Stability of DL-Poly(lactic acid) in Aqueous Solutions

C. S. Proikakis, N. J. Mamouzelos, P. A. Tarantili, A. G. Andreopoulos

National Technical University of Athens, Department of Chemical Engineering, Polymer Technology Laboratory, Iroon Polytechniou 9, Zografou Campus, 157 80, Athens, Greece

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ABSTRACT: DL-poly(lactic acid) of molecular weight about 2500 was prepared by polycondensation of lactic acid and characterized by viscosimetry, infrared spectroscopy, light scattering, GPC, and NMR. Tablets made of the above polymer were immersed in buffer solutions at 37°C, and their swelling behavior was recorded as a function of time, in terms of weight gain. In the same experiments, the hydrolytic stability of PLA was assessed by measuring the weight loss after drying the tablets. To inhibit any degradation due to bacteria, formaldehyde was added to the solution as a biostatic factor. © 2002 Wiley Periodicals, Inc. J Appl Polym Sci 87: 795–804, 2003

Key words: swelling; polyesters; biodegradable; drug delivery systems

INTRODUCTION

Poly(α-hydroxy acid)s derived from glycolic and lactic acids or other higher homologs and their mixtures constitute one of the most widely used classes of degradable biomaterials due to their excellent biocompatibility and variable degradability.[1,2] These polymers are quite useful because they decompose via simple hydrolysis of the ester bond in an aqueous environment and give readily absorbable degradation products.

Their behavior in aqueous media is influenced by several parameters such as crystallinity, molecular weight, glass transition temperature (T_g), and monomer hydrophobicity.[1] The differences in degradation rates of different polymers have been attributed to differences in the access of water to the ester bond rather than to differences in the intrinsic rates of ester cleavage.[3]

DL-Poly(lactic acid) (DL-PLA) is an amorphous polymer with a glass transition temperature of 58°C and a wide range of melting temperatures depending on its molecular weight. Two methods are currently available for PLA synthesis: polycondensation of lactic acids[4] and ring-opening polymerization of lactides.[5] Decomposition of these polymers proceeds essentially via hydrolysis of ester groups, which takes place heterogeneously and shows a higher rate in the inner part of a specimen than at the surface. This fact was attributed to the formation of an outer layer of a slowly decomposing polymer that entraps the degrading macromolecules in the interior of the matrix, allowing only relatively low molecular weight oligomers to diffuse and dissolve to the surrounding medium. As a result, a bimodal distribution is observed because the rate of ester bond cleavage in the inner part of the degrading specimen is accelerated due to autocatalysis.[6–8] In fact, hydrolysis is autocatalyzed by carboxy groups and, therefore, its rate increases exponentially with time since the rupture of the first few ester bonds is very slow compared with the final rate of bond cleavage. The presence of residual monomer, low molecular weight compounds, and oligomers accelerates the degradation rate tremendously because their presence tends to increase chain flexibility and polymer hydrophilicity and, moreover, provides carboxylic groups for the autocatalytic reaction.[9] A kinetic equation to describe the autocatalytic effect of the increasing carboxyl acid end-group concentration was suggested by Siparsky et al.[10] who studied the hydrolysis of PLA in aqueous acetonitrile solutions.

Additional parameters that influence the hydrolysis process are the temperature and pH of the solution.[11–13] The mode of scission during hydrolysis of biodegradable polymers could be completely random regarding the backbone bonds or it could be "chain-end-unzipping" mechanisms. It was found that the base-catalyzed hydrolysis of DL-PLA demonstrated a random process, while the acid-catalyzed hydrolysis followed a fast chain-end scission.[12] It was also established that the hydrolysis rate is higher in acidic than in neutral media.[11] Degradation becomes a bulk process above the T_g, while at temperatures below T_g, degradation of the polymer matrix was restricted to its surface.[13] Hausberger et al.[14] studied the influence of gamma-irradiation on biopolymers and they reported that the onset times for degradation decreased with increasing irradiation dose.

Correspondence to: A. G. Andreopoulos.

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The progress of polymer degradation is assessed by measuring the weight loss of the sample, using a microbalance within a selected time interval, by determining the molecular weight reduction of the polymer using gel permeation chromatography (GPC) or by calculating the M_w from the intrinsic viscosity data.[1] A qualitative graphical method was also developed for studying the mode of the hydrolysis of biodegradable polymers. This approach requires the determination of the molar fraction of the monomer by nuclear magnetic resonance (H-NMR) or high-pressure liquid chromatography (HPLC) as well as the degree of degradation by H-NMR.[12] Differential scanning calorimetry (DSC) is another method suitable for determination of the T_g that might be a measure of the polymer degradation.[13]

PLA decomposes to its monomer, lactic acid, which is a normal metabolite of the human body. This characteristic makes this polymer suitable for many important biomedical uses such as the preparation of resorbable sutures and production of implants for orthopedic surgery or blood vessels. Very interestingly, PLA has been used in the sustained release of drugs, for the delivery of antimycobacterial drugs, quinolones, antimalarial and anti-inflammatory drugs, antitumor agents, and hormones, and as the base of fluoride-containing tablets for oral use.[15–17]

It is evident that the degradation process of PLA is very critical for drug-delivery behavior in the controlled-release systems based on this polymer. In fact, the already-reported surface and bulk erosion combined with the autocatalytic effect of carboxy groups probably disturb an even rate of release and, more specifically, zero-order release, which is well desired when designing the above systems.[18] Furthermore, the drug diffusion through the polymeric matrix is an additional mechanism contributing to the complexity of the phenomenon. This latter mechanism is obviously influenced by the swelling characteristics of the polymeric matrix. Therefore, the study of the stability of PLA specimens immersed into various aqueous media seems to be important as a means for providing useful information to further explore the performance of biodegradable controlled-release systems.

In a previous work,[15] an investigation was made of the swelling behavior of high molecular DL-PLA prepared by ring-opening bulk polymerization of DL-lactide. It was observed that hydrolytic degradation starts in a few days for the low molecular weight material, whereas for the higher molecular weight product, it takes much longer and probably follows a two-stage mechanism. The results obtained showed that swelling tends to obey Fick's law, especially for the specimens with high molecular weight where biodegradation proceeds slowly.

In this work, the study was expanded to low molecular weight PLA prepared by polycondensation of lactic acid. This polymer was characterized by viscosimetry, infrared spectroscopy, light scattering, GPC, and NMR, and then the weight change of the tablets immersed into buffer solutions was recorded. At the same time, the hydrolytic stability of PLA was assessed by measuring the weight loss of the dry tablets.

EXPERIMENTAL

Materials

Three types of DL-PLA prepared by polycondensation of lactic acid: (a) with xylene as an azeotropic solvent, (b) at 180°C and reduced pressure, and (c) at 220°C under nitrogen, were used. The experimental details for their preparation were described in a previous work.[19] For the viscosimetry measurements, PLA was dissolved in chloroform (chemically pure, Fluka, Buchs, Switzerland).

Buffer solutions of citric acid ($C_6H_8O_7 \cdot H_2O$) and disodium hydrogen phosphate ($Na_2HPO_4 \cdot 2H_2O$) were prepared according to the following composition:

Buffer	$C_6H_8O_7 \cdot H_2O$	$Na_2HPO_4 \cdot 2H_2O$	Deionized water
pH 5.4	9.3g	19.8g	to 1 L
pH 7.4	1.9g	32.3g	to 1 L

Formaldehyde (chemically pure, Fluka) was used a biostatic agent.

Methods

Viscosity measurements

The molecular weight of the polymerized materials was determined by viscosimetry, using a Ubbelohde capillary viscometer placed in a water bath thermostatically controlled at 25°C. The polymers were dissolved in chloroform at concentrations ranging from 0.2 to 2 g dL^{-1}. Calculations were made using the well-known Mark–Houwink equation:

$$n = KM_v^a$$

where $K = 2.21 \times 10^{-4}$ and $a = 0.77$.

Small-angle light scattering

A KMX-6 LALLS photometer at 25°C, operating at 633 nm, was used for the measurements of the weight-average molecular weight M_w and the second virial coefficients A_2. THF refluxed over sodium and cyclohexane refluxed over CaH_2 distilled just prior to use were the solvents. All the solutions were clarified by filtration through 0.22-μm pore-size nylon filters. The M_w and the A_2 values were obtained from the plots of $K_c/\Delta R_\theta$ versus c, where K is a combination of optical

TABLE I
Molecular Weight and Polydispersity Index of PLA Obtained via Different Polymerization Procedures

DL-PLA	Viscosimetry (M_v)	Light scattering (M_w)	Polydispersity index
Polycondensation with azeotropic solvent	1300	—	—
Polycondensation at reduced pressure	1470	1500	—
Polycondensation at high temperature	2550	2600	1.87

—: Not measured.

constants, and ΔR_θ, the excess Rayleigh ratio. The refractive index increments, dn/dc, required for the determination of the optical constant for the light-scattering experiments, were measured at 25°C with a Chromatix KMX-16 differential refractometer, operating at 633 nm and calibrated with NaCl solutions.

Gel permeation chromatography (GPC)

The molecular weight distribution and polydispersity Index (M_w/M_n) of PLA were determined by GPC. A Waters 501 system was used equipped with a Waters HPLC solvent pump, three gel columns (10^3, 10^4, 10^5 Å) connected in series, and a Waters differential refractometer. Chloroform was used as an eluent and was delivered at a flow rate of 1.0 mL/min. All samples were tested at 30°C after dissolution in chloroform and the injection volume was 200 μL

FTIR spectroscopy

Infrared spectra were obtained by employing a Nicolet Magna 750 FTIR spectrophotometer equipped with a DTGS detector and interfaced with a personal computer. A Spectra Tech microcup DRIFTS accessory was used, and the background spectra were recorded using pure dried KBr in powder form. Spectra were acquired and manipulated with the use of Omnic (ver. 3.1) FTIR software at 4-cm^{-1} resolution and 100 scans per sample.

NMR

For the NMR analyses, a Varian (Model Gemini 2000) instrument was used, with a magnet frequency of 300 MHz. ^{1}H- and ^{13}C-NMR spectra were obtained using deuterated chloroform ($CDCl_3$) as a solvent and tetramethylsilane (TMS) as the internal standard.

Swelling

Swelling experiments were run in deionized water and buffer solutions of pH 5.4 and 7.4. Tablets of PLA were prepared by forming the polymer melt into the appropriate steel molds, at 100°C and 200 mbar pressure for 1 h. The specimens were immersed into the solutions and placed in an oven at 37°C. At various intervals, the specimens were removed, gently wiped to clean the surface water, and reweighed. The degree of swelling (SI) was calculated using the following equation:

$$\mathrm{SI} = \left(\frac{M_1 - M_0}{M_0}\right) \times 100 \qquad (1)$$

where M_0 is the initial weight of the tablet and M_t denotes the weight of the tablet at time t. Swelling was recorded as a function of weight gain of the specimen.

RESULTS AND DISCUSSION

The data relevant to the magnitude of the molecular weight, obtained by viscosimetry and small-angle light scattering, in terms of viscosity-average molecular weight (M_v) and weight-average molecular weight (M_w), respectively, can be seen in Table I.

Furthermore, the polydispesity index shown in Table I is a measure of the molecular weight distribution obtained by GPC. From the data of the table, it is clear that polymerization via the azeotropic distillation of water produced during the polycondensation reaction leads to low molecular weight oligomers, since the reaction temperature is restricted by the boiling point of xylene (140°C). Some improvement can be observed by running the reaction at higher temperature and facilitating water removal by applying a vacuum. However, in that case also, the molecular weight remained below 2000, which is the lower limit for a product with potential use as biomaterial and, more specifically, in controlled-release applications.[15–17] Finally, polycondensation at the temperature of 220°C, which requires an inert atmosphere to avoid oxidation, gave PLS with an acceptable molecular weight. Therefore, it seemed reasonable to focus on this material and explore its behavior after immersion into various liquids. Moreover, the polydispersity index of the above polymer, although higher than unity, suggests that a rather narrow molecular weight distribution was achieved by running the polymerization process at that high temperature. This can also be observed by looking at the graph of Figure 1, which shows a relatively narrow curve of the molecular weight distribution.

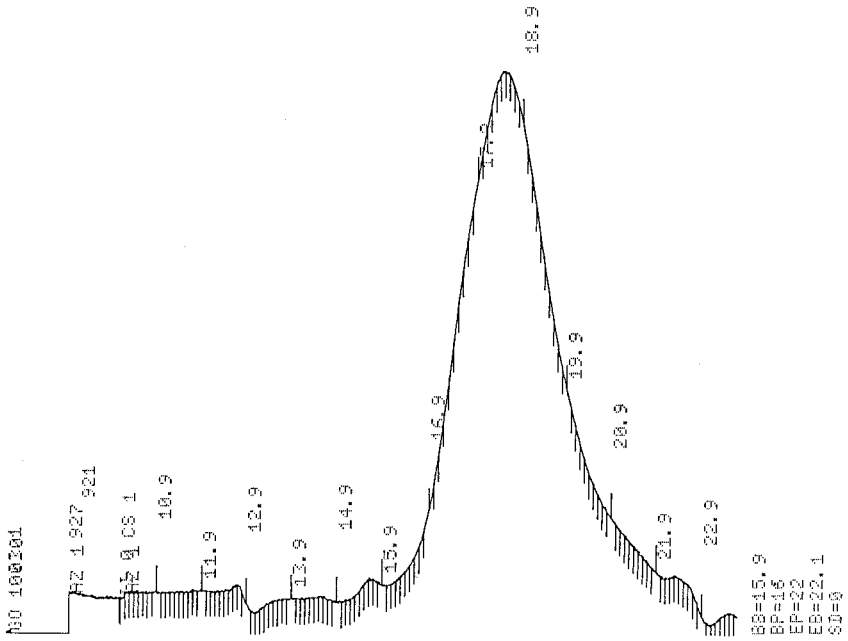


Figure 1 GPC diagram of DL-PLA.

In general, physical and biological properties as well as degradation kinetics of poly(α-hydroxy acid)s are strongly related to the molecular weight, chemical structure of repeating units, and macromolecular characteristics such as chiral unit distribution, crystallinity, and tacticity. It seems therefore worthwhile to identify the chemical and morphological characteristics of the PLA samples obtained by various polymerization techniques in order to explore the interrelation between the preparation procedure and the performance of the final product. Spectrometry, especially ^{13}C-NMR and vibrational spectroscopies such as infra-

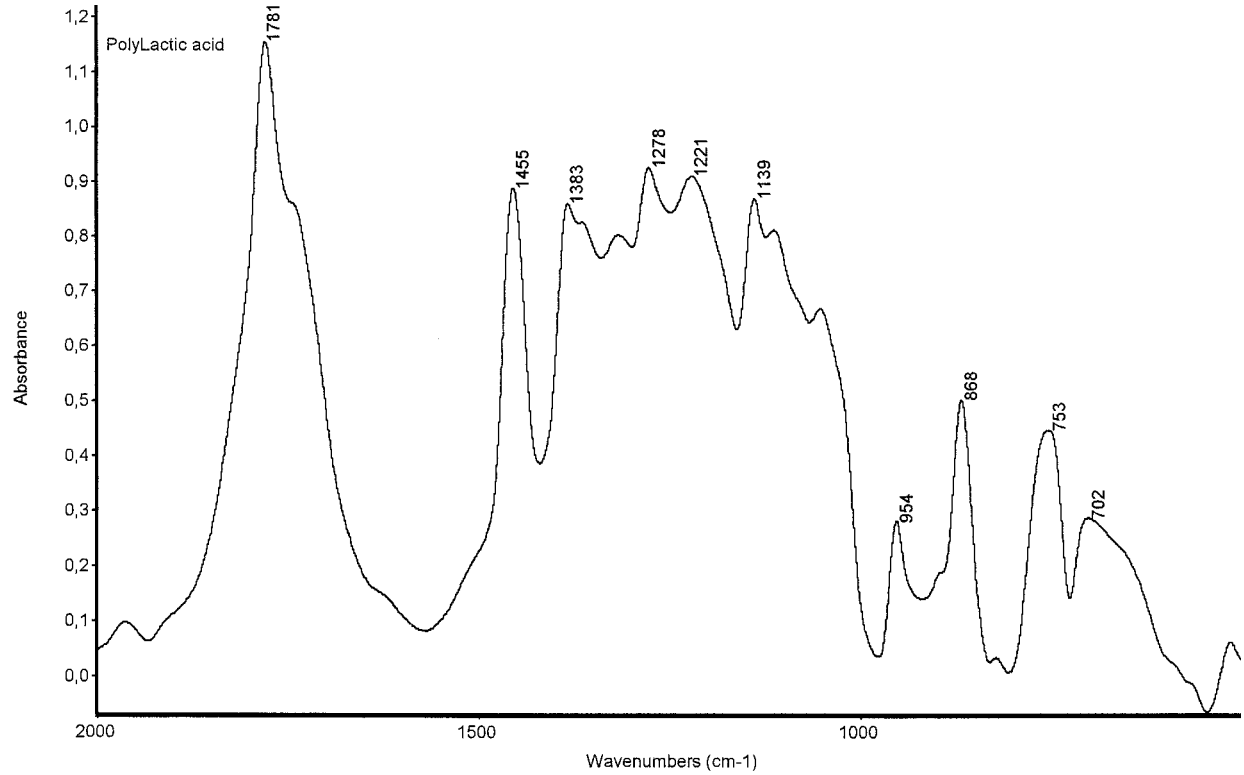


Figure 2 FTIR spectrum of DL-PLA.

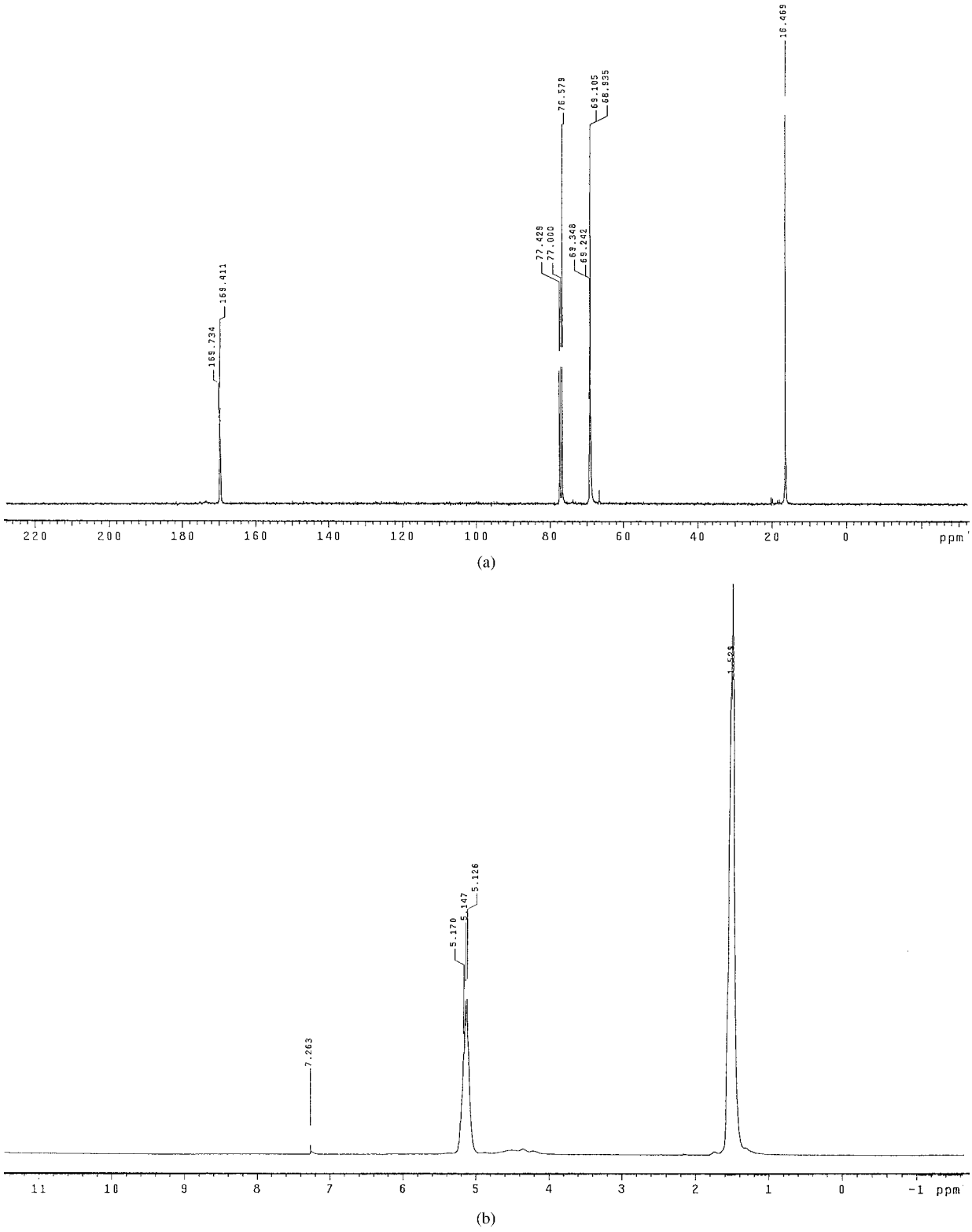


Figure 3 (a) ^{13}C-NMR and (b) ^{1}H-NMR spectra of DL-PLA.

red (IR) and Raman, are powerful techniques for the study of polymer structures. IR spectroscopy revealed the typical pattern of DL-PLA, as Figure 2 illustrates, for the three examined products.[20] More specifically, the peak at 1781 cm^{-1} corresponds to the carboxyl group stretching mode and the CH_3 asymmetric deformation modes appeared at 1455 cm^{-1}. Frequency minor shifts were observed at 1383 and 1300 cm^{-1} and

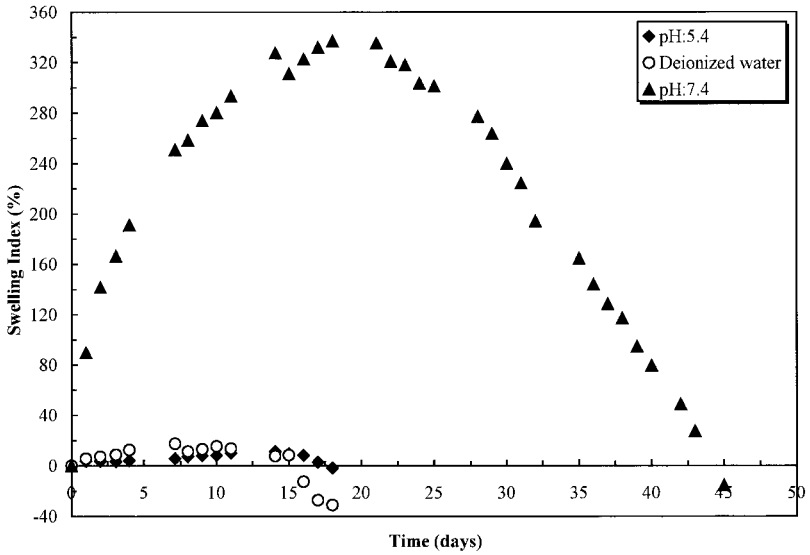


Figure 4 Swelling index of DL-PLA tablets immersed in various liquids.

assigned to $\delta_s CH_3$ symmetric deformation and δCH bendings, respectively. The peak at 1215 cm^{-1} could be attributed to the stretching modes of C—O—C ester groups. The peaks at 1139 and 1045 cm^{-1} have also been observed with lactic acid oligomers and were assigned to rCH_3 rocking and $\nu C—CH_3$ stretching, respectively.

^{13}C-NMR was expected to provide useful and reliable information about any monomer units remaining unreacted into the polymer after polycondensation. Furthermore, the presence of dimers or different optical isomers in the samples of DL-PLA could be revealed by the same analytical technique. Figure 3(a) presents a typical pattern deriving from the ^{13}C-NMR analysis. The bands appeared at 169.5725, 69.1575, and 16.469 ppm to the carbon of —COOH, —C—OH, and —CH_3 groups, respectively. It should be noted that the graph is identical to the original spectrum of PLA and no signs exist suggesting the presence of a monomer. On the other hand, ^{1}H-NMP gave a multiple peak at 5.147 ppm owing to —CH groups in the polymer chain, a doublet at 1.529 ppm due to —CH_3, and a peak at 7.263 ppm which was assigned to —OH groups. This spectrum, shown in Figure 3(b), is again the same as that corresponding to pure PLA[21] and further acts as evidence of the purity of the examined polymer.

These results are very useful for the establishment of the above-described direct polymerization method, since it appears suitable for the preparation of PLA at reasonable yield and with the appropriate molecular weight for biomedical uses. In addition, this proce-

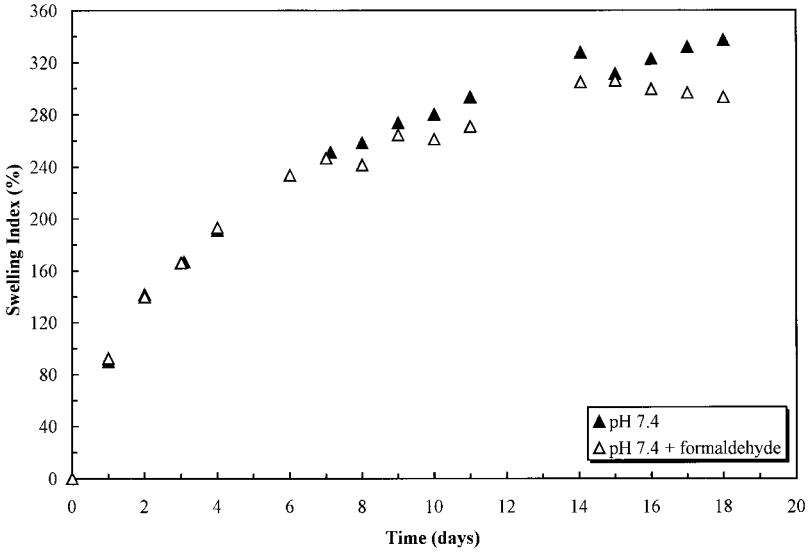


Figure 5 Swelling index of DL-PLA at pH 7.4 and 37°C.

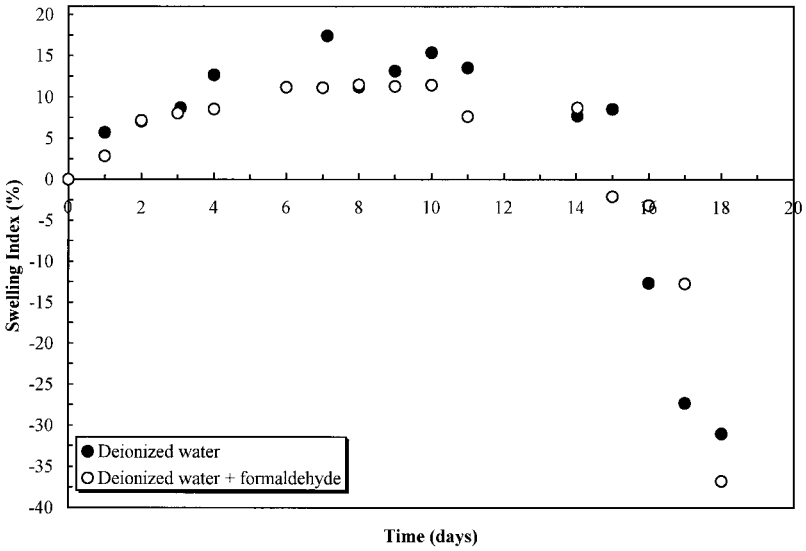


Figure 6 Swelling index of DL-PLA in deionized water and 37°C.

dure leads to pure products, that is, free from solvents, catalysts, monomer, and thermal degradation by-products.

The swelling capacity and overall behavior of PLA specimens immersed into various liquids is shown in Figure 4. Very interestingly, the graphs of that figure display a similar response for the PLA samples that remained in the deionized water and acidic solution, whereas those immersed into the rich disodium hydrogen phosphate buffer gave about a 10 times higher swelling index. This difference could probably be due to some interactions between the carboxylic end groups of PLA and the sodium cations present in this solution. Such interactions are likely to lead to the formation of carboxy anions, as reported in a study of poly(methacrylic acid) and sodium hydroxide systems.[22] Carboxy anions are responsible for strong repulsive electrostatic forces among the polymer chains, which contribute to a higher degree of swelling.

From the curves of Figure 4, it is clear that sorption reaches a maximum and then a continuous weight decrease can be recorded, leading to values even lower than those of the original dry PLA specimens. This is obviously due to degradation of the polymer because of hydrolytic cleavage of ester bonds, which is known to occur with PLA in aqueous solutions at a rate depending on various parameters. The graphs of Figures 5–7, incorporating also plots corresponding to solutions containing 1% formaldehyde as biostatic agents, clearly show the differences among the immersion liquids. In fact, swelling is more prolonged in the pH 7.4 buffer followed by that in pH 5.4 and deionized

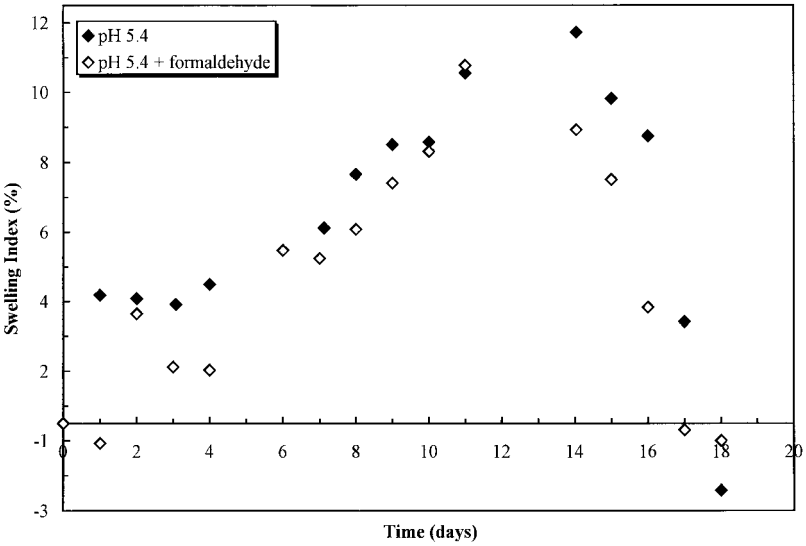
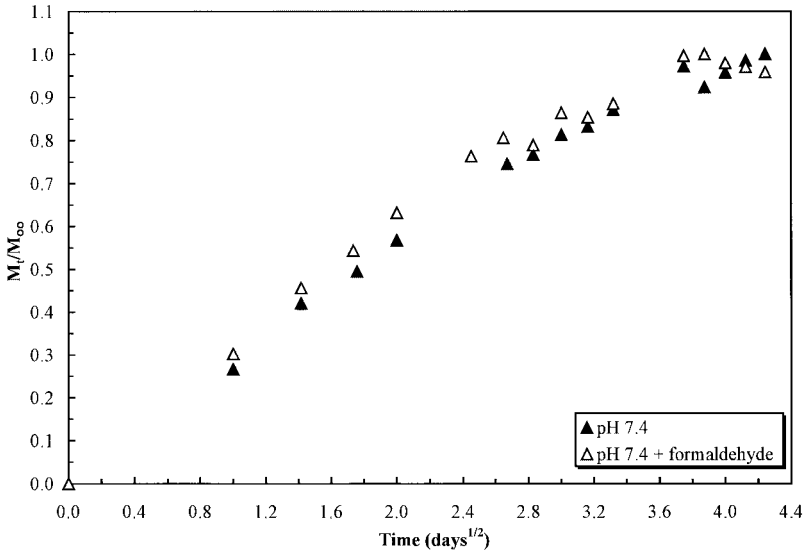
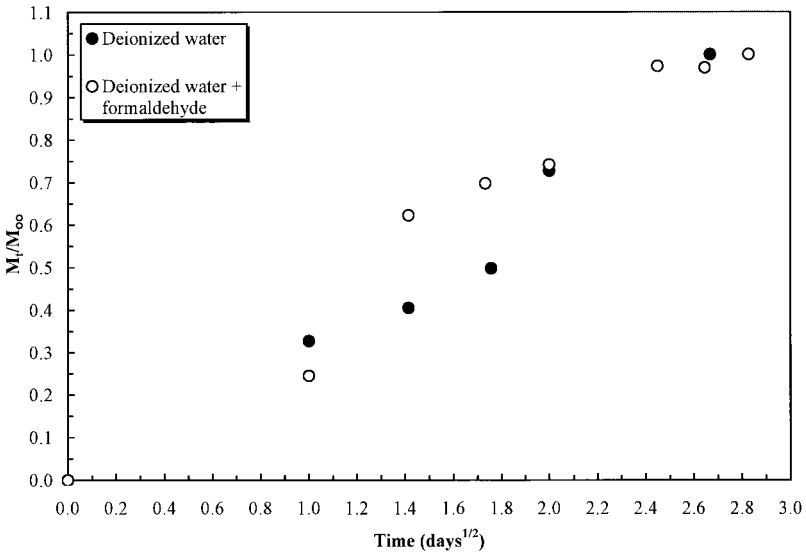


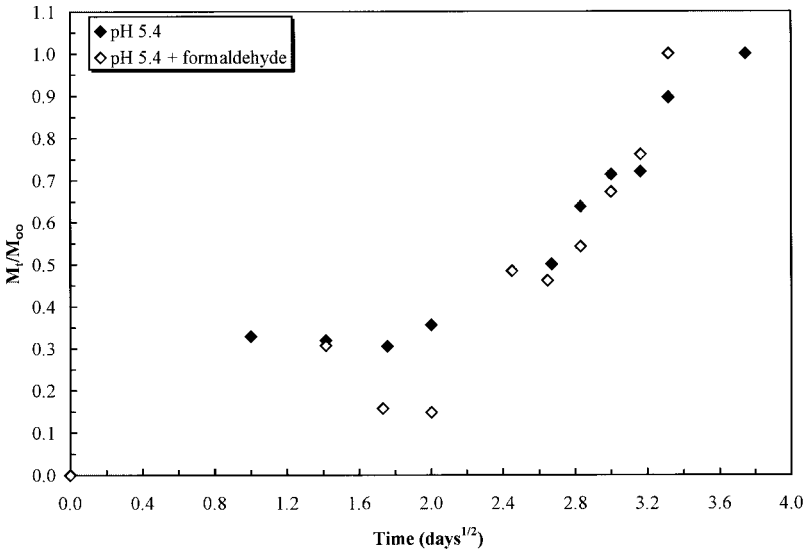
Figure 7 Swelling index of DL-PLA at pH 5.4 and at 37°C.



(a)



(b)



(c)

Figure 8 Swelling of DL-PLA as a function of the square root of time: (a) pH 7.4; (b) deionized water; (c) pH 5.4.

water, which, however, gives a higher swelling index compared with the buffer with pH 5.4. On the other hand, formaldehyde not only is unable to inhibit degradation but also seems to promote weight loss, especially in the case of the pH 7.4 buffer and deionized water. This shows that, within the experimental conditions followed in this work, the degradation of PLA is a purely chemical interaction without the interference of biological factors. It should be noted that differences in the rate of degradation could also be a reason for the higher weight uptake of specimens immersed in the pH 7.4 solution. In fact, it was observed that, for low molecular weight polymers, hydrolytic degradation starts in a few days depending on the pH, whereas for the higher molecular weight products, it takes much longer and probably follows a two-stage mechanism.[15] Therefore, the lower swelling index of the PLA samples immersed in deionized water and the pH 5.4 solution could be explained by that the balance swelling/degradation gives a much lower weight increase than that observed in the specimens into the pH 7.4 buffer.

The data presented in Figures 5–7 were also plotted in terms of M_t/M_∞ versus the square root of time, where M_∞ was assumed as the weight uptake at the maximum of each curve. The results are presented in Figure 8(a–c), where a linear dependence can be seen in the case of the pH 7.4 buffer, which does not apply for the other solutions. This appears reasonable since PLA samples that degrade slowly were found to obey Fick's law when immersed in water.[15]

The above results are very significant for the prediction of the rate of drug delivery in controlled-release systems, based on PLA with similar characteristics with the samples studied. In fact, surface and bulk erosion of PLA accompanied with drug diffusion through the polymeric matrix are the mechanisms which control the rate of release. Some models have been proposed for the prediction of the delivery rate from various geometries, one of the most simplified being the following:

$$M_t/M_\infty = 1 - [1 - K_0 t/C_0\alpha]^n$$

where M_t is the amount of drug release at time t; M_∞, the total amount of drug in the device; K_0, a constant; and α, the radius of a cylinder or a sphere or the half-thickness of a slab; $n = 1$ for a slab, 2 for a cylinder, and 3 for a sphere, which gives a linear relationship versus time in the case of a slab:

$$M_t/M_\infty = K_0 t/C_0\alpha$$

This equation should apply to devices using the polymer studied in this work, operating in contact with liquids of the same consistency and pH with deionized water of the acidic buffer, since degradation is dominant in these cases. On the other hand, in the case of the pH 7.4 buffer, PLA samples show much better stability and Fickian diffusion seems to play an important part and, therefore, drug delivery from those systems should follow more sophisticated models. This behavior, depending also on the nature of drug, should be the objective of future work in this area.

CONCLUSIONS

From the discussion of the results obtained in this work, the following conclusions can be drawn:

(a) The direct polymerization of lactic acid at 220°C under an inert atmosphere of nitrogen leads to PLS with a molecular weight around 2500, which is recommended for biomedical uses. In addition, this procedure leads to pure polymers, that is, free from solvents, catalysts, monomer, and thermal degradation by-products.
(b) PLA tablets immersed in buffers and deionized water display differences in their stability depending on the pH of the liquid. Degradation seems to be purely chemical and proceeds faster in deionized water and an acidic buffer. A linear relationship between release and time would be expected for PLA systems in contact with the above types of liquids.
(c) PLA samples immersed in the pH 7.4 buffer show much better stability and water transport tends to obey Fick's law. Controlled release from such systems would be complex due to the two parallel mechanisms of degradation and diffusion.

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