Degradation of PLA, PLGA homo- and copolymers in the presence of serum albumin: a spectroscopic investigation

E Çatıker,[1] M Gümüşderelioğlu[2]* and A Güner[3]
[1]*Abant İzzet Baysal University, Chemistry Department, 14280, Bolu, Turkey*
[2]*Chemical Engineering Department, Hacettepe University, 06532, Beytepe, Ankara, Turkey*
[3]*Chemistry Department, Hacettepe University, 06532, Beytepe, Ankara, Turkey*

Abstract: Poly(D,L-lactide-*co*-glycolide) samples with different glycolide contents, ie 85:15, 75:25 and 50:50 mole ratios of lactide to glycolide, and poly(L-lactide) were obtained from a commercial source. Polymer films of 15 μm thickness were prepared by a solvent-casting method. Degradation studies were conducted at 37 °C in pH 7.4 phosphate buffered saline and in bovine serum albumin solution. The degradation behaviour of the films was followed by UV and FTIR spectrophotometry and viscometric and gravimetric measurements. Spectroscopic investigations showed that during the first month of degradation, both the chain length and the chain chemical structure changed, especially in the presence of bovine serum albumin. The short chains are attributed to the formation of new ester groups; however, the end-groups may be aldehyde- or ketone-like structures. While the intrinsic viscosities of all polymers decreased continuously after being exposed to the degradation media, insignificant mass loss occurred during the experimental investigation.

Keywords: degradation; spectroscopy; poly(lactide); poly(glycolide)

INTRODUCTION

Poly(α-hydroxy acids), mainly polylactides (PLAs) and polyglycolide (PGA), are the most widely known synthetic polymers used for the production of degradable biomaterials[1,2] (Fig 1). Their potential applications include self-regulated drug delivery systems, orthopaedic fixing devices and scaffolds for soft and hard tissue repair.[3–6] PGA is the simplest linear aliphatic polyester. Because PGA is highly crystalline, it has low solubility in organic solvents and a high melting point. Lactic acid is a chiral molecule and therefore it exists in two stereoisomeric forms, D and L; a racemic form, D,L-PLA, is also available. The polymers derived from the optically active D and L monomers are semicrystalline materials, while the optically inactive D,L-PLA is always amorphous. It is possible to control the degree of crystallinity in polymers by copolymerization of glycolide with lactide at different compositions.[7]

PLA and PLGA biomaterials degrade *in vivo* by hydrolysis into lactic acid and glycolic acid, which are then incorporated into the tricarboxylic acid cycle and excreted.[8] The degradation process is completed in four steps: (1) water penetrates the amorphous region of the polymer and disrupts the secondary forces; (2) cleavage of the covalent bonds in the polymer backbone begins by hydrolysis and, as hydrolysis proceeds, more and more carboxylic end-groups may autocatalyse the hydrolysis reaction; (3) significant mass loss begins to occur by massive cleavage of the backbone covalent bonds; (4) the polymer loses weight. (This step is called 'erosion' which designates the loss of material owing to monomers and oligomers leaving the polymer.[9])

Chemical and physical changes accompany the degradation of lactide/glycolide polymers. Thus, degradation can be followed by monitoring some of those changes. The most important parameter for monitoring degradation is molecular weight.[9] Besides loss of molecular weight, other parameters, such as loss of mechanical strength, have been proposed as a measure of degradation. Although a number of reports which describe degradation behaviour of these polymers are available in the literature, they do not give adequate detail based on the spectroscopic investigation.

This paper describes the *in vitro* degradation of the PLA and PLGA films prepared from poly(L-lactide) (PLA) and poly(D,L-lactide-*co*-glycolide, 85:15, 75:25 and 50:50) in the presence of phosphate buffered saline (PBS) and bovine serum albumin (BSA). The studies were carried out by UV-spectrophotometry, FTIR and intrinsic viscosity–mass loss measurements.

* Correspondence to: M Gümüşderelioğlu, PK 137, Maltepe, 06571, Ankara, Turkey
E-mail: menemse@hacettepe.edu.tr
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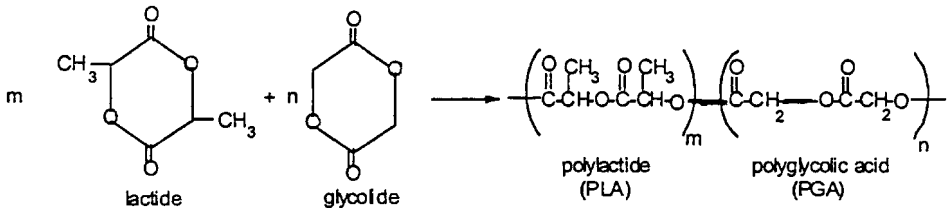


Figure 1. Structural formulae of trimers and copolymers.

MATERIALS AND METHODS

Materials

PLA (100 L-lactide) and PLGA (D,L lactide:glycolide, 85:15, 75:25, and 50:50) were obtained from Du Pont (Medisorb, Wilmington, DE). Their composition and inherent viscosities determined by the manufacturer are given in Table 1. Solvent, methylene chloride and Sigmacote were purchased from Sigma Chemical Company (St Louis, MO). Phosphate buffered saline (PBS, pH 7.4) and bovine serum albumine (BSA) solution were used as *in vitro* degradation media. BSA solution was prepared in PBS (concentration 4.6 mg ml^{-1}) from BSA, fraction V (Sigma, St Louis, MO).

Preparation of polymer films

Polymeric films were prepared by a solvent-casting method. After complete dissolution of the polymers (300 mg) in methylene chloride (4 ml), films were cast by pouring this solution into glass Petri dishes prelubricated with Sigmacote and allowing them to dry initially at room temperature for 24 h, then in a vacuum oven for a further 48 h. After drying, films were cut into strips (6.0 cm long and 2.0 cm wide) and each strip was placed in a stainless-steel frame for spectral investigation. The thickness of the polymeric film was adjusted to be 15 μm.

In vitro degradation studies

Degradation of the PLA and PLGA films was investigated in PBS and BSA solution under physiological conditions ie pH 7.4 and 37 °C. In a typical degradation experiment, preweighed polymer films were placed in 25 ml of degradation medium. Samples were periodically taken from the medium, washed with distilled water three times and dried overnight in a vacuum oven at room temperature. Intrinsic viscosities [η] were then determined by a single-point method described by Solomon and Ciuta.[10] Therefore, the flow time of 0.2 % (w/w) polymer solution in chloroform was measured at 25 °C using an Ubbelohde viscometer. Mass loss was determined gravimetrically, and the UV and FTIR spectra of each sample were taken by a Hitachi 100-60 model UV–vis double beam spectrometer and a Nicolet 510 model FTIR spectrophotometer at room temperature, respectively.

RESULTS AND DISCUSSION

As indicated before, PLA and PLGA structures contain hydrolysable ester bonds; therefore, their most important degradation mechanism is chemical degradation via hydrolysis or enzyme-catalysed hydrolysis. There are several factors that influence the velocity of this reaction: pH, copolymer composition and the presence of enzymes are the most important. However, enzymatic involvement in the biodegradation of PLA/PGA polymers has been the subject of some controversy. Most early reports concluded that biodegradation of these materials occurred strictly through hydrolysis, with no enzymatic involvement. Other investigators suggest that enzymes do play a significant role in the breakdown of PLA/PGA materials.[11] The objective of the present study is to characterize spectroscopically the degradation by BSA, which is one of the important proteins of blood plasma, of PLA and a series of PLGA copolymers When these materials are exposed to blood, interaction occurs between the material and plasma proteins. As indicated in the literature, serum albumin has an esterase-like activity such that aspirin and *p*-nitrophenyl esters are hydrolysed.[12] Therefore, BSA was used as a component of the degradation medium.

Viscosimetric studies

Intrinsic viscosity is one of the common properties used to characterize PLA and PLGA structures. Previous studies showed that these polymers satisfy the Mark–Houwink equation quite well.[13] Because the process of 'degradation' describes the chain-scission process during which polymer chains are cleaved to form oligomers and finally to form monomers, it can

Polymer/copolymer (MEDISORB® Grade)				*Mole ratio (%) Lactide*	*Glycolide*	*Inherent viscosity ($dl\,g^{-1}$)*
100	L	Low	IV	100	0	0.80–1.00
8515	DL	High	IV	85	15	0.66–0.80
7525	DL	Low	IV	75	25	0.50–0.65
5050	DL	Low	IV	50	50	0.50–0.65

Table 1. Properties of PLA and PLGA copolymers given by manufacturer

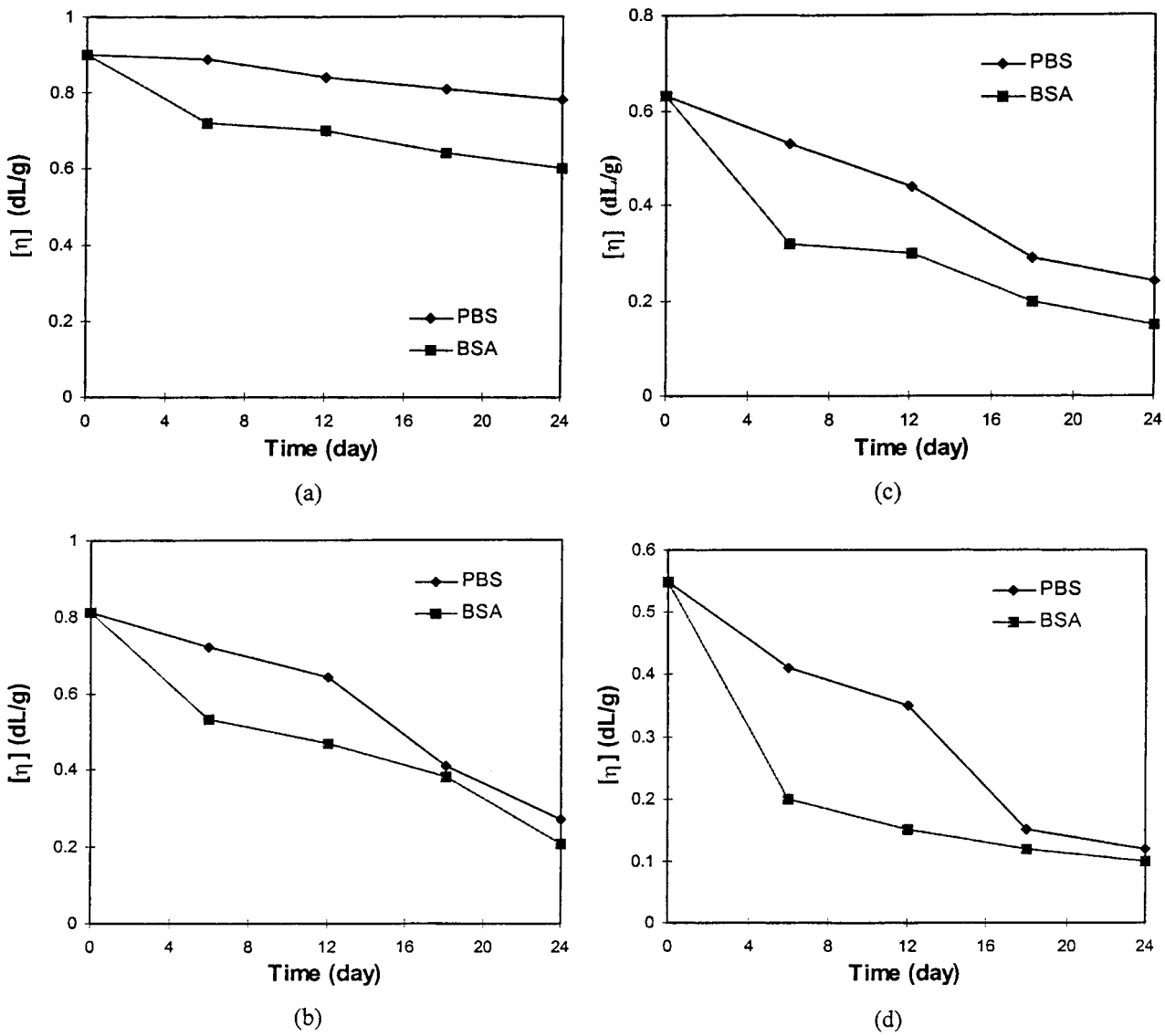


Figure 2. Changes in intrinsic viscosity of: (a) PLA; (b) 85:15 PLGA; (c) 75:25 PLGA; (d) 50:50 PLGA during the *in vitro* degradation in PBS (◆) and BSA (■).

be followed by molecular weight measurements. Therefore, in this study, intrinsic viscosities which are directly related to the molecular weight of polymers were determined and used to clarify the *in vitro* degradation process. Figure 2(a–d) shows the decrease in intrinsic viscosities with time in both of the media for PLA and PLGA 85:15, 75:25 and 50:50, respectively. These figures clearly indicate that the intrinsic viscosities of all polymers decreased continuously after being exposed to PBS at 37 °C. This behaviour is more significant in the presence of BSA than PBS. In particular, during the first week of degradation a sharp decrease of intrinsic viscosity was observed for all types of samples. This result indicates that the protein, (BSA) speeds up the degradation or, in other words, the hydrolytic de-esterification process. It is noteworthy that copolymers degraded at a slightly faster rate, depending on the glycolide ratio, than did the homopolymer. These differences may be attributed to the morphological differences between the homopolymer and copolymers. Whereas PLA is semicrystalline, crystallinity is rapidly lost in copolymers of glycolic acid and lactic acid. Also, PLA is more hydrophobic than the copolymers of PGA. These structural differences lead to an increase in the rates of hydration and hydrolysis. Thus, as can be clearly seen from Fig 2(d), 50:50 PLGA degrades more rapidly than the other structures. This is consistent with the results of a previous study which showed that degradation of 50:50 PLGA was complete in approximately 2 months and the degradation period for PLA was 18–24 months and for 85:15 PLGA was 5 months.[14]

Mass loss studies

Mass loss experiments for all polymers showed that the films exposed to PBS lost a small amount of mass during the 1-month study. In the case of PLA films, mass loss was less than 7%. The onset of mass loss (defined as 10% mass loss) was observed in all PLGA films near the end of 3 weeks. Similar results were obtained by Hyon *et al*[15] for PLA and PLGA

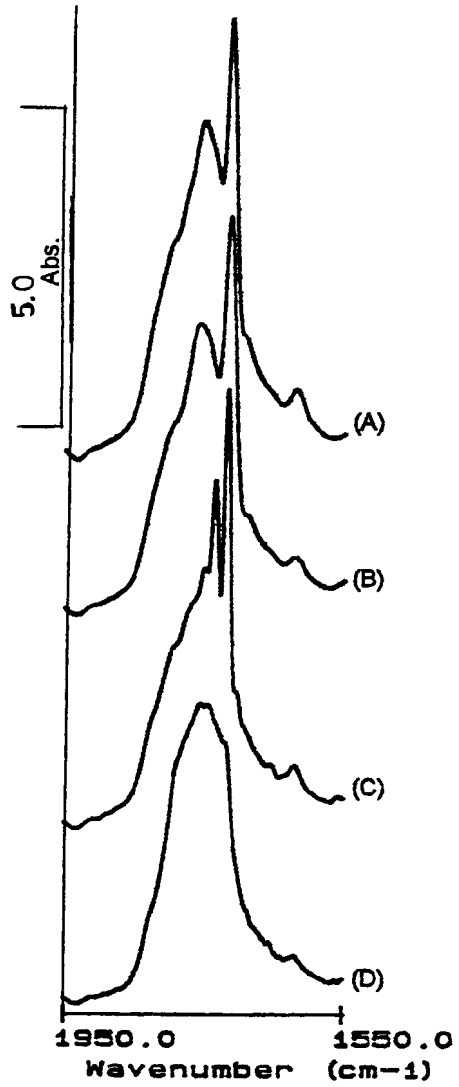


Figure 3. FTIR absorbance spectra of PLA: (A) original sample; (B) after 12 days in PBS; (C) after 32 days in PBS; (D) after 12 days in BSA.

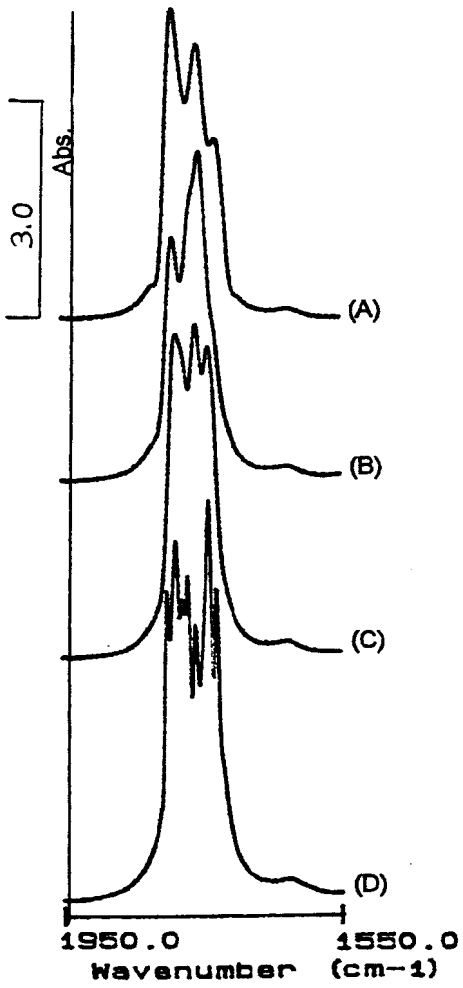
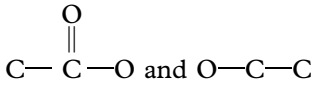


Figure 4. FTIR absorbance spectra of 50:50 PLGA: (A) original sample; (B) after 12 days in PBS; (C) after 32 days in PBS; (D) after 12 days in BSA.

copolymers in PBS at pH 7.4 and 37 °C. Where the films were in contact with BSA in the medium, the onset of mass loss was found to begin 1 week earlier than in PBS. Comparison of mass loss and viscosity measurements indicates that the polymers studied here undergo bulk hydrolysis, as expected.

FTIR results

IR studies were carried out in three different regions. The first one is the aliphatic C—H stretching region between 3000 and 2850 cm^{-1}; the second and third regions include C=O stretching bands (1850–1650 cm^{-1}) and the



asymmetric stretching vibrations at 1300–1000 cm^{-1}, respectively. The absorbances of the aliphatic C—H stretching bands at 2997, 2950–2947 and 2883–2881 cm^{-1} were observed to decrease on all four different samples during the degradation period. The decrease in absorbances is greater in the BSA solution than in PBS. This situation becomes most clear with increasing glycolide content.

The original polymer and copolymers have characteristic peaks at 1267, 1185, 1129, 1092 and 1044 cm^{-1}. The intensity of these absorption bands changed, some of them shifted and some new bands formed when they exposed to the degradation media. However, large changes in spectral features were observed in the presence of BSA.

The spectral evaluation was mainly interpreted in the region of carbonyl stretching vibrations between 1850 and 1650 cm^{-1}. The absorbance FTIR spectra of PLA and 50:50 PLGA samples in PBS and BSA at different time periods are given in Figs 3 and 4, respectively. Also, the main spectral changes of all polymers observed in this region are summarized in Table 2. The data in Table 2 belong to the samples incubated in PBS for 1 month and in BSA for 12 days and show several differences between the original samples and PBS/BSA treated samples. In the degradation medium, some of the characteristic ester bands have decreased in magnitude

Table 2. Spectral changes in C=O stretching region (1850–1650 cm^{-1}) during *in vitro* degradation[a]

Sample	Frequencies of C=O stretching peaks (cm^{-1})								
PLA									
Original	1754	–	1717						
in PBS	1754 (ad)	1736 (a)	1717 (nc)						
in BSA	1754 (ad)	1745 (a)	1717 (sad)						
85:15 PLGA									
Original	–	–	–	1766	–	1750	–		
in PBS	–	1781 (a)	–	da	1756 (a)	da	1742 (a)		
in BSA	1789 (a)	–	1773 (a)	da	1759 (a)	da	1742 (a)		
75:25 PLGA									
Original	–	1784	–	–	–	1745	–	1733	–
in PBS	–	1787 (ad)	–	1764 (a)	–	da	1737 (a)	da	–
in BSA	1793 (a)	da	1778 (a)	–	1758 (a)	1748 (a)	–	da	1731 (a)
50:50 PLGA									
Original	–	1788	–	–	1752	–	1726		
in PBS	–	1789 (ad)	–	1756 (a)	1750	–	s		
in BSA	1798 (a)	1786 (ad)	1769 (a)	1757 (a)	da	1737 (a)	1727 (ad)		

[a] appeared; da, disappeared; ad, absorption decreased; s, shoulder; nc, no change; sad, sharp absorption decreased.

while some of them partially disappeared. In addition, formation of new bands at near frequencies, ie $\pm 10\,cm^{-1}$ is obvious. It was known that C=O stretching vibrations of aldehyde and ketone groups were observed at lower frequencies (ie 1750–1700 cm^{-1}) than ester bands. Therefore, the new bands observed at 1742 cm^{-1} for 85:15, at 1737 and 1731 cm^{-1} for 75:25, at 1737 and 1727 cm^{-1} for 50:50 PLGAs and at 1745 and 1736 cm^{-1} for PLA could be attributed to the aldehyde and ketone groups. The other bands formed at 1765–1750 cm^{-1} may be considered as short-chain ester bands.

However, formation of new bands at higher frequencies (1790–1780 cm^{-1}) than the original ester bands is more obvious. In general, the carbonyl stretching vibrations observed at high frequencies belong to the acid halides, anhydrides and cyclic ketones.[16,17] In our polymeric system, the bands which appear between 1790 and 1781 cm^{-1} are shifted to higher frequencies with increasing glycolide content

	Sample			
Time (day)	*PLA 100*	*PGLA 85/15*	*PGLA 75/25*	*PGLA 50/50*
Absorption decrement[a] *(%)*				
In PBS				
13	4.0	5.0	5.0	5.0
25	5.0	5.0	5.0	7.0
32	5.0	6.0	8.0	9.0
In BSA				
4	2.0	9.0	12.0	20.0
8	2.0	15.0	17.0	24.5
12	9.0	20.0	31.0	52.0
Absorption increment[b] (%)				
In PBS				
13	<1.0	10.0	15.0	26.0
25	<2.0	30.5	21.0	32.5
32	<2.0	40.0	34.5	58.0
In BSA				
4	11.0	15.5	24.0	40.0
8	33.0	40.0	60.0	85.0
12	55.0	70.0	115.0	nd

[a] Measured at 220 nm, 223 nm, 225 nm and 227 nm for 100 PLA, 85/15, 75/25 and 50/50 PLGA samples, respectively.
[b] Measured at 270 nm.

Table 3. Results obtained from the UV studies

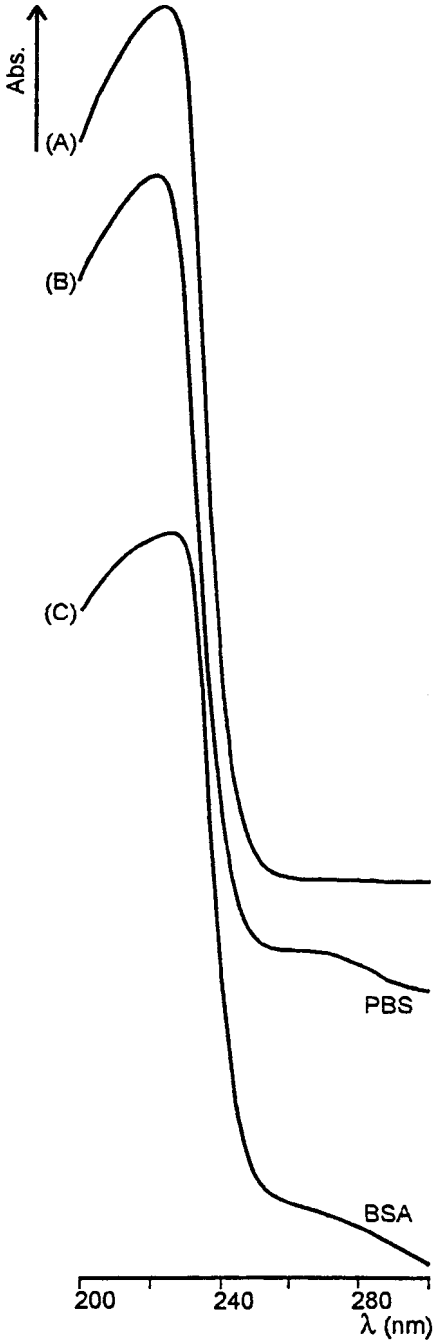


Figure 5. UV spectra of 85:15 PLGA copolymers: (A) original sample; (B) after 32 days in PBS; (C) after 12 days in BSA.

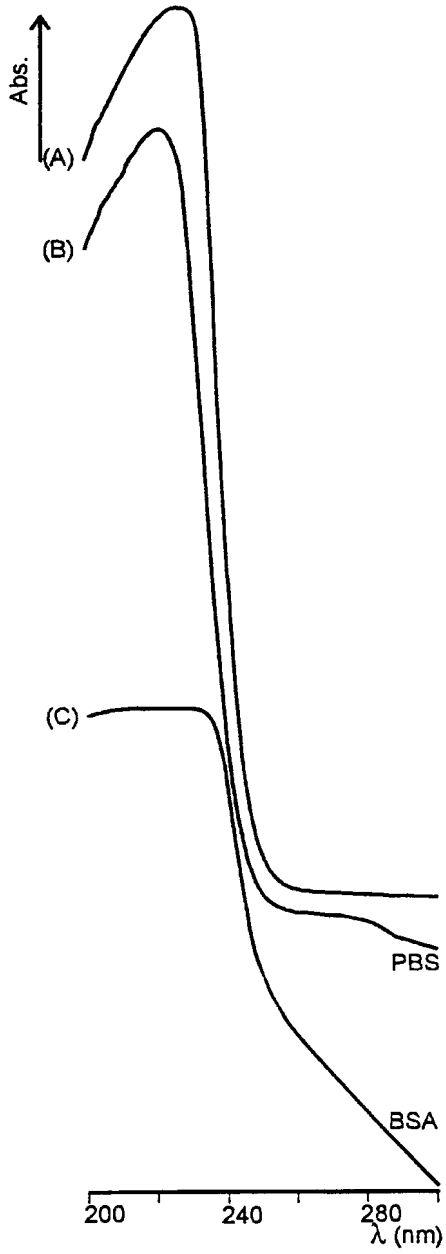


Figure 6. UV spectra of 75:25 PLGA copolymers: (A) original sample; (B) after 32 days in PBS; (C) after 12 days in PBS.

and are mainly interpreted as resulting from the formation of cyclic ester, ie lactone, structures.

UV results

The electronic spectral behaviour of the samples exposed to PBS and BSA solution were followed in the UV region between 340 and 200 nm at different time periods. All spectral features observed by UV are summarized in Table 3. Figures 5 and 6 also show the UV spectra of two copolymer samples in both media at different time periods. During the degradation process, absorbances of characteristic peaks which belong to the original samples, ie at 220 nm for PLA, 223 nm for 85/15, 225 nm for 75/25 and at 227 nm for 50/50 PGLA decreased with time. Increasing glycolide content and the presence of BSA in the degradation medium increases the decrement of the original absorbances. Here the effect of BSA is more important than the composition of films. This situation can be explained as resulting from the cleavage of ester bonds. Short chains formed contain repeatable ester units and/or new ester structures may be formed. These results are in agreement with those obtained by FTIR.

During the digital scanning between 280 and 260 nm the highest absorption was observed at 270 nm for all samples. The absorbances at 270 nm increased with time, increasing glycolide content and the presence of BSA in the degradation medium. This behaviour indicates that end-groups of chains are

oxidized to the aldehyde- or ketone-like structures. Because it was well known that, ketone or aldehyde structures have absorbances at higher wavelengths (>260 nm) than esters (≈220 nm), it can be considered that dissolved O_2 present in the degradation medium has an active role in this oxidation reaction. While breakdown of poly(lactide) chains causes formation of ketone-like structures, breakdown of poly(glycolide)chains leads to aldehyde-like end-groups.

CONCLUSIONS

The degradation study results provide quantitative and qualitative evidence to show that hydrolytic breakdown of PLGA copolymers is strongly influenced by the presence of BSA in the degradation media. This is attributed to the esterase-like activity of serum albumin. While mass loss is insignificant for all polymers during the 1 month degradation period (approximately 6% for PLA and 14% for 75:25 PLGA), intrinsic viscosities decreased greatly (eg from $0.55\,dl\,g^{-1}$ to $0.12\,dl\,g^{-1}$ for 50:50 PLGA), suggesting that erosion is not important during the first month of degradation. Spectroscopic studies indicate that it is possible to follow the degradation process by taking UV and FTIR spectra of the polymeric samples. The presence of BSA does not change the main degradation mechanism, it only increases the initial velocity of the hydrolytic de-esterification process, especially for glycolic-acid-containing copolymers. It is well known that PGA and its copolymers with PLA are degradable by enzymes such as esterases.[18] Further investigations should be carried out on the role of other proteins having esterase-like activity in the degradation of PLGA copolymers.

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