

Degradation kinetics of poly(lactic-co-glycolic) acid block copolymer cast films in phosphate buffer solution as revealed by infrared and Raman spectroscopies

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ABSTRACT

Poly(lactic-co-glycolic) acid (PLGA) is an important copolymer used in drug delivery platforms where controlled release is required. In this work we investigated the *in vitro* degradation of four PLGA copolymers with *L/G* molar compositions of 50/50, 65/35, 75/25 and 95/5. ATR-IR and Raman spectroscopies were used to differentiate and quantify the degradation rates of glycolic and lactic units. Both techniques were used to determine the polymer composition as a function of degradation time and the degradation rate constants for the hydrolysis of glycolic and lactic units were calculated using a 1st order kinetics approach. Our results revealed a two stage process for the degradation of PLGA cast films in PBS in agreement with our previous work. The degradation rate constant for glycolic unit was found to be 1.3 times higher than for lactic units. In addition the degradation rate constants for *L* and *G* units were shown to decrease proportionally with increasing initial lactic content of the copolymer used to prepare the films.

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1. Introduction

Poly(lactic-co-glycolic) acid (PLGA) is a copolymer with regulatory acceptance for use in biomedical devices and drug delivery platforms. It has excellent biocompatibility properties as do its hydrolytic breakdown products, lactic and glycolic acids. Control of the polymer degradation rate is an important parameter in the use of PLGA for controlled drug release. There is a significant body of work that describes the impact of the chemical and physical design of the material/device on the *in vivo* and *in vitro* hydrolysis mechanisms of PLGA, see for examples references [1–12]. In a previous paper we have described the *in vitro* degradation of solvent cast PLGA 50/50 films in phosphate buffer solution (PBS) at 37 °C [12]. We have shown that the degradation process takes place in two stages. As soon as the films are immersed in media water was found to diffuse throughout the samples. Swelling and

wrinkling of the surface layer was observed with time. The degradation of the polymer in the bulk and in the surface layer was found to occur at different rates. Chain scission in the bulk of the films was observed as soon as the samples were immersed in the media resulting in an increase in the bulk acidity of the films. In acidic condition the autocatalysis of the hydrolysis reaction of PLGA is known to result in an increased rate of degradation of the polymer. On the other hand degradation of the swelled surface layer was found to be slow due to the buffering effect of the media that results in the absence of auto catalysis reaction. The second stage of the film degradation process was found to occur when the weight average molecular weight (M_w) of the polymer in the bulk of the film became low enough so as to allow the short polymer fragments, in other word oligomers, to diffuse in the media resulting in a decrease of the media pH and a shrinking of the films. For more details on this previous work we refer the reader to reference [12]. For greater size sample the degradation mechanism has been described in some detail by Vert et al. [11,13–15] and the processes and kinetics are modified compared to thin film degradation. In the large majority of cases published work on the degradation mechanisms of PLGA describes the overall chemistry of the process and do not distinguish between lactic and the glycolic units. In this paper, we discuss the degradation of PLGA as

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revealed by attenuated total reflectance infrared (ATR-IR) and Raman spectroscopies. The aim of this work is to characterise the *in vitro* degradation of PLGA cast films in PBS by following the hydrolysis of the individual lactic and glycolic acid units and to determine the kinetics for each. First, the spectroscopy of the films before degradation will be discussed. Then, the *in vitro* degradation in PBS of four polymers with different L/G ratios: 50/50, 65/35, 75/25 and 95/5 will be compared. Finally, the kinetics of degradation of lactic and glycolic units will be investigated.

2. Experimental

2.1. Materials and film preparation

Acid-terminated PLGA random copolymers with lactic to glycolic mole percentage ratios of 50/50, 65/35, 75/25, 95/5, 100/0 (PLA) and 0/100 (PGA) were supplied by AstraZeneca (Macclesfield, UK). The polymers were amorphous; the number average (M_n) and the weight average (M_w) molecular weights are summarised in Table 1. Dichloromethane (CH_2Cl_2 , $M = 84.93 \text{ g mol}^{-1}$, $T_b = 39.8^\circ\text{C}$, 99.9%), sodium chloride (NaCl , $M = 58.44 \text{ g mol}^{-1}$), sodium phosphate dibasic (Na_2HPO_4 , $M = 141.96 \text{ g mol}^{-1}$), potassium phosphate monobasic (KH_2PO_4 , $M = 16.09 \text{ g mol}^{-1}$) and hydrochloric acid (HCl , $M = 36.46 \text{ g mol}^{-1}$) were purchased from Aldrich. Sodium azide (NaN_3 , $M = 65.01 \text{ g mol}^{-1}$) was purchased from Fluka. All the chemicals were used as-received.

The PLGA films were prepared by solvent casting from dichloromethane. A 0.5 g ml^{-1} solution of PLGA in dichloromethane was prepared and stirred for 2 h at room temperature to ensure full dissolution of the polymer. A fixed volume ($600 \mu\text{L}$) of solution was dispensed using a pipette and spread on a glass slide inside a glass ring of 1.3 cm internal diameter. The samples were then dried in an oven at 100°C for 24 h. This methodology ensured that all films contained the same amount of polymer ($0.30 \pm 0.02 \text{ g}$) and were of the same thickness ($0.3 \pm 0.05 \text{ mm}$).

Phosphate buffered saline (PBS) solution was prepared by dissolving 8 g of sodium chloride (NaCl), 1.38 g of sodium phosphate dibasic (Na_2HPO_4), 0.19 g of potassium phosphate monobasic (KH_2PO_4) and 0.2 g of sodium azide (NaN_3) in 1 L of distilled water. The pH was adjusted to 7.4 using hydrochloric acid (HCl). The *in vitro* degradation studies were carried out by placing the polymer films into 20 mL of PBS solution in sealed jars, which were then placed into an incubator at $37.0 \pm 0.1^\circ\text{C}$.

2.2. Mass loss measurements

Samples were weighed before being placed in the degradation medium in order to measure the initial mass of the films, m_{ini} . At regular time intervals the films were recovered, gently washed with distilled water, swabbed with blotting paper and dried in an oven at

100°C for a week and weighed to obtain the dry mass left after degradation, m_{dry} . The mass remaining (%) of the film was then calculated using Equation (1).

$$\text{Mass remaining(\%)} = 100 - \frac{m_{\text{ini}} - m_{\text{dry}}}{m_{\text{ini}}} \times 100 \quad (1)$$

2.3. Attenuated total reflectance-infrared spectroscopy (ATR-IR)

ATR-IR spectra were recorded on a Thermo Nicolet Magna 860 FTIR spectrometer using a Golden Gate™ ATR accessory (Thermo Nicolet). Spectra of PLGA samples were taken during the *in vitro* degradation at different time points. The software OMNIC Version 7.2 was used to acquire and process the data.

2.4. Raman spectroscopy

This measurement was performed on a Thermo Almega dispersive Raman spectrometer (Thermo Nicolet), using a laser excitation wavelength of 633 nm and an Olympus BX51 microscope. Spectra of PLGA samples were obtained with a $10\times$ objective lens during *in vitro* degradation at different time points. Data acquisition and analysis was performed using OMNIC Version 7.2 software.

Fitting of both infrared and Raman spectral data was performed by non-linear least squares and using a Voigt line shape function (GRAMS32 AI Version 6.00 Peak Fit).

3. Results and discussion

3.1. Spectroscopy of dry films and band assignment

Before analysing the spectra of PGA, PLA and their copolymers, it is important to know which chemical groups are present in each polymer. The chemical structures are shown in Fig. 1. The chemical difference between lactic and glycolic units is the presence of a methyl group in the lactic units. Infrared and Raman spectroscopies are sensitive to the local chemical architecture of these polymers. The presence of the methyl group in the lactic units gives rise to additional characteristic bands that can be used to differentiate between lactic and glycolic units. The infrared and Raman spectra of each polymer studied are shown in Figs. 2 and 3, respectively. Assignments of vibrational infrared and Raman bands are explained on the basis of the spectra presented and previous work done by Vert [16–19] and Taddei [20,21] and are summarised in Table 2.

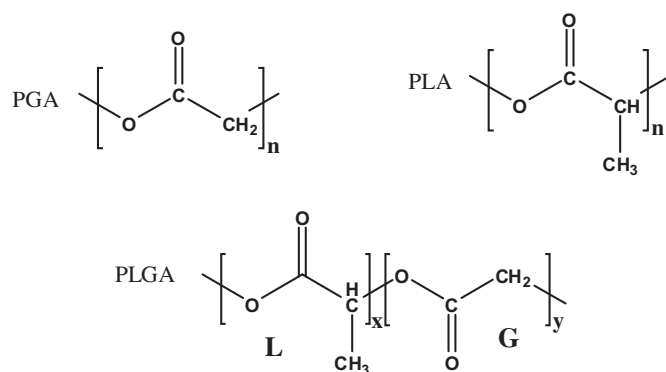


Table 1

List of samples' characteristics: L/G is the copolymer lactic/glycolic units molar ratio; M_n is the number average molecular weight; M_w is the weight average molecular weight; M_w/M_n is the polydispersity.

Samples	L/G	M_n (g mol ⁻¹)	M_w (g mol ⁻¹)	M_w/M_n
PLGA 50/50	50/50	4475	8965	1.78
PLGA 65/35	65/35	4705	8285	1.76
PLGA 75/25	75/25	6140	11,300	1.84
PLGA 95/5	95/5	5875	11,850	2.01
PLA	100/0	7535	10,200	1.36
PGA	0/100	—	—	—

Fig. 1. Chemical structures of PGA, PLA and PLGA polymers. (n : number of repeat units in PLA and PGA; x and y : number of lactic and glycolic units in PLGA respectively).

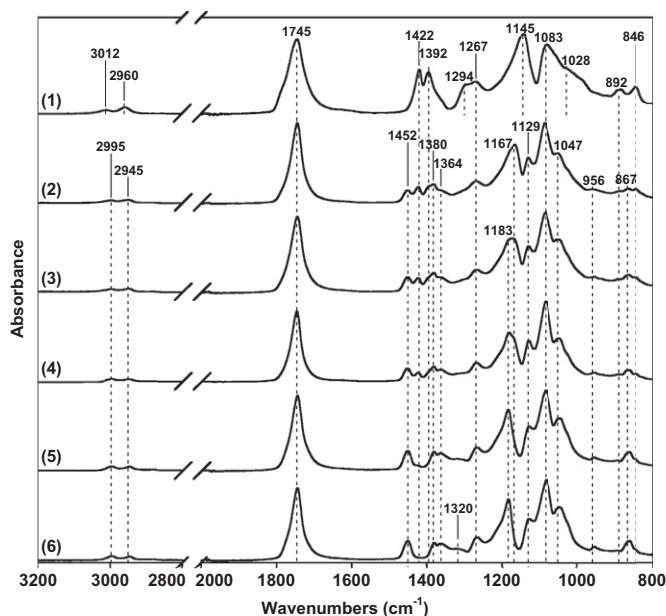


Fig. 2. Infrared spectra of (1) PGA, (2) PLGA 50/50, (3) PLGA 65/35, (4) PLGA 75/25, (5) PLGA 95/5 and (6) PLA dry films.

In the 1500–1300 cm^{-1} region, the deformation bands of CH_2 and CH_3 can be observed. The band at 1450 cm^{-1} in the infrared spectrum and at 1452 cm^{-1} in the Raman spectra corresponds to the anti-symmetric vibration of CH_3 from the lactic unit. The bending of CH_2 from the glycolic unit is observed at 1422 cm^{-1} in the infrared spectrum and at 1424 cm^{-1} in the Raman spectrum. The ratio of intensity of $\delta_{\text{as}} \text{CH}_3$ over intensity of δCH_2 increases when the content in lactic units increases. These two bands appear to be well separated in both the infrared and the Raman spectra and therefore should allow the distinction of lactic and glycolic units. In the infrared spectra, three additional bands are observed at 1392, 1380 and 1364 cm^{-1} which are assigned to the wagging mode of CH_2

Table 2

Wavenumbers, $\bar{\nu}$, and vibrational assignments for PLGA 50/50, PLGA 65/35, PLGA 75/25 and PLGA 95/5. $\bar{\nu}$, stretching; δ , bending; ω , wagging; τ , twisting; ρ , rocking; s, symmetric; as, anti-symmetric; VS, very intense; S, intense; M, medium; w, weak; sh, shoulder.

PGA	PLA		PLGA		Assignments
Infrared	Infrared	Raman	Infrared	Raman	
3012 w				3000 S	$\nu_{\text{as}} \text{CH}_2$
	2995 w	3000 S	2995 w		$\nu_{\text{as}} \text{CH}_3$, $\nu_{\text{as}} \text{CH}_2$
2960 w				2960 sh	$\nu_{\text{s}} \text{CH}_2$
	2945 w	2944 VS	2948 w	2945 VS	$\nu_{\text{s}} \text{CH}_3$
	2882 w	2880 sh	2882 w	2882 sh	νCH
1745 VS	1745 VS	1768 S, 1749 sh	1745 VS	1766 M, 1748 sh	$\nu \text{C=O}$
	1450 S	1449 S	1452 M	1452 M	$\delta_{\text{as}} \text{CH}_3$
1420 S			1422 M	1424 M	δCH_2
1396 S			1392 M		ωCH_2
	1380 M, 1362 M, 1320 sh/ 1267 M	1387 M, 1358 sh, 1300 M	1380 M, 1364 M, 1320 M	1387 M, 1358 w, 1300 w	$\delta_{\text{s}} \text{CH}_3$
					δCH
1294 sh/ 1267 M			1269 S	1272 w	τCH_2
1145 VS	1183 VS	1180 w	1183 VS, 1167 VS	1180 w	$\nu_{\text{as}} \text{COC}$
	1127 sh	1129 S	1129 VS	1127 M	$\rho_{\text{as}} \text{CH}_3$
1079 VS, 1028 sh	1086 VS	1092 S	1083 VS	1090 M, 1030 sh	$\nu_{\text{s}} \text{COC}$
	1045 S	1043 S	1047 sh, 1040 sh	1046 M	$\nu \text{C-CH}_3$
	955 w		956 sh	949 sh	ρCH_3
950 w/ 887 M	863 w	872 VS	892 sh	890 S	$\nu \text{C-COO}$, ρCH_2
			867 M	872 VS	$\nu \text{C-COO}$, ρCH_3
					ρCH_2
846 M			846 sh	848 S	

(1392 cm^{-1}) from the glycolic units and the symmetric bending of CH_3 (1380 and 1364 cm^{-1}) from the lactic units. The band at 1392 cm^{-1} relative intensity decreases when the content of lactic units in the polymer increases.

In the 1300–1000 cm^{-1} region of the infrared spectra, two initial peaks are observed at 1167 and 1183 cm^{-1} that are assigned to the anti-symmetric stretching of COC for glycolic and lactic units respectively. The symmetric stretching of COC is observed at 1083 cm^{-1} for both glycolic and lactic units. $\nu_{\text{s}} \text{COC}$ is observed at similar wavenumbers in PLA and PGA. The two other peaks observed in this region at 1129 and 1047 cm^{-1} are assigned to the vibration of CH_3 from the lactic units. These two bands are therefore more intense in PLGA 95/5 than in PLGA 50/50. In the Raman spectra, the two bands at 1180 and 1090 cm^{-1} correspond to the anti-symmetric and the symmetric stretching of COC for the lactic units respectively. The corresponding bands of the glycolic units are not clearly observed due to the low intensity of the bands in this region ($\nu_{\text{s}} \text{COC}$ from the glycolic units is at 1030 cm^{-1}). The two bands at 1127 and 1046 cm^{-1} are assigned to the vibrations of CH_3 from the lactic units.

In the 1000–800 cm^{-1} region in the infrared spectra, four bands are observed at 956, 892, 867 and 846 cm^{-1} . By comparing these bands to the ones observed in PGA and PLA, they can be assigned to ρCH_3 (956 cm^{-1}) and $\nu \text{C-COO}$ (867 cm^{-1}) for lactic units and ρCH_2 (846 cm^{-1}) and $\nu \text{C-COO}$ (892 cm^{-1}) for glycolic units. In the Raman spectra, the stretching of C-COO appears as two sharp bands at 890 and 875 cm^{-1} . The band at 875 cm^{-1} is the most intense and corresponds to the vibration of C-COO in the lactic units of the polymer, as seen in PLA at 872 cm^{-1} . The $\nu \text{C-COO}$ band was observed in PGA at 890 cm^{-1} . Therefore, in PLGA, the band at 890 cm^{-1} is assigned to $\nu \text{C-COO}$ of the glycolic units. Moreover,

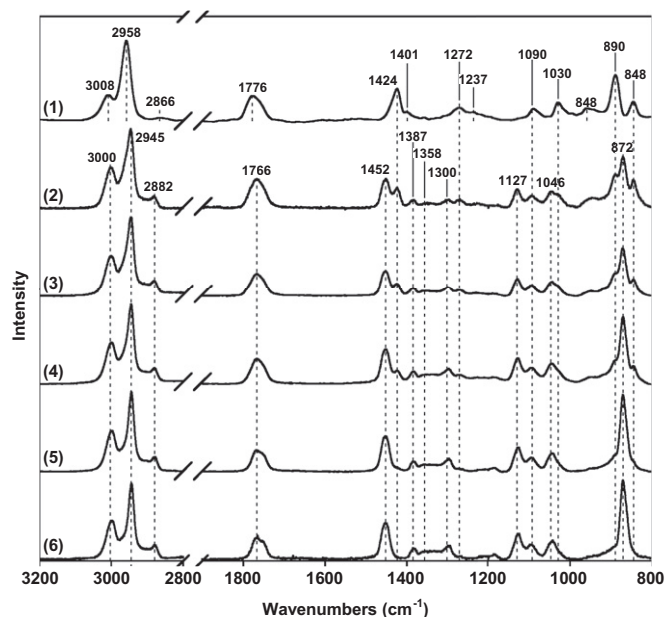


Fig. 3. Raman spectra of (1) PGA, (2) PLGA 50/50, (3) PLGA 65/35, (4) PLGA 75/25, (5) PLGA 95/5 and (6) PLA dry films.

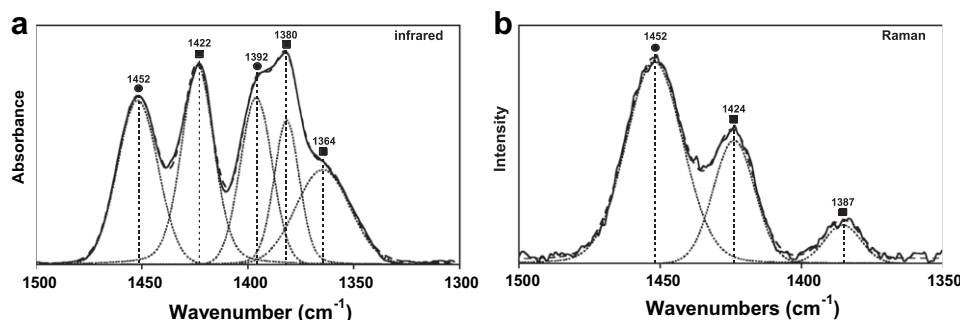


Fig. 4. Examples of peak fit for PLGA 50/50: — original trace; - - fitted trace; peaks. a) infrared spectra from 1500 to 1300 cm^{-1} and b) Raman spectra from 1500 to 1350 cm^{-1} . The symbols indicate the bands due to ● lactic and ■ glycolic units.

the band at 890 cm^{-1} can be observed clearly in the Raman spectrum of PLGA 50/50; it becomes a shoulder in the Raman spectra of PLGA 65/35, PLGA 75/25 and PLGA 95/5 due to their lower content in glycolic units of these polymers. These two bands in the Raman spectra should also allow the clear differentiation of lactic and glycolic units.

In the CH (3200–2800 cm^{-1}) and C=O (2000–1600 cm^{-1}) stretching regions of infrared and Raman spectra the differentiation of bands from lactic and glycolic units is not clear enough to be used to determine the proportion of monomers within the polymer. Bands due to the stretching vibrations of CH_3 and CH_2 or C=O from lactic and glycolic units are observed at the same wavenumbers.

In summary in the infrared spectra, two regions show clear differences between lactic and glycolic units: 1500–1300 cm^{-1} which correspond to the region of bending of CH_2 and CH_3 , and 1300–1000 cm^{-1} which corresponds to the region of stretching of COC. In the Raman spectra again two regions allow the identification of bands typical of lactic and glycolic units: 1500–1300 cm^{-1} which is the region of the bending of CH_2 and CH_3 , and 1000–800 cm^{-1} which is the region of the stretching of C–COO.

3.2. Calibration curves

The relative intensity ratio, $I_{G/L}$, between bands assigned to lactic and glycolic units can be calculated through Equation (2):

$$I_{G/L} = \frac{I^G}{I^G + I^L} \quad (2)$$

where I^G and I^L are the intensity of the bands due to glycolic and lactic units respectively. The plot of $I_{G/L}$ vs. %G (molar percentage of glycolic units) for the dry PLGA samples with known compositions should give a calibration curve that can be used to evaluate the composition of the copolymers during degradation. From the previous section two infrared and two Raman regions have been identified allowing the differentiation between the lactic and glycolic units. In the analysis of the intensities of the bands, I^G and I^L , only the regions 1500–1300 cm^{-1} in infrared and Raman gave reproducible statistics. Examples of peak fittings for PLGA 50/50 in these two regions are shown in Fig. 4. The bands for which consistent fitting is obtained are the bands observed at 1452 and 1422 cm^{-1} in the infrared spectra and the bands observed at 1452 and at 1424 cm^{-1} in the Raman spectra. These are assigned to $\delta_{\text{as}} \text{CH}_3$ (lactic units) and δCH_2 (glycolic units) respectively in both infrared and Raman. The intensity of each peak can be measured and the relative intensity ratios calculated through Equations (3) and (4):

$$I_{1422/1452}^{\text{IR}} = \frac{I_{1422}^G}{I_{1422}^G + I_{1452}^L} \quad (3)$$

$$I_{1424/1452}^{\text{RAMAN}} = \frac{I_{1424}^G}{I_{1424}^G + I_{1452}^L} \quad (4)$$

where I_{1422}^G and I_{1452}^L are the intensities of the δCH_2 and $\delta_{\text{as}} \text{CH}_3$ infrared bands respectively and $I_{1422/1452}^{\text{IR}}$ the corresponding infrared relative intensity ratio and I_{1424}^G and I_{1452}^L are the intensities of δCH_2 and $\delta_{\text{as}} \text{CH}_3$ Raman bands respectively and $I_{1424/1452}^{\text{RAMAN}}$ the corresponding Raman relative intensity ratio. In Fig. 5 the relative intensity ratios, infrared and Raman, are reported as a function of the molar percentage of glycolic units (%G). A linear correlation is obtained in both cases. These calibration curves allow the content of glycolic and lactic units in the PLGA copolymer to be determined through Equation (5),

$$I = a \times \%G + b \quad (5)$$

where a and b are the slope and intercept, respectively, of the best linear fits. The values determined for a and b are summarised in Table 3. Vert [17] proposed a similar relationship between the relative Raman intensity ratio of several P(L)GA copolymers (Table 3). It should be kept in mind these authors used a different polymer and a different experimental procedure.

3.3. Films in vitro degradation in PBS

The hydrolytic degradation of PLGA cast films, prepared as outlined earlier, was studied in PBS (pH 7.4) at 37 °C. Infrared

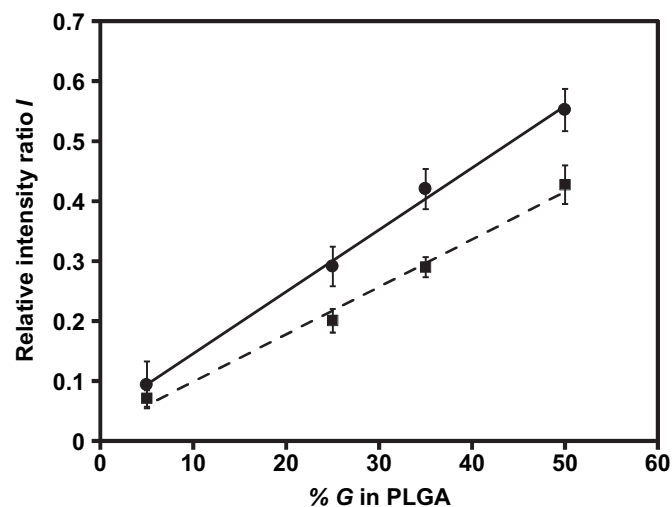


Fig. 5. Calibration curves: relative intensity ratio, ● $I_{1422/1452}^{\text{IR}}$, ■ $I_{1424/1452}^{\text{RAMAN}}$, vs. %G in PLGA dry films.

Table 3

Values of the slope, a , and the intercept, b , for the different calibration curves; R^2 is the correlation coefficient.

Parameters measured	$I_{IR}^{1422/1452}$	$I_{RAMAN}^{1424/1452}$	$I_{RAMAN}^{1424/1452}$ Vert et al. [17]
a	0.0109 ± 0.0005	0.0079 ± 0.0007	0.0069
b	0.031 ± 0.002	0.019 ± 0.003	0.093
R^2	0.996	0.992	—

analysis of PLGA 50/50, 65/35, 75/25 and 95/5 and Raman analysis of PLGA 50/50, 75/25 and 95/5 wet films were performed as a function of degradation time. First, we will present the mass loss and the infrared and Raman data obtained. We will do a qualitative description of the changes in the spectra focusing on PLGA 50/50 and the 1300–1500 cm^{-1} infrared and Raman regions for which, as discussed above, reliable calibration curves allowing the evaluation of the copolymer composition were obtained. Then we will discuss the evolution of the molar ratio of glycolic and lactic units as a function of degradation time for all polymers studied.

Residual mass of degrading films vs. degradation time for the PLGA samples in PBS is presented in Fig. 6. The rate of mass loss depended on the polymer L/G ratio and was found to decrease with increasing lactic unit content. After 10 days PLGA 95/5 lost only 3% of its mass while PLGA 50/50 lost 20%. It is only after 30 days that the mass loss of PLGA 95/5 begins to increase significantly. This is due primarily to the difference in hydrophobicity between *L* and *G* units, *L* units being more hydrophobic, and to the higher glass transition temperature of the copolymers with higher lactic content. Indeed higher hydrophobicity and glass transition will result in a slower diffusion of water in the films resulting in an overall slowing of the film degradation kinetics. This aspect will be discussed further in a forthcoming article.

The infrared spectra of PLGA 50/50 at $t = 0$ and $t = 33$ days (degradation time) in the 1500–1300 cm^{-1} region are shown in Fig. 7. In order to visualise the relative band intensity changes occurring during the *in vitro* degradation, the spectra are normalised to the $\delta_s \text{CH}_3$ band at 1380 cm^{-1} . The two bands at 1452 and 1422 cm^{-1} represent the anti-symmetric bending of CH_3 from the lactic units and the bending of CH_2 from the glycolic units. The relative intensity of the band at 1422 cm^{-1} , which is an intense band at the beginning, decreases with increasing degradation time. At the same time, the band found at 1452 cm^{-1} increases in relative intensity while degradation proceeds. The same evolution in bands

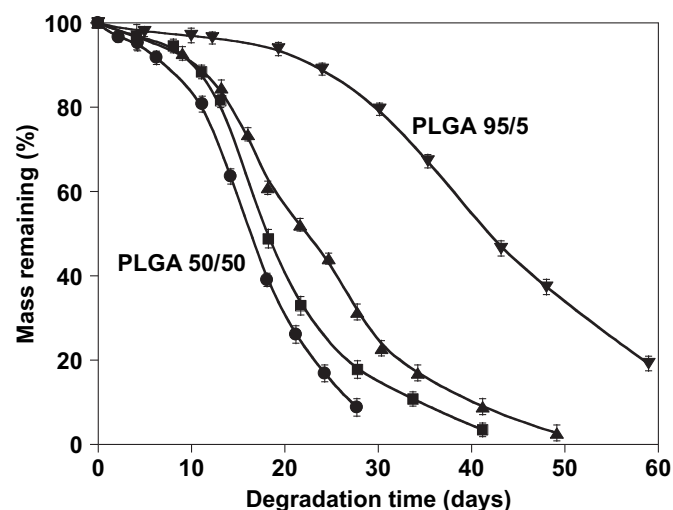


Fig. 6. Mass remaining vs. degradation time for PLGA films degraded in 20 mL PBS at 37 °C: ● PLGA 50/50, ■ PLGA 65/35, ▲ PLGA 75/25, ▼ PLGA 95/5.

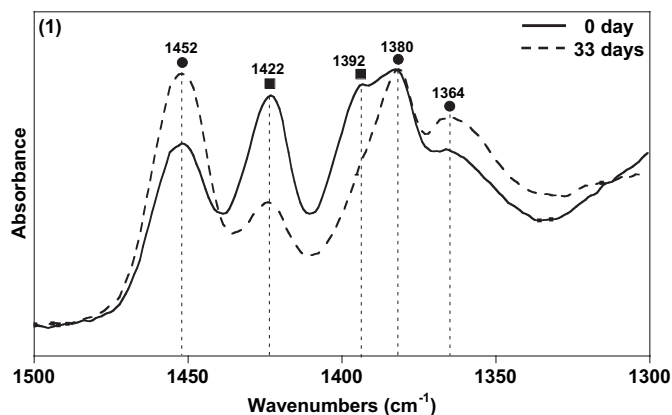


Fig. 7. Infrared spectra from 1500 to 1300 cm^{-1} for PLGA 50/50 films degraded in 20 mL PBS at 37 °C. The symbols indicate the bands due to ● lactic and ■ glycolic units.

relative intensities is observed for the other PLGA copolymers studied (Figure S1). Due to the lower glycolic unit content in PLGA 75/25 and PLGA 95/5, the changes in bands relative intensities during the *in vitro* degradation are less prominent. For PLGA 95/5, the changes in intensity are difficult to assess, but it is apparent that the shoulder found at 1422 cm^{-1} (δCH_2) from the glycolic units completely disappears after 40 days of degradation (Figure S1). More generally the relative intensities of all the bands assigned to glycolic units across the full infrared spectra (Figure S2) decrease with increasing degradation time confirming the preferential degradation of the glycolic units.

A similar trend was observed for the Raman spectra. The Raman spectra obtained for PLGA 50/50 at $t = 0$ and $t = 33$ days in the 1500–1300 cm^{-1} region are shown in Fig. 8. All spectra are normalised to the band at 1452 cm^{-1} for ease of visualisation of the changes occurring during the *in vitro* degradation. The first band at 1452 cm^{-1} is assigned to $\delta_{as} \text{CH}_3$ (lactic units) and that at 1425 cm^{-1} corresponds to δCH_2 (glycolic units). The last band observed in these spectra, at 1387 cm^{-1} , corresponds to the symmetric bending of CH_3 in the lactic units. After 33 days of *in vitro* degradation, the band due to the bending of CH_2 is present only as a shoulder. This indicates that a lower number of CH_2 groups are present in the polymer, due to the preferential hydrolysis of the glycolic units of the polymer. In this case too the same trend was observed for the other copolymers (Figure S3). More generally in the case of Raman spectra too the relative intensities of all the bands assigned to

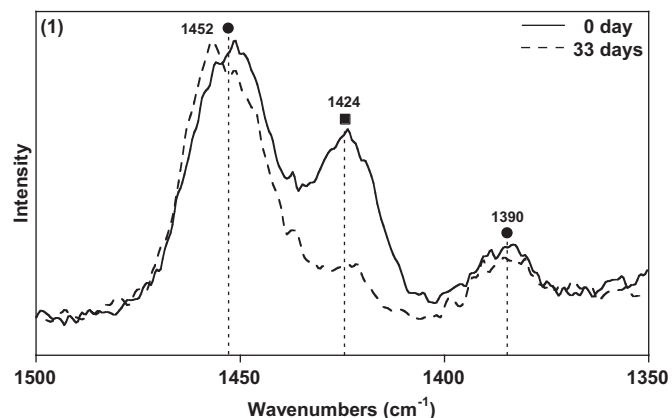


Fig. 8. Raman spectra from 1500 to 1300 cm^{-1} for PLGA 50/50 films degraded in 20 mL PBS at 37 °C. The symbols indicate the bands due to ● lactic and ■ glycolic units.

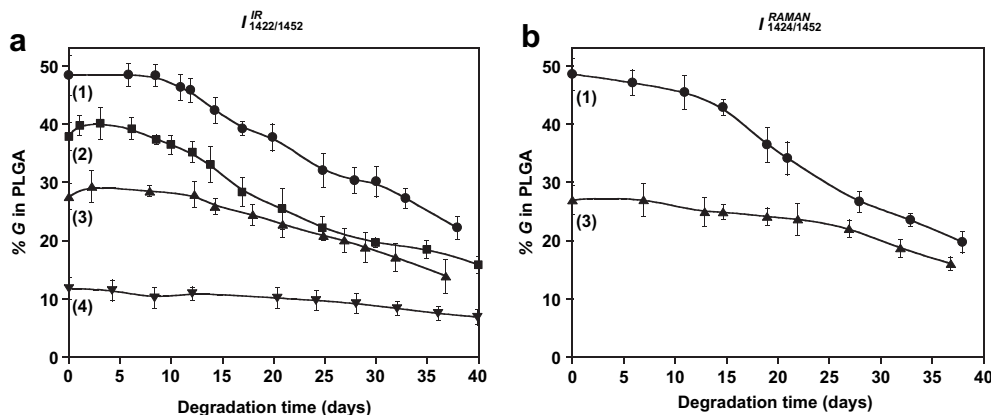


Fig. 9. %G units in PLGA versus degradation time for different PLGA films degraded in 20 mL PBS at 37 °C, calculated from: a) $I_{1422/1452}^{IR}$ and b) $I_{1424/1452}^{RAMAN}$. (1) PLGA 50/50, (2) PLGA 65/35, (3) PLGA 75/25, (4) PLGA 95/5.

glycolic units were found to decrease with increasing degradation time (Figure S4).

In order to follow the *in vitro* degradation kinetics of the polymers, all spectra were analysed. The intensity of the bands assigned to the lactic and glycolic units were measured and the relative intensity ratios $I_{1422/1452}^{IR}$ and $I_{1424/1452}^{RAMAN}$ calculated through Equations (3) and (4) (Figure S5). Due to the low intensity of the bands in the Raman spectra of PLGA 95/5, the relative intensity ratio could not be calculated for this polymer. Using the calibration curves obtained previously (Fig. 5 and Table 3) the molar percentage of glycolic units was calculated. In Fig. 9 the evolution of the glycolic content of each copolymer investigated is presented as a function of degradation time. A very good agreement between the compositions calculated from the infrared and Raman spectra was obtained. From these data it is clear that the percentage of lactic and glycolic units stays approximately constant up to 10 days for PLGA 50/50 and up to 15 days for PLGA 75/25. As degradation proceeds, the percentage of lactic units in the remaining polymer increases with time relatively to the percentage of glycolic units. After about 38 days, the L/G composition of PLGA 50/50, PLGA 65/35, PLGA 75/25 and PLGA 95/5 is 78/22, 85/15, 86/14 and 96/4 respectively.

3.4. Kinetics of degradation of glycolic and lactic units

The percentage of lactic and glycolic units calculated above is a percentage in moles. The percentage in weight, %G (wt.) and %L (wt.), can be calculated through Equations (6) and (7).

$$\%G(\text{wt.}) = \frac{\frac{\%G}{100} \times M_G}{\frac{\%G}{100} \times M_G + \frac{\%L}{100} \times M_L} \quad (6)$$

$$\%L(\text{wt.}) = \frac{\frac{\%L}{100} \times M_L}{\frac{\%G}{100} \times M_G + \frac{\%L}{100} \times M_L} \quad (7)$$

where M_G is the molecular weight of glycolic acid (76.05 g mol⁻¹) and M_L the molecular weight of lactic acid (90.08 g mol⁻¹). Because the mass of polymer remaining (mass remaining (%)) is known at all times during degradation (Fig. 6), the relative mass of glycolic units, m_G (%), and lactic units, m_L (%) remaining can be calculated through Equations (8) and (9).

$$m_G(\%) = \%G(\text{wt.}) \times \text{mass remaining}(\%) \quad (8)$$

$$m_L(\%) = \%L(\text{wt.}) \times \text{mass remaining}(\%) \quad (9)$$

A semi-logarithmic plot of the mass remaining of glycolic and lactic units vs. degradation time is presented in Fig. 10 (Only data obtained from the infrared spectra are presented as the full set of polymer is available. Raman data are presented in Figure S6). As can be seen from Fig. 10 a two stage degradation process is revealed in agreement with our previous work. During the 1st stage both the mass of glycolic and lactic units remain relatively constant

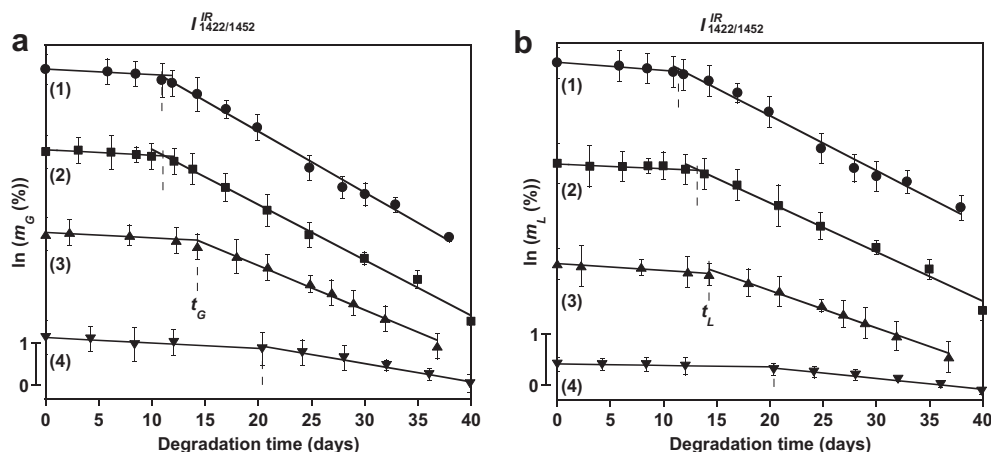


Fig. 10. Semi-logarithmic plot of mass remaining of glycolic units m_G (a) and lactic units m_L (b) vs. degradation time for different PLGA films degraded in 20 mL PBS at 37 °C calculated from $I_{1422/1452}^{IR}$. (1) PLGA 50/50, (2) PLGA 65/35, (3) PLGA 75/25, (4) PLGA 95/5. Graphs have been shifted along the y-axis by an arbitrary value for ease of visualisation.

suggesting that few glycolic and lactic units are hydrolysed and released. The length of this first stage depends on the lactic content of the polymer. For PLGA 50/50 significant mass loss of lactic and glycolic units is observed to start after 11 days of degradation while for PLGA 95/5 significant mass loss is observed to start after 20 days. The start of this second stage of the degradation process correlates well with the sudden decrease in pH of the media described in our previous work. For PLGA 50/50 films we showed that after 8–10 days of degradation the media becomes highly acidic probably resulting in the autocatalysis of the hydrolysis reaction and as a consequence the rate of degradation of the polymer increases as suggested by Fig. 10.

A linear behaviour for m_G and m_L is observed for both stages of the degradation process suggesting that the degradation kinetics of glycolic and lactic units can be described using a first order kinetics approach. The degradation rate constants of glycolic and lactic units, k_G and k_L can therefore be estimated through Equations (10) and (11):

$$\ln(m_G(\%)) = -k_G t + b \quad (10)$$

$$\ln(m_L(\%)) = -k_L t + b \quad (11)$$

The degradation rate constants for each stage of the degradation process are presented in Fig. 11 as a function of the copolymers initial lactic content. For PLGA 50/50 and PLGA 75/25 the rate constants presented are an average of the rate constants obtained from the analysis of the different regions in the infrared and Raman spectra. In the first step, both the mass of glycolic and lactic units remain relatively constant and therefore the rate constants obtained for this first stage, k_{G1} and k_{L1} , are very low. The degradation rate constant of the glycolic units ($k_{G1} = 0.016 \pm 0.005 \text{ day}^{-1}$) was found to be slightly higher compared to the degradation rate constant of the lactic units ($k_{L1} = 0.010 \pm 0.003 \text{ day}^{-1}$). In the second stage the mass of glycolic and lactic units both decrease significantly with time. The rate constants of degradation of glycolic and lactic units in the second stage, k_{G2} and k_{L2} were found to decrease consistently in proportion to the percentage of lactic units present in the copolymer at the start. However the rate of hydrolysis of glycolic units was found to be consistently 1.3 times greater than the rate of hydrolysis of lactic units for all copolymers. The origin of this difference in degradation rates stems from the difference in hydrophobicity between L and G units. Glycolic units are more hydrophilic and are therefore attacked preferentially by

water resulting in an increase in the proportion of lactic units in the remaining copolymer. As said previously our results also indicate that the degradation rate constant decreases with increasing initial lactic content of the copolymer. This effect is thought to result from the experimental design used. The overall hydrophobicity of the films investigated will depend on the copolymer used: the higher the lactic content the higher the films overall hydrophobicity. As a result the ability of water to diffuse into the films decreases with increasing lactic content resulting in a lower amount of water molecules being available inside the films for degradation. As a result the overall kinetic of degradation of the films decreases. This issue of water availability affects both the degradation of glycolic and lactic units and explains why the degradation rates constants for both units are seen to decrease in a similar fashion with increasing initial lactic content of the copolymer. These results show that the ability of water to diffuse through the sample can affect significantly the rate of degradation of the copolymer.

These results are consistent with those from the literature [22–25]. Alexis et al. [22] studied the *in vitro* degradation of PLGA 50/50 ($M_w = 550 \times 10^3 \text{ g mol}^{-1}$) and quantified the composition of the polymer during degradation by nuclear magnetic resonance (NMR). After an initial period of 25 days where the percentage of glycolic and lactic units was constant, the relative content of glycolic units decreased rapidly while the relative content of lactic units increased. The difference in time, in comparison with our results, is due to the difference of molecular weight of the polymer studied. Other studies [23,24] reported that the content of glycolic and lactic units was decreasing and increasing respectively as soon as the samples were in contact with the medium. Hakkarainen et al. [23] studied PLGA 50/50 ($M_n = 76 \times 10^3 \text{ g mol}^{-1}$) and PLGA 75/25 ($M_n = 41 \times 10^3 \text{ g mol}^{-1}$) and observed that only a few percentage of lactic units were hydrolysed after 40 days of degradation, while the percentage of glycolic units hydrolysed increased as soon as the polymer was in contact with the medium. The difference found is again likely to be due to the difference in molecular weight of the polymers studied. Alternatively it could also be due to differences in sample configuration. Porous films [24] or powder form polymers [23], which were used by Cai et al. and Hakkarainen et al. respectively, are known to degrade at a higher rate than the films used in this work due to the higher surface area accessible to water in these materials. However, these studies [22–25] support the conclusion reported herein, that glycolic units are preferentially degraded. Only Fredericks et al. [26] suggests that no preferential hydrolysis occurs; this is probably due to that fact the authors studied PLGA 8/92, in which the composition is changing only slightly during degradation.

4. Conclusions

ATR-IR and Raman spectroscopies have been used to differentiate and quantify the degradation rates of glycolic and lactic units in a family of PLGAs. It has been shown that both techniques can be used to determine the polymer composition as a function of degradation time and that rate constants for the hydrolysis of glycolic and lactic units can be calculated. The $1500\text{--}1300 \text{ cm}^{-1}$ regions in both the infrared and Raman spectra were found to yield the best results. In particular the use of the bands at 1452 and 1422 cm^{-1} in the infrared spectra and the bands at 1452 and 1424 cm^{-1} in the Raman spectra, assigned to the anti-symmetric bending of CH_3 from the lactic units and the bending of CH_2 from the glycolic units respectively, were used to establish calibration curves allowing the composition of the copolymer during degradation to be evaluated. The data revealed a two stage process for the degradation of PLGA cast films in PBS in agreement with our previous work [12]. The degradation rate constant for glycolic unit was found to be 1.3 times higher than for lactic units in all

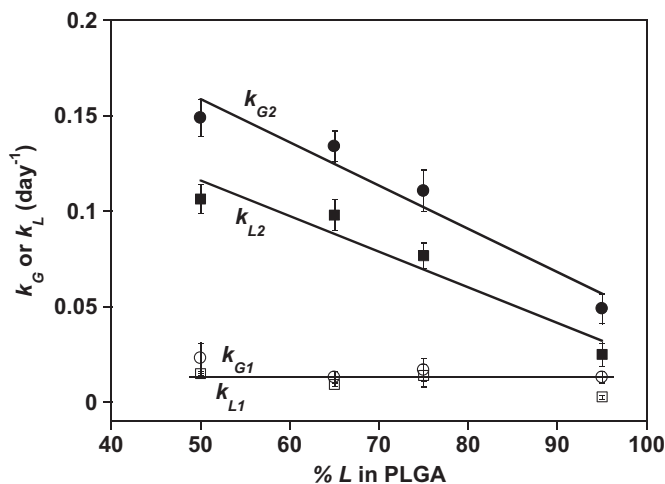


Fig. 11. Rate constants of glycolic and lactic units mass vs. %L in PLGA: \circ k_{G1} and \square k_{L1} degradation rate constants for the first stage of the degradation process; \bullet k_{G2} and \blacksquare k_{L2} degradation rate constants for the second stage of the degradation process.

copolymers. In addition the degradation rate constants for *L* and *G* units were shown to decrease with increasing initial lactic content of the copolymer suggesting that the ability of water molecule to diffuse in the sample and therefore their availability for the hydrolysis process has a significant effect on the kinetics of degradation of the copolymers within the film.

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Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version, at [doi:10.1016/j.polymdegradstab.2011.07.011](https://doi.org/10.1016/j.polymdegradstab.2011.07.011).

References

- [1] Kovalchuk A, Fischer W, Epple M. *Macromol Biosci* 2005;5(4):289–98.
- [2] King TW, Patrick Jr CW. *J Biomed Mater Res, Part A* 2000;51(3):383–90.
- [3] von Burkersroda F, Schedl L, Göpferich A. *Biomaterials* 2002;23(21):4221–31.
- [4] Schlieker G, Schmidt C, Fuchs S, Wombacher R, Kissel T. *Int J Pharm* 2003;266(1–2):39–49.
- [5] Bigg DM. *Adv Polym Technol* 2005;24(2):69–82.
- [6] Liu L, Li S, Garreau H, Vert M. *Biomacromolecules* 2000;1(3):350–9.
- [7] Li S, Liu L, Garreau H, Vert M. *Biomacromolecules* 2003;4(2):372–7.
- [8] Lee WK, Iwata T, Gardella Jr JA. *Langmuir* 2005;21(24):11180–4.
- [9] Kranz H, Ubrich N, Maincent P, Bodmeier R. *J Pharm Sci* 2000;89(12):1558–66.
- [10] Friess W, Schlapp M. *J Pharm Sci* 2002;91(3):845–55.
- [11] Li S, Vert M, editors. *Encyclopedia of controlled drug delivery*. New York: John Wiley and Sons; 1999.
- [12] Vey E, Rodger C, Meehan L, Booth J, Claybourn M, Miller AF, et al. *Polym Degrad Stab* 2008;93(10):1869–76.
- [13] Li SM, Garreau H, Vert M. *J Mater Sci: Mater Med* 1990;1(3):123–30.
- [14] Li SM, Garreau H, Vert M. *J Mater Sci: Mater Med* 1990;1(3):131–9.
- [15] Li SM, Garreau H, Vert M. *J Mater Sci: Mater Med* 1990;1(4):198–206.
- [16] Kister G, Cassanas G, Vert M. *Spectrochim Acta, Part A* 1997;53(9):1399–403.
- [17] Kister G, Cassanas G, Vert M. *Polymer* 1998;39(15):3335–40.
- [18] Kister G, Cassanas G, Vert M, Pauvert B, Terol A. *J Raman Spectrosc* 1995;26(4):307–11.
- [19] Kister G, Cassanas G, Vert M. *Polymer* 1998;39(2):267–73.
- [20] Taddei P, Tinti A, Fini G. *J Raman Spectrosc* 2001;32(8):619–29.
- [21] Taddei P, Monti P, Simoni R. *J Mater Sci: Mater Med* 2002;13(1):59–64.
- [22] Alexis F, Venkatraman S, Rath SK, Gan LH. *J Appl Polym Sci* 2006;102(4):3111–7.
- [23] Hakkarainen M, Albertsson AC, Karlsson S. *Polym Degrad Stab* 1996;52(3):283–91.
- [24] Cai Q, Shi GX, Bei JZ, Wang SG. *Biomaterials* 2003;24(4):629–38.
- [25] Kamei S, Inoue Y, Okada H, Yamada M, Ogawa Y, Toguchi H. *Biomaterials* 1992;13(13):953–8.
- [26] Fredericks RJ, Melveger AJ, Dolegiewitz LJ. *J Polym Sci, Part B: Polym Phys* 1984;22(1):57–66.