Revista Mexicana de Ingeniería Química
ISSN: 1665-2738
amidiq@xanum.uam.mx
Universidad Autónoma Metropolitana
Unidad Iztapalapa
México

Rueda, C.; Vallejo, I.; Corea, M.; Palacios, E. G.; Chairez, I.
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Revista Mexicana de Ingeniería Química, vol. 14, núm. 3, 2015, pp. 813-827
Universidad Autónoma Metropolitana Unidad Iztapalapa
Distrito Federal, México


Polímeros

Revista Mexicana de Ingeniería Química
Vol. 14, No. 3 (2015) 813-827

DEGRADATION STUDY OF POLY(LACTIC-L(+)-CO-GLYCOLIC ACID) IN CHLOROFORM

ESTUDIO DE LA DEGRADACIÓN DE POLI(ÁCIDO-L(+)-LÁCTICO-CO-GLICÓLICO) EN CLOROFORMO

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Recibido 16 de Abril, 2014; Aceptado 15 de Julio, 2015

Abstract

Polymer/solvent interaction has been of great interest in many fields, such as scaffold processing, polymer recycling and drug delivery system (DDS), among others. It has been reported that the effect of solvent on polymers could determine the final physical and chemical properties in specific applications. Thus, this article describes a degradation study of poly(lactic-L(+)-co-glycolic acid), or PLGA, in the presence of chloroform. The PLGA polymer was synthetized by ring opening polymerization (ROP). Five lactic/glycolic acid volumetric ratios (90/10, 80/20, 70/30, 60/40 and 50/50) were used to prepare PLGA. Copolymers were characterized using scanning electron microscopy (SEM), differential scanning calorimetry (DSC), Fourier transform infrared (FTIR) and static light scattering (SLS). Copolymer degradation was induced using chloroform as the solvent. FTIR was used to follow variations on the functional groups of copolymers during the PLGA degradation process. The study was conducted using the evidence provided from the modifications of OH wavelength bands (3000-3500 cm^{-1}) obtained from FTIR. A viable reaction scheme to explain the degradation of the copolymer was developed based on the monomolecular constant reaction rates generated by the decomposition dynamic of OH groups.

Keywords: poly(lactic-L(+)-co-glycolic acid), kinetic study, chloroform, degradation, biopolymers.

Resumen

La interacción polímero/solvente es de mucho interés en diversas áreas, como la ingeniería de tejidos, el reciclado de plásticos y la industria de los sistemas de liberación de fármacos, entre otras. Se ha reportado que el efecto del solvente sobre los polímeros puede determinar las propiedades físicas y químicas en aplicaciones específicas. Así, este artículo describe un estudio sobre la degradación del poli(ácido-L(+)-láctico-co-glicólico), PLGA, usando cloroformo como medio de degradación. El PLGA se sintetizó por polimerización por apertura de cadena en 5 relaciones volumétricas de ácido láctico/glicólico (90/10, 80/20, 70/30, 60/40, 50/50). Estos co-polímeros se caracterizaron usando microscopia electrónica de barrido, calorimetría diferencial de barrido, espectroscopia de infrarrojo por trasformada de Fourier y dispersión estática de luz. En la degradación se utilizó cloroformo como solvente, en donde las variaciones de los grupos funcionales se analizaron mediante espectroscopia de infrarrojo. El estudio se realizó a partir de la evidencia proporcionada por las modificaciones de las bandas características de los grupos OH (3000-3500 cm^{-1}). Con estos resultados se propuso un esquema de reacción de PLGA, basado en las constantes cinéticas de degradación uni-moleculares, obtenidas en la dinámica de descomposición de los grupos OH.

Palabras clave: poli(ácido-L(+) láctico-co-glicólico), estudio cinético, cloroformo, degradación, biopolímeros.

1 Introduction

Natural and synthetic biocompatible and biodegradable polymers are often used for the preparation of particulate systems (Raval *et al.*, 2007). They have attracted much attention due to the environmental concerns and sustainability issues associated with petroleum-based polymers (Rasal *et al.*, 2010). Although natural polymers possess high degradability and negligible toxicity, synthetic polymers can be produced under controlled conditions and exhibit reproducible and predictable

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physical and mechanical properties, such as porosity, degradation time, tensile strength and elastic modulus (Dhandayunthapani *et al*., 2011). Therefore, these polymers are well suited for biomedical applications such as sutures (Williams, 1981; Gupta *et al*., 2007), regenerative medicine (Nair and Laurencin, 2007; Zellin *et al*., 1996), tissue engineering scaffolds (Seyednejad *et al*., 2011; Place *et al*., 2009) and drug delivery systems (DDS) (Xu *et al*., 2013; Sackett and Narasimhan, 2011; Saito *et al*., 2005).

Polyesters used for medical applications are mainly derived from glycolide (GA), lactide (LA), β-butyrolactone (β-BL), ε-caprolactone (ε-CL), 1,5-dioxepan-2-one(DXO) and trimethyl carbonate (TMC) (Albertsson and Varma, 2003; Buttafoco *et al*., 2006; Regnier-Delplace *et al*., 2013; Maharana *et al*., 2009). In this field, the major interest has focused on poly(lactic acid) (PLA), poly(glycolic acid) (PGA) and their copolymer Poly(lactic-co-glycolic acid) (PLGA) because they have been commercially synthesized at different ratios and they are approved by FDA for many clinical applications (Nair and Laurencin, 2007; Fredenberg *et al*., 2011; Engineer *et al*., 2011).

The physicochemical characteristics of PLGA copolymers are strongly affected by the synthesis mechanism used. The polycondensation of LA and GA at temperatures above 120 °C under water-removal conditions produces PLGA with low molecular weight. Direct polycondensation of hydroxy acids, carried out by azeotropic distillation of high boiling point solvents, allows the synthesis of PLGA copolymers with higher molecular weight (Wang *et al*., 2006). However, the solvent introduces higher levels of complexity of both process control and purification of the end product. Moreover, high molecular weight polymers can be synthesized by ring opening polymerization (ROP) of cyclic dimers, such as lactide and glicolide, using a coordinative catalyst at high temperatures (130-220 °C) (Wang *et al*., 2006; Jérôme and Lecomte, 2008; Madhavan *et al*., 2010; Chisholm, 2010). Several metal complexes have been used as catalyst; in particular, stannous octoate ($SnOct_2$) is a highly efficient commercial catalyst and a permitted food additive in numerous countries (Storey and Sherman, 2002; Dong *et al*., 2001).

PLGA as a bulk-degrading polyester degrades by the non-specific scission of the ester backbone (Sackett and Narasimhan, 2011). In the human body, this polyester breaks down into the original monomers glycolic and lactic acid, which can be excreted in the urine or converted into carbon dioxide and water via the citric acid cycle (Maurus and Kaeding, 2004; Vert, 2005; Ford *et al*., 2013).

Polyesters are very soluble in organic solvents and hydrolyze in water because of hydrogen bonding interactions (Nair and Laurencin, 2007; Etter, 1990; Lucas *et al*., 2008; Wade, 2004). Some methods as evaporation, solvent casting/particulate leaching, or phase separation have been used in order to produce porous scaffolds and nanoparticles as carries in DDS (Rasal *et al*., 2010; Ito *et al*., 2009). From these methods, the most popular method is the solvent casting/particulate leaching. This technique involves casting polymer dissolved in solvent over a leachable porogen, followed by solvent evaporation and porogen removal (Sander *et al*., 2004; Lanza *et al*., 2000). The major drawback of this technique is the toxic nature of the solvents used. It was proved that a scaffold designed by this method retains some of the solvent and their toxicity that can damage cells and nearby tissues (Rasal *et al*., 2010). It was found that under glass transition temperature, polymer dissolution could experiment a crack process running into the polymer matrix, when these cracks coalesce small blocks of the polymer are caused and eruption process appears on the polymer surface. In addition, it was observed that cracking of polymer begins faster with compatible solvents than poorer solvents, because of higher diffusion rates and swelling power of their molecules (Miller-Chou and Koenig, 2003).

Chang and Seok (2006) have made studies about the effect of using different organic solvents with several stabilizers in the formation of PLGA nanoparticles. They prepared nanoparticles by the emulsification-diffusion method and reported the influence of organic solvents on the particle size distribution by photon correlation spectroscopy. They found that the solubility of organic phase solvents in water was an important parameter affecting the mean size of PLGA nanoparticles, when an ionic stabilizer was used. All particles showed a large mean diameter above 110 nm, irrespective of the type of organic solvents.

Sander and collaborators (2004) have manufactured 75/25 PLGA scaffolds using solvents casting/particulate leaching technique with three different solvents: acetone, chloroform and methylene chloride. Static light scattering experiment was used to find out the morphology of the polymer in each solvent. Results showed that the polymer is well solvated in acetone and methylene chloride, but forms aggregates in chloroform. They found that solvent choice creates small but significant differences in scaffold properties, and that the rate of evaporation is more important in affecting scaffold microstructure than polymer/solvent interaction.

None of the aforementioned studies have presented any reaction mechanism explaining the nature of the interaction between copolymer and solvent. Thus, the aim of this paper was to evaluate the effect of chloroform as solvent on different ratios of monomers used to synthetize PLGA.

For this purpose, a series of PLGA with different composition of L(+)-lactic acid and glycolic acid were synthesized by ring opening polymerization (ROP) using $SnOct_2$ as catalyst. PLGA materials were dissolved in chloroform and their degradation process was followed by FTIR at intervals of 24h. A feasible mechanism of degradation was proposed and reaction rate constants were calculated.

2 Materials and methods

2.1 Materials

L(+)-lactic acid (L-LA, 80 wt%) and glycolic acid (GA, 70 wt% and relative density $d = 1.25$) in solution were obtained from Sigma Aldrich. Stannous octoate ($SnOct_2$, Sigma Aldrich) was used as a catalyst. ACS grade chloroform was obtained from Format Company and used in all copolymers dissolutions. Toluene (J. T. Baker, 99.5%) was used as a reference standard for molecular weight measurements by static light scattering.

2.2 Experimental

2.2.1. Copolymer synthesis

A series of PLGA copolymers were synthesized with different L-LA/GA ratios (90/10, 80/20, 70/30, 60/40, 50/50 $\%V_{L-LA}/\%V_{GA}$), using a variation of the method described by Wang and Zhao (2006). The reaction system consisted of a round bottom flask with a mechanical stirrer and a condenser connected to a flask ball. The monomers mixture at the desired L-LA/GA volume ratios was dehydrated from room temperature to 135°C under a N_2 atmosphere. Water was removed to the flask ball and the mixture obtained was weighed. The dry product was heated at 165°C, and 0.5 wt% $Sn(Oct)_2$ was added to the mixture as a catalyst. Polymerization was carried out at 0.3 mmHg in a Schlenk line for 10 h. The stirring and temperature were kept constant to increase the yield of high molecular weight PLGA.

2.2.2. Copolymer degradation

250 mg of PLGA samples of each volume ratio were dissolved in 50 mL of chloroform. The solutions were placed in glass vials at room temperature and subjected to constant shaking for 4 days. Samples of 0.2 mL were withdrawn at intervals of 24 h, and analyzed by FTIR to follow the polymer degradation.

2.2.3. Mathematical modeling

Based on pseudo-monomolecular dynamics, a mathematical model is proposed for the decomposition of the PLGA copolymers. The decaying exponential function [eq (1)] selected fits the variation of the OH stretching IR peak area observed in the degradation experiments:

$$[OH(t)] = [OH(0)]e^{-bt} \tag{1}$$

where [OH(t)] is the concentration of -OH measured during the reaction, [OH(0)] is the initial concentration of the organic compound, and b is the pseudo-monomolecular reaction rate constant obtained using a common nonlinear least-squares method and yielded a statistically significant result.

2.3 Characterization

2.3.1. Static Light Scattering (SLS)

The average molecular weight (Mw) of the copolymers was determined by static light scattering. A series of solutions of each copolymer was prepared using chloroform as the solvent. The concentration (C) was varied between 0.25 and 1 g/L from a stock solution. Toluene was used as the standard reference liquid. PTFE membranes (0.20 μm pore size) were used to filter the standard and polymer solutions. The polymer solutions were measured by triplicate at 25 °C in a Zetasizer Nano ZS (Malvern Instruments). Measurements of scattered light (KC/R_θ) for each sample were plotted as a function of concentration in a Debbye plot diagram. For these plots, the y-intercept is the inverse of the average molecular weight expressed in Daltons, K is the optical constant, and θ is the Rayleigh ratio.

2.3.2. Differential Scanning Calorimetry (DSC)

Glass transition temperature (T_g) was determined using a Metler-Toledo Differential scanning calorimeter. All the samples (20-30 mg) were placed in standard aluminum oxide vessels and were scanned from 30 to 300 °C under nitrogen atmosphere at a heating rate of 10 °C/min. All the DSC thermograms were obtained from the first heating cycle. The glass transition temperature was determined using the first derivative obtained from the heat flow curve.

2.3.3. Attenuated Total Reflectance-Fourier Transformed Infrared Spectroscopy (ATR-FTIR)

The ATR-FTIR spectra (ATR-FTIR Pike Technologies spectrometer) of monomers, intermediate products, synthesized materials and degraded copolymers were recorded in transmission mode over the mid infrared spectroscopy region (4000-400 cm^{-1}), using a KBr prism as internal reflection cell. Each sample was scanned 64 times at room temperature.

2.3.4. Scanning Electron Microscopy (SEM)

The morphology of the PLGA was characterized by SEM using a high resolution JEOL JSM6701F microscope. 1000x magnification images were obtained at an accelerating voltage of 5 kV. The PLGA films were prepared by dissolving the copolymers in chloroform (5 g/L). Solutions were then placed on copper stubs, and the samples were dried under vacuum pressure and coated with graphite by electro-sputtering.

3 Results and discussion

3.1 Copolymer synthesis

The FTIR spectra of the L-LA and GA dimers, together with the corresponding to the intermediate lactide and glycolide species are shown in Fig 1. Furthermore, characteristic band absorptions are listed in Table 1. Carboxylic acids exist predominantly as hydrogen bonded dimers in condensed phases (Bettelheim *et al.*, 2010; Ratajczak-Sitarz and Katrusiak, 2011; Swiderski *et al.*, 2011). The carboxyl group is mainly associated with two characteristic infrared stretching absorptions νC=O and νO-H, which change markedly with hydrogen bonding. The O-H stretching absorption for such dimers is very strong and broad. Besides, L-LA and GA species contain two different kinds of O-H bond, the one in the acid at 2500-3300 cm^{-1} and the simple alcohol type in α carbon absorbing at 3230-3550 cm^{-1} (Table 1). Regarding the carbonyl group, the dimer νC=O band is observed at 1680-1725 cm^{-1}; the monomer absorbs at a wavenumber 50 cm^{-1} higher.

As can be seen in Fig 1-a and c (L-LA and GA respectively) both spectra show a wide band, due to the νO-H, extending from 2250 to 3550 cm^{-1}. This absorption overlaps the sharper C-H stretching peaks, which may be seen extending beyond the O-H envelope at 2990 cm^{-1} (methyl group, figure 1-a), 2940 and 2927 cm^{-1} (methylene group, figures 1-a and 1-c respectively). The smaller peaks protruding at 2500-2600 cm^{-1} are characteristic of the dimer in both cases. The carbonyl asymmetric stretching frequency of the dimer is found at 1716 cm^{-1} and 1720 cm^{-1} for lactic acid and glycolic acid. In both cases, the presence of water is evidenced by the shoulder appearing around 1645 cm^{-1}, corresponding to the bending O-H vibration mode. The bands appearing at 1223 and 1450 cm^{-1} in both spectra might correspond to the C-O stretching and O-H bending vibrations respectively, although the latter may not be distinguishable from C-H bending bands in the same region. Finally, the strong, sharp band observed at 1125 and 1085 cm^{-1} (Figs 1-a and 1-c) may be due to the C-O rocking vibration mode, νCO (Colthup *et al.*, 1975).

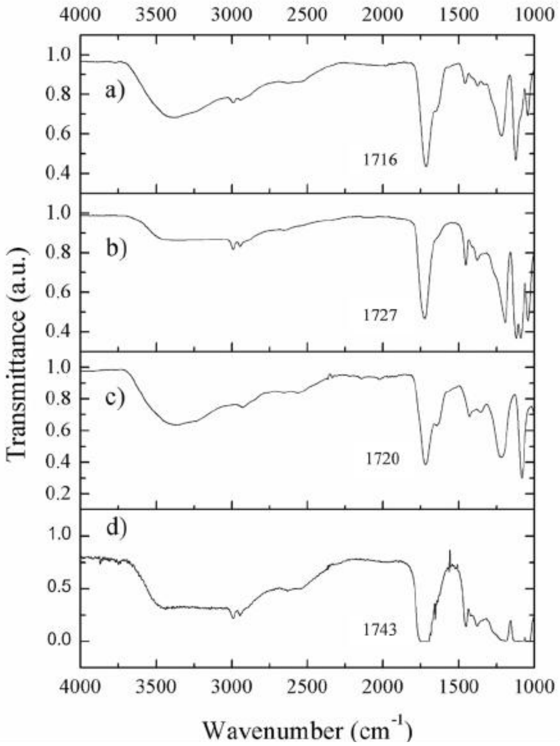


Fig. 1. FTIR spectra for a) lactic acid, b) lactide, c) glycolic acid and d) glycolide

Table 1 Band shifting of characteristic IR absorptions acquired from Fig 1

Lactic acid	Lactide	Glycolic acid	Glycolide	Bond	Functional group
Wavenumber (cm^{-1})					
3300-2500	3480	3300-2500	3480	O-H stretch	Carboxylic acid
3550-3230	-	3550-3230	-	O-H stretch, H bonded	Alcohol
1716	1727	1720	1743	C=O stretch	Carboxylic acid
2990	2990	2990	2990	C-H stretch	Alkane
1223	-	1223	-	C-O stretch	Carboxylic acid
1125	1000-1300	1085	1000-1300	C-O rock	Ester, carboxylic acid

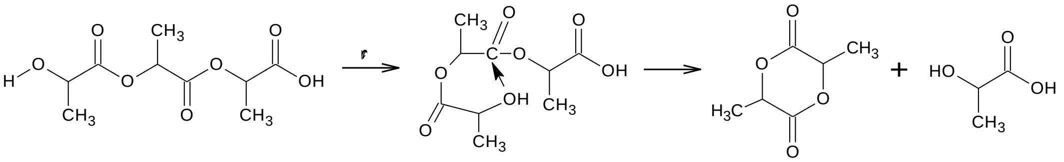


Fig. 2. Transesterification reaction for the synthesis of lactide.

The reactions taking place during the synthesis of the PLGA copolymers are described as follows. First, the carboxylic dimers are dehydrated at 135°C for 6 h, under low pressure to form lactide and glycolide by back-bitting. With regard to the lactide and glycolide species (Figs 1-b and 1-d respectively), three main features are clearly identified (Kohn *et al.*, 1983) (Table 1): (1) The shifting of the νC=O band to higher wavenumbers (to 1727 cm^{-1} for the lactide and to 1743cm^{-1} for the glycolide); (2) the appearance of two new bands at 1000-1100 cm^{-1}; (3) depletion and shifting towards higher wavenumbers of the νO-H band. Those changes indicate the formation of the cyclic di-esters, characterized by a strong band at 1730-1750 cm^{-1} (νC=O) and a doublet appearing from 1000 to 1300 cm^{-1}, corresponding to the stretching O-CO absorption, νO-CO. The presence of the weakened νO-H band at 3480 cm^{-1} in both spectra might be attributed to moisture in the samples. At this point the color of solution turns from colorless to light yellow; this fact, along with the evidence from the spectra (Figs1-b and d) suggest that the first stage of the synthesis is already completed (Fig 2).

In the second stage of synthesis, the temperature is increased gradually to 165 °C, under low pressure to remove by-products. At this time, the catalyst $Sn(Oct)_2$ is added to start the copolymerization reaction. According to Chisholm (2010), this commercially available catalyst precursor must be mixed with 5 equivalents of H_2O because formation of a Sn-OH bond is necessary for the reaction (Fig 3-a).

The Sn-OH bond initiates ring opening of the lactide and glycolide yielded in the first stage. This forms the Sn-alkoxide groups: SnOCHMeC(O)OCHMeC(O)OH (Figs 3-b) and SnOCHC(O)OCHC(O)OH (Fig 3-c). This reaction called ring opening copolymerization (ROP) is considered as a transesterification reaction that follows at temperatures near the melting point or at long reaction times. Intermolecular transesterification reactions serve to modify the sequences of copolylactones and prevent the formation of block copolymers (Stridsberg, 2000).

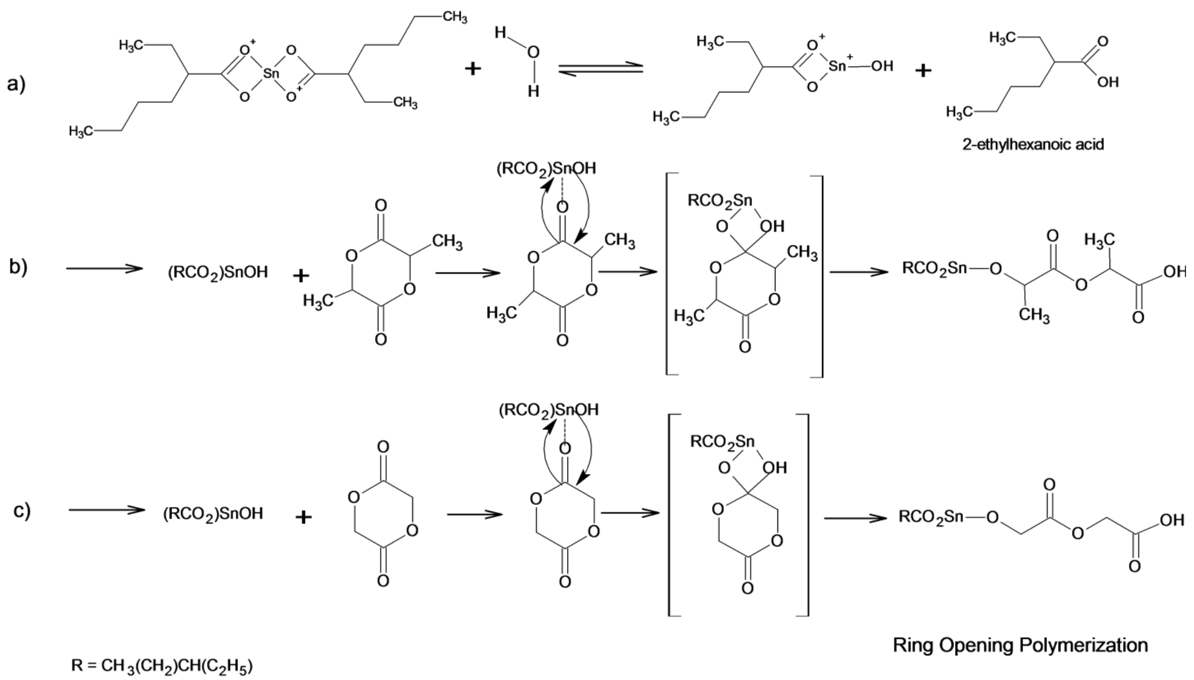


Fig. 3. Reaction scheme of a) Sn-OH bond formation, b) lactide ROP and c) glycolide ROP.

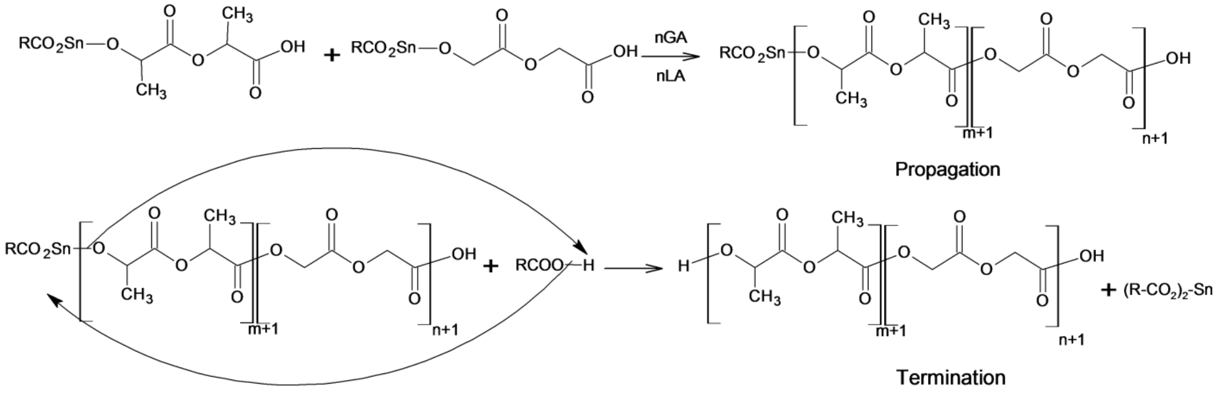


Fig. 4. Scheme of PLGA termination.

In this step, the viscosity of the solution increases due to the increase in the molecular weight of the polymer.

Finally, the carboxylic groups of polymeric chains react reversibly with oligomers that contain a Sn-alkoxide group (Fig 4). This results in the formation of PLGA due to the reaction of the labile Sn-alkoxide group and the release of a proton from the RCOO-H group (Chisholm, 2010). The polymeric solution changes from light yellow to brown during this step, and the material changes from liquid to brown solid when the temperature decreased to room temperature.

3.2 Copolymer characterization

In Table 2, the glass transition temperature (Tg) for each of the PLGA samples obtained from the DSC thermograms is presented. The experimental results were contrasted with the theoretical Tg, calculated from the Fox equation:

$$\frac{1}{T_g} = \frac{x_{L-LA}}{T_{gL-LA}} + \frac{x_{GA}}{T_{gGA}} \tag{2}$$

Where x stands for mass fraction and the subscripts L-LA and GA denote poly(L-lactic acid) and poly(glycolic acid) respectively. The theoretical glass transition temperature values (T_{gL-LA} = 62.5 °C,

Table 2 Theoretical and experimental T_g values of PLGA copolymers

L-LA/GA Ratio	Theoretical T_g (°C)	Experimental T_g (°C)
90/10	57.69	56.66
80/20	53.57	50.67
70/30	50.00	49.96
60/40	46.88	49.36
50/50	44.12	48.61

T_{gGA} = 37.5 °C) of the homopolymers were obtained from literature (Nair and Laurencin, 2007) and the relative density of glycolic acid (d = 1.25) in solution was used for determining the mass fractions. Table 2 shows the experimental and theoretical T_g values for all the PLGA copolymers synthesized. As it can be seen, the experimental T_g values obtained are very close to the corresponding values calculated from eq (2).

Tg decreases when the contents of glycolic acid in the copolymer is increased. Since this study was carried out in the glassy state ($T < Tg$), these results indicate that, when the lactic acid contents in the material is increased, the mobility of the macromolecules is diminished, leading to lower degradation rates.

The crystallinity degree of the copolymers were calculated from eq. (3), where ΔH_{exp} is the experimental heat of fusion measured by DSC and ΔH_c^0 is the theoretical value, estimated from 100% crystalline polylactide and polyglycolide species.

$$\alpha = \frac{\Delta H_{exp}}{\Delta H_c^0} \times 100 \tag{3}$$

The estimated heat of fusion of 100% crystalline PGA is 190 J/g, while a calculated value of 93.7 J/g has been reported for PLLA. As can be seen in Table 3, the exothermic ΔH_{exp} increases when glycolic acid concentration increases. The calculated crystallinity values are in agreement with those reported for commercial PLGA products (Domb *et al.*, 1998).

The FTIR spectra for the synthesized copolymers are presented in Fig 5. The copolymerization spectra show that the OH band of water disappeared and OH end groups centered at 3516 cm^{-1} appeared. Carbonyl peaks of monomers (1716 and 1720 cm^{-1}) are shifted to form a single strong band at 1744 cm^{-1}.

Table 3 Crystallinity degree of PLGA copolymers calculated from eq (3)

PLGA (%V_{L-LA}/%V_{GA})	ΔH_{exp} (J/g)	ΔH_c (J/g)	α (%)
50/50	-54	141.8	38
70/30	-52	122.6	42
90/10	-40	103.3	38

Further, alkyl groups are found at 2997 and 2947 cm^{-1}. Both groups remain unchanged when the %V_{L-LA}/%V_{GA} ratio is varied. However, new bands appear in the 1280-1270 cm^{-1} and 1180-1167 cm^{-1} region, which is due to the formation of C-O groups during copolymerization. Specific regions in the spectra of lactic acid at 1125 and 1044 cm^{-1}, and at 1085 cm^{-1} for glycolic acid remain unchanged in copolymers.

The surface of all PLGA was examined by SEM before degradation. Micrographs corresponding to PLGA samples 90/10, 70/30 and 50/50 are shown in Fig 6. In all cases, smooth surfaces are observed, while porosity is increasing with the GA contents. However, the samples with higher concentrations of lactic acid show a surface with more amounts of cracks. These results are in agreement with the average molecular weight obtained for the samples, i.e. the increment of glycolic acid concentration in the copolymer produces longer chains and, consequently smoother surfaces.

3.3 Degradation process

The average molecular weights (M_w) for the initial PLGA compositions and their coefficients of determination (R^2) were obtained from the Debye plot (Fig 7). The results are summarized in Table 4. It can be seen that when the GA monomer ratio in the copolymer is increased, the Mw is increased too. This behavior has been associated with the melting point and degree of crystallinity of each homopolymer (Nair and Laurencin, 2007; Makadia and Siegel, 2011). Some authors have reported that the melting point of L-LA homopolymer is 175 °C, and its crystallinity degree is 45-55%, while the melting point of a GA homopolymer is 200 °C, and its crystallinity degree is 37%. That means, the increment of glycolic acid proportion allows the formation of polymer chains with high molecular weights.

After 80 h immersed in chloroform (Fig 8), the PLGA samples display the same trend that the initial samples (Fig 7) in the Debye plot, indicating that the effect of the monomer ratio on the molecular

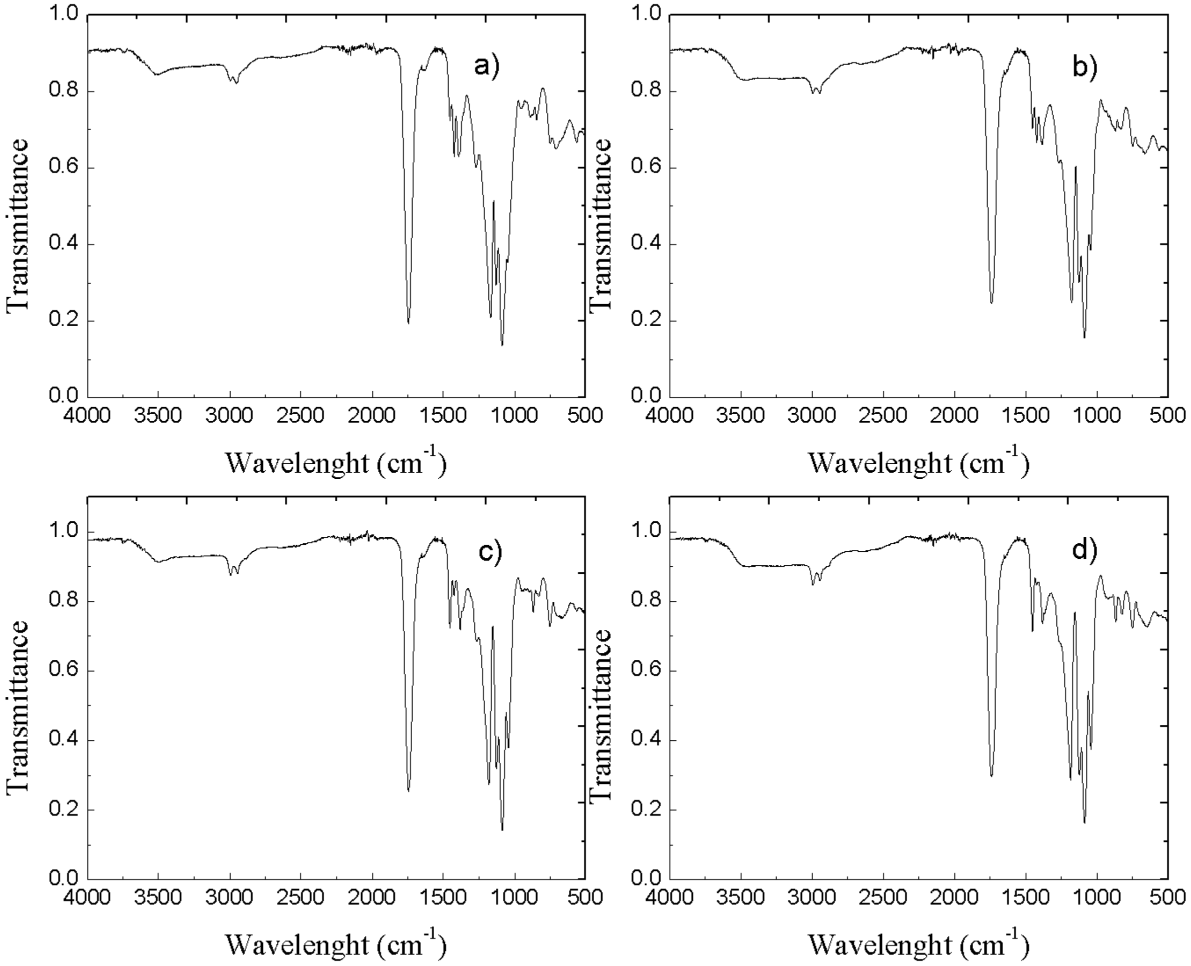
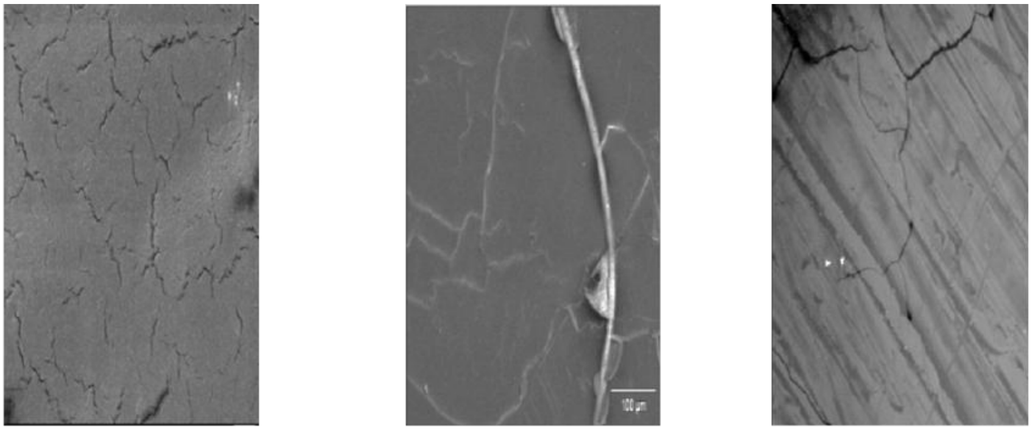


Fig. 5. FTIR spectra of the PLGA copolymers: a) 50/50, b) 60/40, c) 80/20, and d) 90/10.



a) 90/10 **b) 70/30** **c) 50/50**

Fig. 6. SEM images of a) 90/10, b) 70/30, and c) 50/50 PLGA

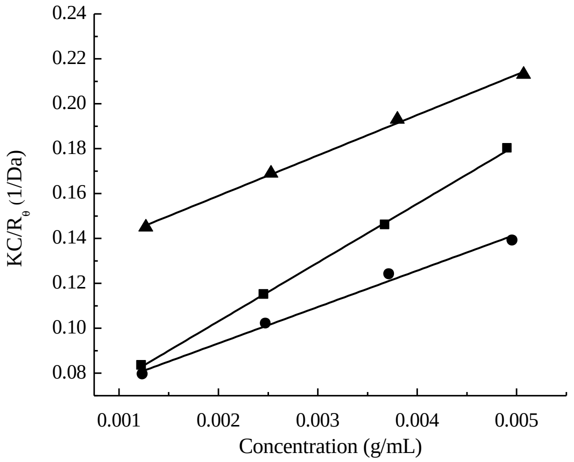


Fig. 7. Debye plot of initial PLGA samples for (■) 50/50, (●) 70/30, (▲) 90/10 %(V_{L-LA}/V_{GA})

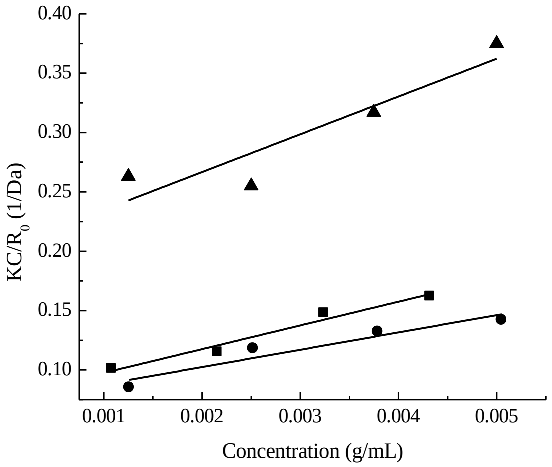


Fig. 8. Debye plot of final PLGA degradation for (■) 50/50, (●) 70/30, (▲) 90/10 %(V_{L-LA}/V_{GA})

weight of the copolymers is maintained even after their degradation. The final molecular weights, shown in Table 5, are lower to the corresponding initial values, evidencing the rupture in the polymer backbone.

Fig 9 shows the spectra of the standard chloroform and decomposition of the 70/30 copolymer at 0, 24, 48, 72, 80 h. At all intervals of time, the characteristic bands due to the νC=O, νO-H, νC-O groups remained without displacement. However, the intensity of the C=O band at 1744 cm^{-1} increase and decrease depending of the monomer ratio, but at final times the intensity returned to its initial level. This behavior suggests that competing reactions occurring and the carboxyl groups tend to regenerate. Also, the intensity of the OH end groups at 3500 cm^{-1} showed depletion of the band; both results were time dependent. This behavior was found for all PLGA compositions (Fig 9-a to 9-e).

During all the degradation process, pH of the polymeric solutions in chloroform was measured. Chloroform pH was 7.91 before dilution with PLGA. After 2 hours of prepared solutions, pH decreased until 1 and remained constant throughout the whole experiment. The results of FTIR spectra and pH measures suggest that degradation process do not affect the C=O bond as it is typically the case in ester hydrolysis under acidic conditions (Gorrasi and Pantani, 2013).

3.4 *Estimation of monomolecular reaction rate constants*

To support the ideas presented above, dynamics of the PLGA degradation was followed by normalizing and calculating the area under the curve (AUC) corresponding to the main functional groups in the FTIR spectra. As an example, Table 6 summarizes experimental and normalized AUC of main bands from Fig 9. It can be seen that the OH AUC tend to zero and the rest of the bands remain practically unchanged.

Table 4 Average molecular weight of the PLGA copolymers before degradation

L-LA/GA Ratio	Mw (KDa)	R^2
90/10	8.13	0.9980
80/20	12.27	0.9472
70/30	16.40	0.9919
60/40	18.05	0.9700
50/50	19.70	0.9996

Table 5 Average molecular weight of the PLGA copolymers after 80 h of degradation

L-LA/GA Ratio	Mw (KDa)	R^2
90/10	4.93	0.8532
80/20	11.70	0.9893
70/30	13.66	0.9228
60/40	11.98	0.9812
50/50	12.90	0.9700

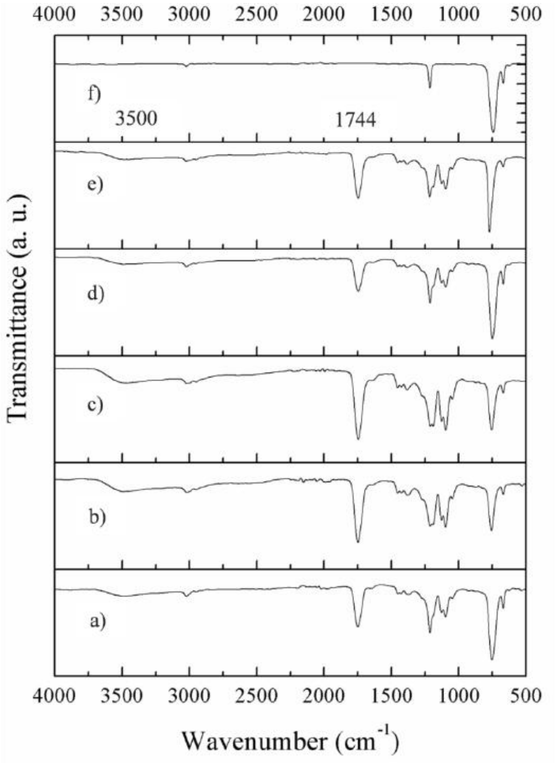


Fig. 9. FTIR spectra of the PLGA 70/30 copolymer degraded in chloroform: a) 0 h, b) 24 h, c) 48 h, d) 72 h, e) 80 h and f) chloroform.

Fig 10 shows the change in the AUC for hydroxyl groups with respect to time of all PLGA compositions. Experimental results were fitted with a pseudo-monomolecular mathematical modeling from the OH bond depletion. The AUC slope of hydroxyl groups decreased when concentration of glycolic acid was increased. As mentioned before, the addition of glycolic acid allowed longer chains formation and consequently, less terminal carboxylic groups are found in the polymeric chains.

To obtain the decomposition constants (b) for each PLGA concentration, AUC of OH groups were used. Constants were calculated with equation (1) and results are listed in Table 7. The trend of the b parameter is related with decomposition dynamic in Fig 10. It shows that the decreasing rate of OH groups was influenced by the monomer ratio. That is, when concentration of glycolic acid increases, the depletion rate of OH groups was higher, as demonstrated in Table 7. It can be explained as consequence of the degradation process being carried out in acidic medium (pH=1.0), making the PLGA less soluble at increasing lactic acid concentration due to the increment of methyl groups. Thus, PLGA with higher lactic acid composition showed the slower degradation rate.

Table 6 AUC of experimental (Exp) and normalized (Norm) data of 70/30 V_{L-LA}/V_{GA} ratio from Fig 9.

70/30 %V_{LA}/%V_{GA}	Functional group Area Under Curve (AUC)*							
Time (h)	OH		CH_2		C=O		CO	
	Exp	Norm	Exp	Norm	Exp	Norm	Exp	Norm
0	8.3851	1.0000	1.0695	0.7393	6.2851	0.7570	3.0241	0.6210
24	4.4281	0.5281	1.3359	0.9234	7.5532	0.9097	3.6396	0.7474
48	2.4966	0.2977	1.4467	1.0000	8.3027	1.0000	4.8696	1.0000
72	0.5785	0.0690	0.9269	0.6407	5.3292	0.6419	3.3517	0.6883
80	0.3114	0.0371	0.9744	0.6735	6.5708	0.7914	3.8337	0.7873

*AUC calculated from $[OH(t)]=[OH(0)]e^{-bt}$

Table 7 Decomposition constants of PLGA after 80 h of degradation in chloroform.

PLGA (%V_{L-LA}/%V_{GA})	Constant b h^{-1}	R^2
50/50	2.325	0.6339
60/40	1.280	0.7745
70/30	0.7314	0.9724
80/20	0.4250	0.9044
90/10	0.591	0.8968

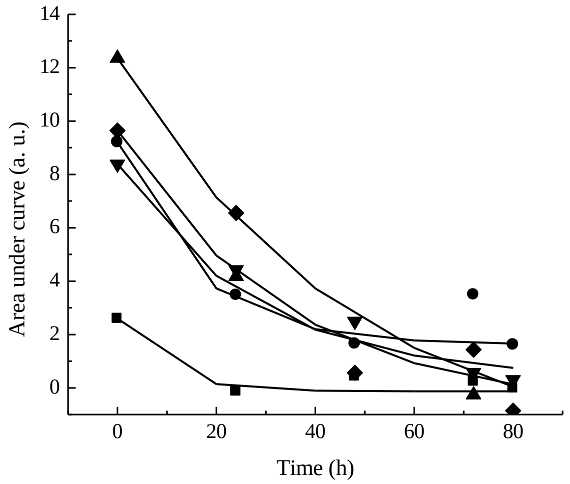


Fig. 10. Decomposition dynamics of the OH groups: a) 50/50 ■, b) 60/40 •, c) 70/30 ▲, d) 80/20▼, and e) 90/10 ◆.

Based on the dynamics of the copolymer decomposition in chloroform and from the FTIR spectra results, nucleophilic attack at the adjacent α-carbon is proposed as a degradation scheme. This behavior is observed because α-carbon atoms are electron deficient due to their position next to a carbonyl group (Wade, 2004).

Fig 11 illustrates the degradation scheme of the PLGA samples at the different volume ratios. In the first bond-breaking step (Fig 11-a), the labile α-carbon of the carboxylic acid may be subtracted due to a nucleophilic displacement reaction causing protonation. Furthermore, a bond break in the polymer chain results in a stable lactic acid monomer. The presence of chloroform as a medium in excess allows the subsequent attack on the remaining protonated polymer chain. Again, the remaining chain is attacked by an interaction with the α-carbon, yielding unstable live groups (Fig 11-b and c), the formation of cyclic glycolide or lactide and regeneration of chloroform, in agreement with the results showed in Fig 12. Decomposition dynamic of chloroform was calculated from AUC of C-Cl band at 700-800 cm^{-1} of FTIR spectra. It can be seen that all compositions reached an AUC minimum value before 50 h of exposure, after it increases until the initial value approximately.

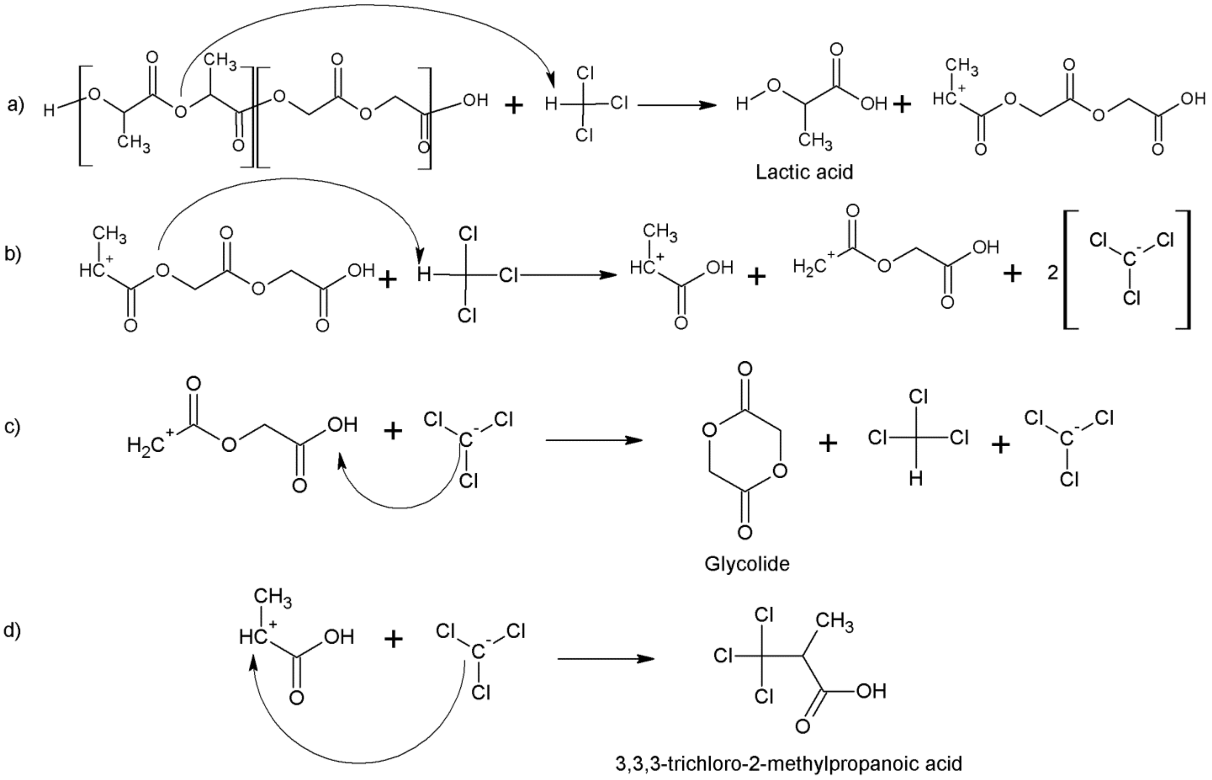


Fig 11 Proposed degradation scheme of PLGA in chloroform.

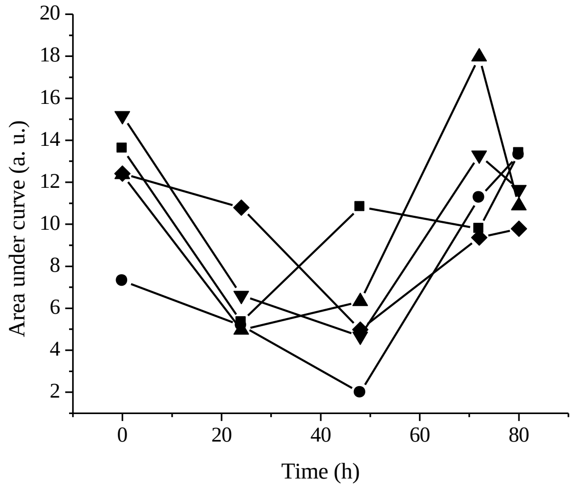


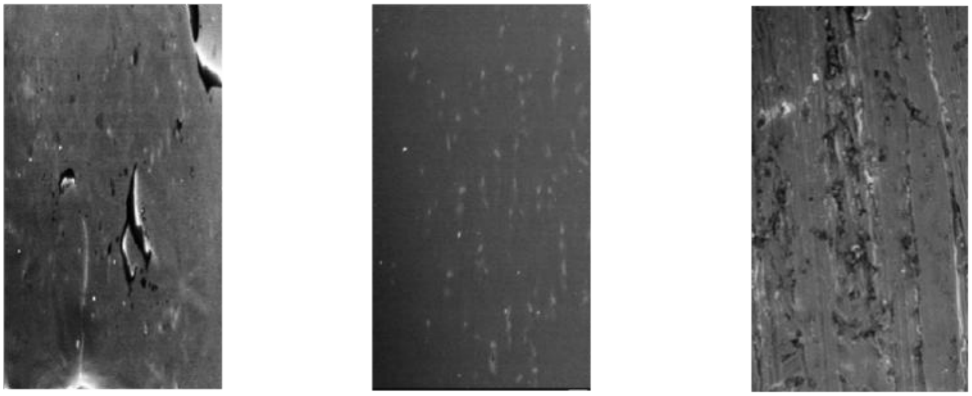
Fig 12 Decomposition dynamics of the chloroform: a) 50/50■, b) 60/40●, c) 70/30▲, d) 80/20▼, and e) 90/10◆.

In the final interaction, protonated open chains may react with live $^{-}CCl_3$ chains to form lactone glycolide, and possible lactide depending on the competing reactions. Accordingly, OH groups tend to disappear and C=O groups remain, forming cyclic chains. 3, 3, 3-trichloro-2-methylpropanoic acid is probably produced, but likely their OH group tends to disappear to form low molecular weight molecules (Fig 11-d).

SEM images of 50/50, 70/30 and 90/10 PLGA after 7 days of degradation in chloroform are shown in Fig 13. Images show an increase in porosity at the surface due to chloroform exposure. These images suggest that the degradation of the PLGA increased the porosity as the GA ratio increased in the copolymer. Thus, when the glycolic acid is present in greater concentrations, the low crystallinity promotes material depletion.

Conclusions

PLGA copolymers with different volumetric ratio of glycolic and lactic acids were synthesized. These materials were characterized using different techniques including DSC, FTIR, SLS, and SEM. They were degraded using chloroform as a solvent and followed by FTIR. Characterization results by DSC confirm an amorphous material, where all PLGA samples presented glass transition temperature. In FTIR spectra of PLGA synthesized, the triple characteristic bands of carboxylic acids (O-H, C=O, C-O) were observed at their corresponding frequency. Average molecular weights determined in SLS were found that increased when glycolic acid ratio increases. Degradation results showed variation in the band at 3000-3500 cm^{-1} associated with OH groups. A kinetic study was used to determine pseudo-monomolecular reaction rate constants from degradation of PLGA. With these values, a reaction scheme was proposed to describe the changes observed by FTIR and SEM of copolymers. Also, they were found to depend on the solvent, time of exposure and monomer ratio. These results could confirm that interaction polymer/solvent originate undesirable time dependent byproducts, that could be harmful in biomedical applications.



a) 90/10 b) 70/30 c) 50/50

Fig 13 SEM images of a) 90/10, b) 70/30, and c) 50/50 PLGA after 7 days of degradation.

Acknowledgements

This work was supported by Consejo Nacional de Ciencia y Tecnología (CONACYT) and Instituto Politécnico Nacional (IPN). The authors warmly thank Dr. Arizbeth Pérez for her support in developing the degradation mechanism.

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