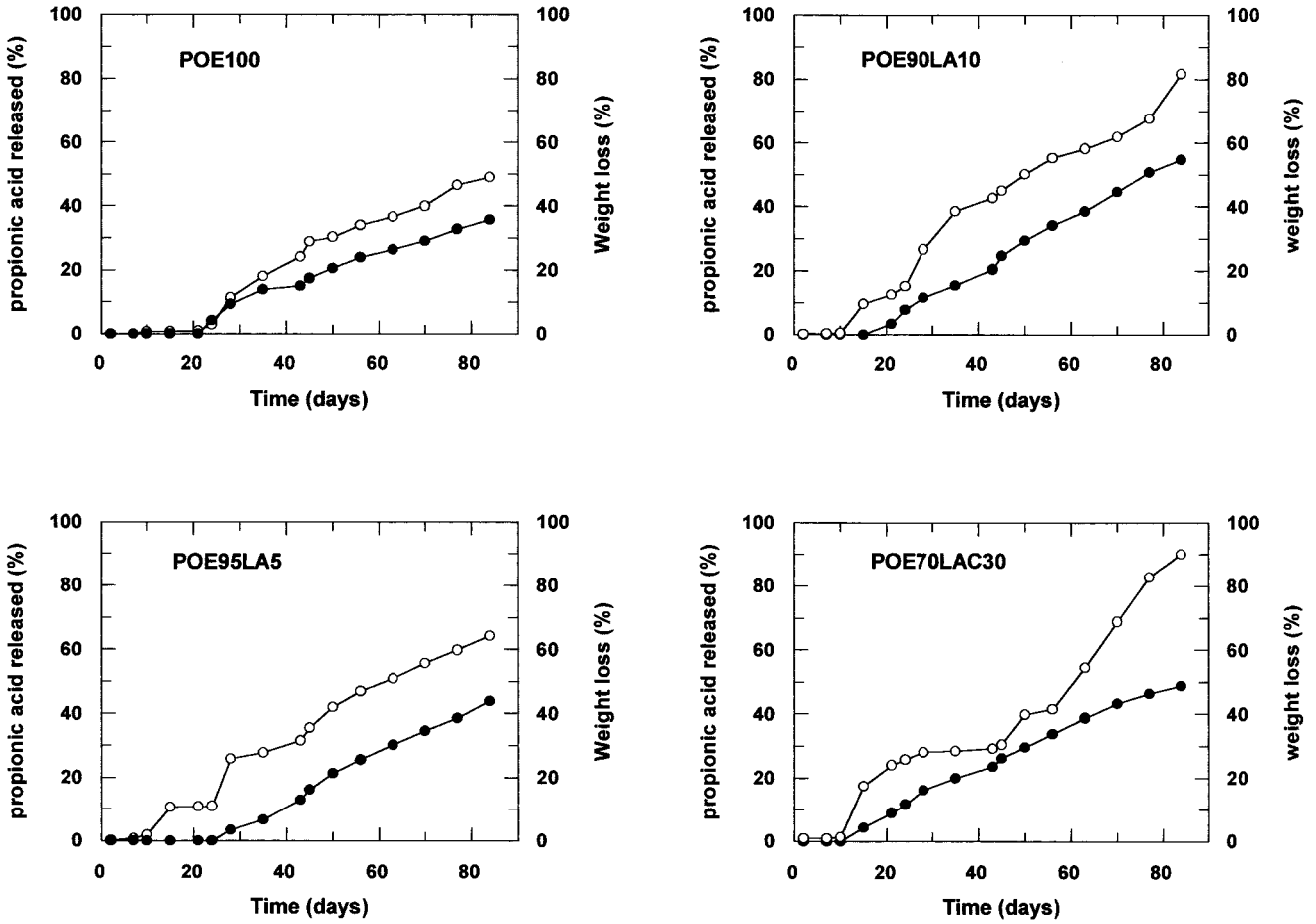

Figure 6. Relationship between weight loss (○) and propionic acid release (●) for a range of autocatalyzed POE_xLA_y during in vitro degradation in 0.13 M sodium phosphate buffer, pH 7.4, at 37 °C.

$POE_{95}LA_5$, the molecular weight continues to decrease after the initial rapid drop, while with the more rapidly eroding polymers $POE_{70}LA_{30}$ and $POE_{90}LA_{10}$, there is little decrease in the second phase. The initial rate of decrease in molecular weight depends on the concentration of the dilactate segment in the polymer; materials with a higher dilactate segment concentration show a faster initial rate of molecular weight decrease. This is as expected, on the basis of the higher production of lactic acid for polymers having higher concentration of lactic acid dimer in the backbone.

Figure 2 shows SEC chromatograms of changes in molecular weight distribution of $POE_{95}LA_5$ with time in PBS buffer. After a certain time in PBS, the chromatogram shows a shift of the polymer molecular weight peak to lower values, a broadening of the molecular weight distribution, and the appearance of a peak at an apparent molecular weight of about 550 Da. The higher the lactic acid content in the polymer, the more rapid is the appearance of the 550 Da peak; for $POE_{70}LA_{30}$, $POE_{90}LA_{10}$, $POE_{95}LA_5$, and POE_{100}, the peak appears, respectively, at 10, 21, 24, and 28 days.

Characterization of the 550 Da Peak. The compounds under the 550 Da peak were characterized by electrospray ionization/mass spectrometry. The spectrum is shown in Figure 3 and molecular weight peak assignments are shown in Table 2.

Mass Loss. Figure 4 shows the relationship between decrease in polymer molecular weight and weight loss for a range of POE_xLA_y having different amounts of the dilactate segment in the polymer backbone. Clearly, both weight loss and molecular weight decrease are dependent on the amount of that segment in the polymer. Weight loss for all polymers shows an induction period, which varies from 20 days for POE_{100} to 8 days for $POE_{70}LA_{30}$. After the induction period, an almost constant erosion rate is observed.

Lactic Acid Release. Figure 5 shows lactic acid release and polymer weight loss as a function of time for various POE_xLA_y. While weight loss and lactic acid release both show an induction period, weight loss precedes release of lactic acid. This result can best be rationalized by assuming that during the dilactate segment cleavage, polymer fragments terminating in an acid function are produced and these fragments will catalyze ortho ester cleavage. Free lactic acid is not liberated until a second cleavage takes place. The difference in induction periods between lactic acid release and weight loss decreases as the concentration of lactic acid dimer in the polymer increases and vanishes with $POE_{70}LA_{30}$.

The most significant observation is that lactic acid is released linearly and concomitantly with weight loss of the polymer, which is also linear, for the entire 90 days and that the rate of weight loss is clearly related to the concentration of lactic acid dimer in the polymer.

Propionic Acid Release. Figure 6 shows propionic acid release and polymer weight loss as a function of time for various POE_xLA_y. These data are similar to those shown in Figure 5 for lactic acid release, in that weight loss and propionic acid release both show an induction period, which decreases as the concentration

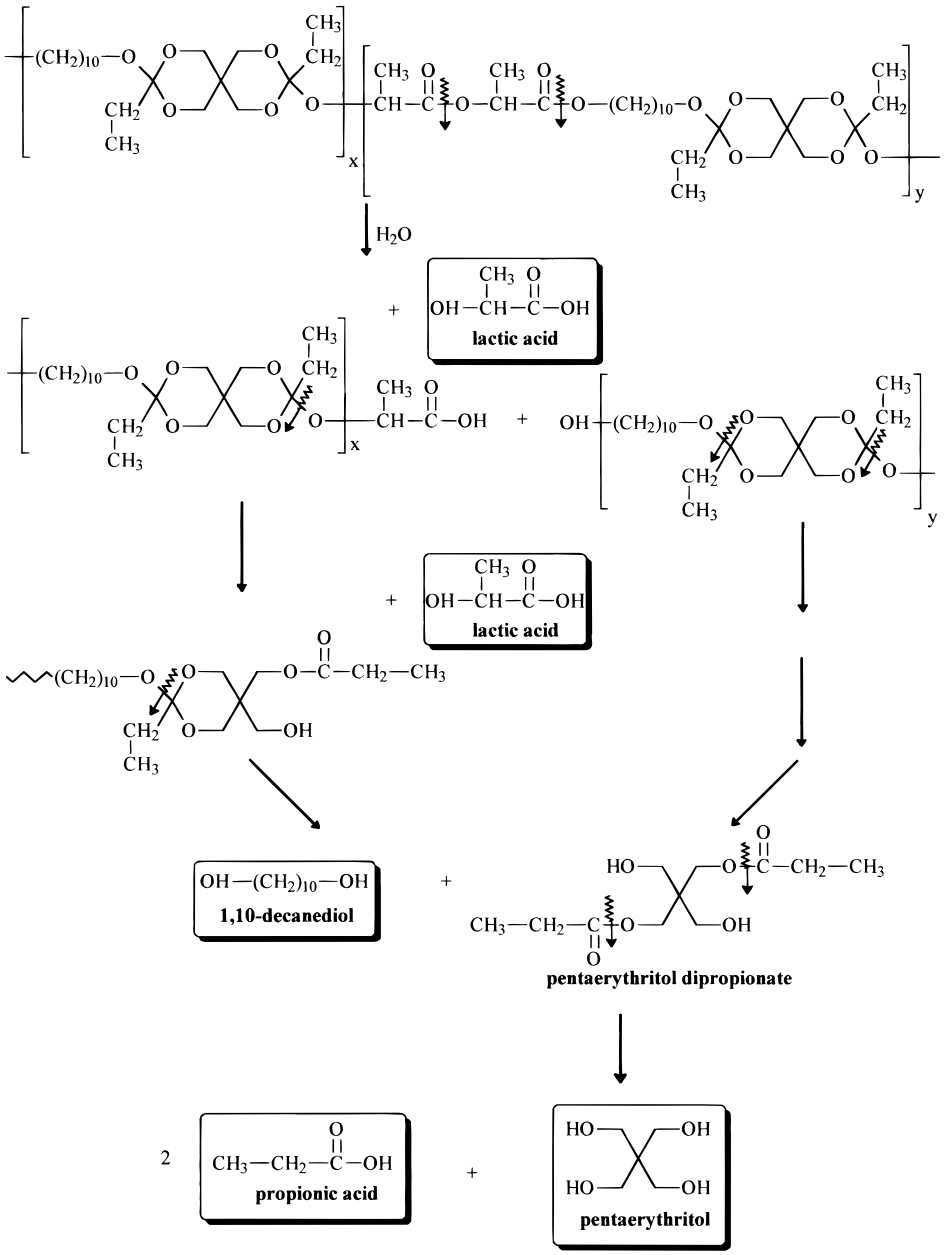

Figure 7. Schematic representation of the hydrolysis pathway for autocatalyzed POE_xLA_y.

of lactic acid dimer in the polymer increases and vanishes with $POE_{70}LA_{30}$.

There is not a significant difference between the induction periods before lactic acid and propionic acid are released. Because propionic acid is one of the ultimate hydrolysis products, this suggests that once lactic acid is formed, rapid hydrolysis of the ortho ester linkages takes place.

Discussion

The proposed hydrolysis mechanism shown in Figure 7 proceeds in three consecutive steps. In the first step, the lactic acid dimer segment in the polymer backbone hydrolyzes at the points indicated by the arrows to generate a polymer fragment containing a carboxylic acid end group, which will catalyze ortho ester hydrolysis. A second cleavage produces free lactic acid, which also catalyzes hydrolysis of the ortho ester links. The hydrolysis of ortho esters then proceeds in two steps, as previously shown, to first generate the diol or mixture of diols used in the synthesis and pentaerythritol dipropionate, followed by ester hydrolysis to produce pentaerythritol and propionic acid.[11]

Taken collectively, the data obtained in this study can be used to arrive at a mechanistic depiction of the erosion process. Perhaps the most significant finding is the linearity of weight loss and the concomitant release of lactic and propionic acid. While linear rate of weight loss alone does not necessarily indicate surface erosion,[12] the concomitant linear weight loss and release of lactic and propionic acids argues convincingly for a process confined predominantly to the surface layers of the polymer matrix. The process is clearly not a pure surface erosion because there is a significant drop in molecular weight of the uneroded polymer, indicating that some hydrolysis is taking place in the bulk material.

Pure surface erosion demands that no water penetrate the matrix or that polymer chain cleavage is prevented by stabilizing the interior of the matrix, as has been done previously with $Mg(OH)_2$.[13] Although not carried

out with these materials, water sorption determinations for similar polymers without the lactoyl−lactyl segment (POE_{100}) yielded values between 0.30% and 0.75% with diffusion coefficients ranging from a high of 4.07×10^{-8} for a low glass transition material based on 1,6-hexanediol to a low of 2.11×10^{-8} for a high glass transition polymer based on *trans*-cyclohexanedimethanol.[14] Thus, water can penetrate the polymer, but the concentration in the bulk will be extremely low.

Another significant finding is that the induction periods before lactic acid and propionic acid release take place are about the same. Because, as postulated in the hydrolysis mechanism shown in Figure 7, propionic acid is generated in the third step of the hydrolysis process, we can conclude that once cleavage of the ortho ester chains takes place, hydrolysis of some, but not all, ester groups proceeds to yield the ultimate hydrolysis products.

The following process seems to account for the observed facts. Initially, due to the highly hydrophobic nature of the polymer surface, no hydrolysis takes place, and an induction period is observed. Gradually, as a result of hydrophilic species generated by the hydrolysis process, sufficient water penetrates the surface layers to induce a more rapid hydrolysis, and once steady state has been reached, constant erosion with concomitant generation of hydrolysis products takes place. The induction period is a function of the lactoyl−lactyl segment in the polymer, and higher amounts of lactic acid generated will accelerate the initial hydrolysis, resulting in decreased induction periods.

In parallel with this process, some water penetrates the bulk material and induces some polymer chain cleavage. However, due to the limited number of polymer chains cleaved, the material remains very hydrophobic and water concentration in the bulk remains very low, despite the fact that some hydrophilic species have been generated.

As shown in Figure 2, as hydrolysis proceeds, a lower molecular weight peak begins to appear in the SEC chromatograms. On the basis of electrospray ionization/mass spectrometry studies, this peak corresponds to a multiplicity of lower molecular weight hydrolysis products, and proposed structures based on their molecular weights are shown in Table 2. The peak was analyzed at 77 days where, as shown in Figure 5, about 80% of the lactic acid has been released but only about 60% of the polymer has eroded. Thus, the residue comprises water-insoluble low molecular weight hydrolysis fragments. Because little lactic acid remains, further hydrolysis of these fragments is relatively slow. When the hydrolysis is allowed to proceed to completion, these fragments will eventually hydrolyze to pentaerythritol dipropionate and decanediol, and pentaerythritol dipropionate will ultimately hydrolyze to pentaerythritol and propionic acid. Such low molecular weight fragments are probably also found in the surface layers of the eroding matrix.

As can be seen in Figure 6, unlike lactic acid release, propionic acid release lags significantly behind the rate of polymer weight loss. This finding is consistent with the formation of segments containing propionic esters such as the ones shown for structure III in Table 2.

Conclusion

We have demonstrated that hydrolytic erosion of autocatalyzed POE_xLA_y occurs via a process predominantly confined to, but not limited to, the surface layers and have also shown that rate of erosion can be controlled by the amount of lactic acid dimer incorporated into the polymer backbone. A mechanistic depiction of the erosion process that seems to fit all experimental data has been developed.

This mechanistic depiction is only valid for the pure polymer and entirely different behavior will be observed once drugs, and particularly hydrophilic drugs, are incorporated into the matrix. Such studies are planned.

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