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Degradation behaviour of microspheres prepared by spray-drying poly(D,L-lactide) and poly(D,L-lactide-*co*-glycolide) polymers

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Abstract

Polymeric microsphere degradation must be taken into account in the design of drug delivery systems to be injected in *in vivo* systems, thus a prior analysis of *in vitro* degradation behaviour of microspheres appears to be necessary. In this study degradation characteristics of poly(lactide-*co*-glycolide) (PLGA) and poly(D,L-lactide) (PLA) microspheres prepared by the spray-drying technique have been examined. It was found that a slow decrease in molecular weight took place during the first stage of degradation, and the value of the rate constant decreased with the increase of the percentage of lactic acid of the polymer in a linear way. Thus, the period of time of this first stage decreased with the increase of content of glycolidyl units of the polymer, and it was the unique stage observed in PLA microspheres after 5 months of study. During this period of time, significant mass loss was not observed in the microspheres. The second stage of degradation of PLGA microspheres showed a larger rate constant, whose value increased with the content of glycolidyl units of the polymer. Mass loss was observed from number-average molecular weight about 6000. A sharp decrease of glass transition temperature (T_g) was observed coinciding with the start of mass loss. This fact was accompanied by a physical change of the samples, fusion of microspheres to form large particles, which also fusion to form a unique mass of polymer; moment from that the degradation process was quicker.

Keywords: Poly(D,L-lactide); Poly(lactide-*co*-glycolide); Spray-dryer; Microspheres; Degradation

1. Introduction

Aliphatic polyesters such as poly(D,L-lactide) and its copolymers with glycolic acid have received considerable attention for the preparation of sustained release drug delivery systems (Okada and Toguchi, 1995; Bala et al., 2004; Matsumoto et al., 2005) and also in the medical field as substrates for tissue engineering (Lu et al., 2001) or osteofixation devices in craniomaxillofacial surgery (Ashammakhi et al., 2001). The different uses of these polymers in pharmaceutical and biomedical fields are based on their biodegradable and biocompatible characteristics, since their degradation products, lactic acid and glycolic acid, can be metabolized by human beings (GenomeNet, 2006). Thus, the degradation characteristics of these polymers are very important for their pharmaceutical and biomedical applications. Their degradation is known to be affected by the preparation method of the systems (Lemoine et al., 1996; Cai et al., 2003), by polymer properties such as initial molecular weight, morphology of the devices and lactide/glycolide ratio of the copolymers (Li et al., 1990a,b,c), as well as by physical and chemical parameters such as temperature and pH of the external medium (Li and MacCarthy, 1999). Studies have been also carried out to evaluate the effect of the particle size (Lemoine et al., 1996; Dunne et al., 2000), the number of carboxylic end groups of the devices (Schliecker et al., 2003) or the presence of enzymes in the external medium (Cai et al., 2003) on the degradation behaviour of poly(α-hydroxy acids).

The release of drugs from microparticulate delivery systems prepared with poly(D,L-lactide) and poly(lactide-*co*-glycolide) is controlled by diffusion and/or erosion mechanisms (Batycky et al., 1997; Wong et al., 2001; Lemaire et al., 2003). The erosion of microparticles depends on the polymer, the size of the system and the process used to obtain the particles (Dunne et al., 2000). Poly(α-hydroxy acids) nano- and microparticles are usually produced by oil in water emulsion solvent evaporation methods (Matsumoto et al., 2005; Kim et al., 2005; Zhang and Zhu, 2004),

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and the degradation studies have been carried out mainly with particles prepared using emulsion techniques (Lemoine et al., 1996; Dunne et al., 2000). However, the spray-drying technique is being also used to encapsulate different substances in order to obtain drug delivery systems (Blanco et al., 2003; Blanco-Príeto et al., 2004; Pamujula et al., 2004). The purpose of this paper is to analyse the *in vitro* degradation of microspheres prepared by spray-drying poly(D,L-lactide) and poly(lactide-*co*-glycolide) polymers with different lactide/glycolide ratio, as a previous step to evaluate their degradation behaviour and the effect of degradation on drug release after *in vivo* injection.

2. Materials and methods

2.1. Materials

Poly(D,L-lactide) (PLA) (Sigma–Aldrich, Barcelona, Spain), Poly(D,L-lactide-*co*-glycolide) (PLGA) [lactide:glycolide 50:50 and lactide:glycolide 75:25] (Sigma–Aldrich), dichloromethane (Panreac, Barcelona, Spain), chloroform (Panreac), potassium monohydrogen phosphate (K_2HPO_4) (Panreac), potassium dihydrogen phosphate (KH_2PO_4) (Panreac), tetrahydrofuran (Sigma–Aldrich), poly(methyl methacrylate) standards (Polymer Lab., Shropshire, USA), were used as received. Milli-Q® water (Millipore, Madrid, Spain) was used.

2.2. Preparation of microspheres

Preparation of microspheres was carried out by the spray-drying process (Mini Spray-dryer B-190, Büchi, Switzerland) (Blanco et al., 2005). To obtain microspheres, poly(D,L-lactide) (PLA), poly(D,L-lactide-*co*-glycolide) 50:50 (PLGA 50/50) or poly(D,L-lactide-*co*-glycolide) 75:25 (PLGA 75/25) was dissolved in dichloromethane (2 wt.%). The polymeric solutions (100 mL) were maintained under constant stirring (900 rpm) and sprayed through the nozzle (0.5 mm diameter) of the spray-dryer. Assay conditions were: (a) PLA microspheres: inlet air temperature 63–66 °C, outlet air temperature 51–53 °C; (b) PLGA 75/25 microspheres: inlet air temperature 62–65 °C, outlet air temperature 50–53 °C; (c) PLGA 50/50 microspheres: inlet air temperature 63–66 °C, outlet air temperature 51–54 °C. Spray flow 5 mL/min, and compressed spray air flow (represented as the volume of the air input) 700 L/h. Microspheres were collected from the spray-dryer cyclone separator, and then they were placed in a vacuum oven (Bioblock Scientifics) for 24 h at 100 mbar of pressure and 37 °C. Microspheres were stored in a desiccator under vacuum condition.

2.3. In vitro degradation of microspheres

For each polymer sample, polyethylene test tubes were prepared with 40 mg of microspheres and 3 mL of phosphate buffer (1 mM, pH 7.4). They were stored in the absence of light at 37 °C. Once a day, test tubes were stirred during 1 min in a vortex, and buffer solution was replaced every week. At suitable intervals of time, phosphate buffer was removed and then the polymer sample was dehydrated using a vacuum concentrator (Speed Vac SAVANT model SVC 200 H) for 12 h. The maximum time of incubation was 5 months. The dehydrated polymer sample was examined for mass loss and then it was stored in dark. Mass loss of the microspheres was determined gravimetrically using the following equation:

$$\%\ \text{mass remaining} = \frac{M_2}{M_1} \times 100 \quad (1)$$

where M_1 is the initial weight of microspheres and M_2 is the weight of the microspheres after the incubation period.

2.3.1. Morphology studies

The morphology of the samples at different times of degradation was studied by scanning electron microscopy (SEM) (Jeol JSM-6400 Electron Microscope, resolution 36 mm of Centro de Microscopia Electrónica "Luis Bru", UCM). The samples were fixed with an adhesive sheet on a rigid support and coated with gold for their later visualisation.

2.3.2. Molecular weight of the polymer

The molecular weight of the samples at different times of degradation was determined. The molecular weight measurements were carried out by size exclusion chromatography (GPC) (Pump Waters model 501; injector Waters model U6K). The separation system was formed by four columns Ultrastyragel with pore sizes of: 10^3, 10^4, 10^5 and 10^6 Å, respectively, connected in series. The detection system was a detector of refraction index (Waters R-401), whose signal is codified and transmitted to the data processing system. Tetrahydrofuran (THF) was the mobile phase. Columns were previously calibrated using poly(methyl methacrylate) standards (Polymer Lab). Samples were dissolved in THF (concentration 0.5 wt.%) and then microfiltered (pore size 0.2 μm).

2.3.3. Calorimetric studies

Thermal analysis of all the above-mentioned samples were carried out using a DSC system (Shimadzu DSC-50 equipment) connected to a computer by an interface (Shimadzu TA-50). A cryostat bath [Julabo, F-10] filled with methanol at −10 °C was used. Samples weighing 5–7 mg were sealed in an aluminium sample pan. An empty sealed aluminium pan was used as the reference. The heating rate was 5 °C/min. Two heating scans were performed, the first one from 15 to 80 °C, to avoid the thermal history of the polymer, and the second one from 15 to 350 °C. The glass transition temperature (T_g) of the samples was determined.

3. Results and discussion

Microspheres of PLGA 50/50, PLGA 75/25 and PLA were prepared by the spray-drying process and their characterization was previously reported (Blanco et al., 2005). Microspheres were of small size (average diameter: 0.9 ± 0.4 μm for PLGA 50/50; 1.4 ± 0.8 μm for PLGA 75/25; 1.3 ± 0.7 μm for PLA), and their surface was smooth and slightly porous. A fractionation process of the raw polymer during the formation of microspheres

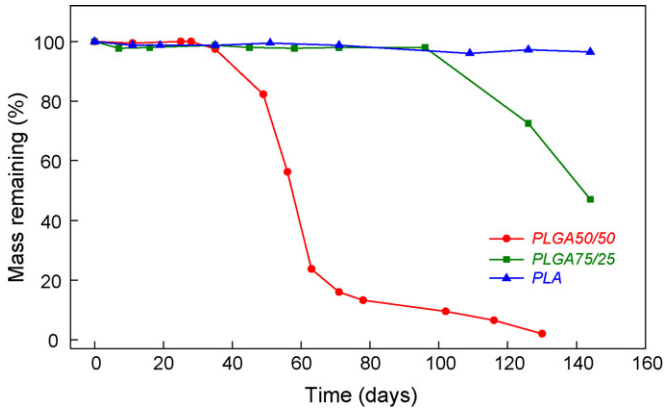

Fig. 1. Percentage of mass remaining at different times of the degradation process of PLGA 50/50, PLGA 75/25 and PLA microspheres.

was observed as an increase of the average molecular weight and also of T_g of the polymer of the microspheres.

The degradation studies of the microspheres in phosphate buffer (1 mM, pH 7.4) at 37 °C showed that the mass loss of the microspheres could be related to their polymer composition (Fig. 1). Thus, for PLGA 50/50 microspheres there were a period of negligible mass loss during the first 35 days. This was followed by a quick decrease of the microsphere mass over the next 30 days after which a residual mass of 16% was slowly lost during the next 2 months. The period of negligible mass loss was the first 100 days for PLGA 75/25 microspheres, and it was also followed by a quick mass loss during more than 50 days. For PLA microspheres mass loss was not observed during the 5 months of study. Degradation studies of PLGA 50/50 microspheres synthesised by oil in water emulsion (Dunne et al., 2000) have shown a similar behaviour, although a small mass loss (≤9%) during the first 24 h of incubation took place, and it was attributed to the dissolution of low molecular weight oligomers produced during the manufacture of the particles at or near the surface of the particles. In the characterization of the microspheres obtained by spray drying methodology that phenomenon was not observed (Blanco et al., 2005). Degradation studies of non-porous PLGA films (Cai et al., 2003) also show the effect of the polymer composition on the weight loss. Thus, PLGA 50/50 films showed significant weight loss after 2 weeks, and for PLGA 70/30 films the rapid weight loss was observed at 6 weeks.

The mass loss from these microspheres could be described by the Prout–Tompkins equation, which has been evaluated to investigate degradation-dependent release from polymers (Ramtoola et al., 1992):

$$\ln\left(\frac{X}{1-X}\right) = kt + m, \quad \text{where } m = -kt_{50} \tag{2}$$

where X is the fractional mass remaining at time t, k the rate constant, and t_{50} is time to achieve 50% mass loss. Mass loss from PLGA 50/50 and PLGA 75/25 microspheres determined from 20 to 60 days of incubation in phosphate buffer for PLGA 50/50 and from 100 to 145 days of incubation for PLGA 75/25, has been evaluated using the Prout–Tompkins equation (Table 1).

Table 1
Values of k (day^{-1}) and t_{50} (day) for the mass loss of PLGA 50/50 and PLGA 75/25 microspheres fitted to Eq. (2)

Type of microspheres	k (day^{-1})	t_{50} (day)	r^2
PLGA 50/50	0.183	56.8	0.996
PLGA 75/25	0.085	140.6	0.987

r^2, regression coefficient.

Experimental data were fitted to first order plots with good correlation coefficients, from which the rate constant (k) and the time to obtain the 50% of mass loss (t_{50}) representing the induction and acceleration of decomposition were determined. These values indicated the high increase of stability of microsphere when the percentage of lactic acid of the copolymer increased. Similar values of the time needed to achieve 50% of mass loss have been described for PLGA 50/50 microspheres ($\bar{M}_n = 26,700 - 26,200$) prepared by oil in water emulsion (Dunne et al., 2000); thus, microspheres of 6.87 μm of average diameter has values of t_{50} of 54.2 days, and the value of the parameter was 59.7 days for microspheres of 0.53 μm of average diameter.

The degradation behaviour of the microspheres was also studied by determining the average molecular weight of the polymer of the microspheres at different period of incubation in phosphate buffer pH 7.4 at 37 °C. (Table 2). The number-average molecular weight ($\bar{M}_n$) was 24,200 for PLGA 50/50 microspheres, 39,800 for PLGA 75/25 microspheres, and 25,400 for PLA microspheres after the obtaining process. When the degradation of the polymers of the microspheres was examined the molecular weight profile was found to decrease with time. The ratio of the molecular weight at time t ($\bar{M}_n^t$) and the initial molecular weight ($\bar{M}_n^0$) of the polymers of the microspheres (Fig. 2) indicated the capability of degradation of each type of microsphere. Thus, the average molecular weight of the microspheres prepared with PLGA 50/50 decreased 94% during the first 2 months, whereas the average molecular weight of PLGA 75/25 and of PLA microspheres decreased 37% and 12%, respectively, during the same period of time. These data were in accordance to those of mass loss, and they reflected the same order

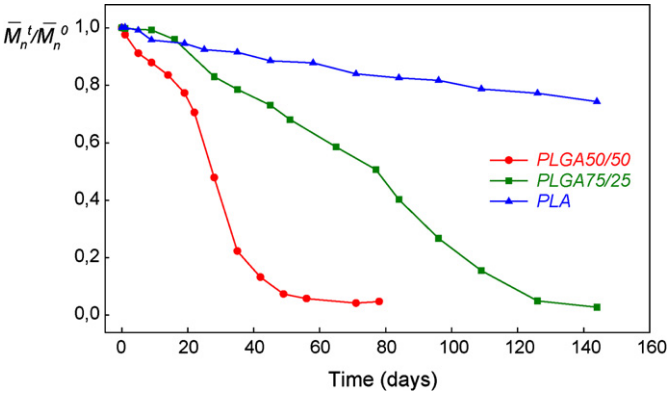

Fig. 2. Evolution of the ratio of number-average molecular weight at different times of the degradation process (M_n^t) and the initial number-average molecular weight (M_n^0) of the PLGA 50/50, PLGA 75/25 and PLA microspheres.

Table 2
Number-average molecular weight ($\bar{M}_n$), weight-average molecular weight ($\bar{M}_W$) and polydispersity indices (PI) of PLGA 50/50, PLGA 75/25 and PLA microspheres after different time of incubation in phosphate buffer 1 mM, pH 7.4 at 37 °C

Incubation time (days)	PLGA 50/50 microspheres[a]			PLGA 75/25 microspheres[b]			PLA microspheres[c]		
	$\bar{M}_n$	$\bar{M}_W$	PI	$\bar{M}_n$	$\bar{M}_W$	PI	$\bar{M}_n$	$\bar{M}_W$	PI
0	24200	38800	1.60	39800	65400	1.64	25400	42600	1.68
1	23900	38200	1.60	39700	64200	1.61	25400	42000	1.65
5	22100	36600	1.65	–	–	–	25200	41600	1.65
9	21300	35400	1.66	39500	64400	1.63	24300	40400	1.66
14	20200	34600	1.71	–	–	–	–	–	–
16	–	–	–	38200	59200	1.55	–	–	–
19	18700	30300	1.62	–	–	–	24000	40200	1.67
22	17100	26900	1.57	–	–	–	–	–	–
25	–	–	–	–	–	–	23500	39800	1.69
28	11600	19300	1.67	33000	52300	1.59	–	–	–
35	5400	8100	1.50	31200	50300	1.61	23300	39500	1.70
42	3200	4900	1.54	–	–	–	–	–	–
45	–	–	–	29100	45400	1.56	22500	38800	1.73
49	1800	2300	1.29	–	–	–	–	–	–
51	–	–	–	27100	44400	1.64	–	–	–
56	1400	1700	1.18	–	–	–	–	–	–
58	–	–	–	–	–	–	22300	39900	1.79
71	1100	1200	1.08	20800	34000	1.64	21300	36700	1.72
78	1000	1100	1.08	–	–	–	–	–	–
84	–	–	–	16000	25800	1.61	21000	36200	1.73
96	–	–	–	10600	17400	1.63	20800	35700	1.71
109	–	–	–	6200	9200	1.50	20000	34700	1.74
126	–	–	–	2000	2100	1.08	19600	33900	1.72
144	–	–	–	1100	1200	1.09	18900	32100	1.70

[a] $\bar{M}_n \pm 300$, $\bar{M}_W \pm 500$, PI ± 0.02.
[b] $\bar{M}_n \pm 600$, $\bar{M}_W \pm 700$, PI ± 0.03.
[c] $\bar{M}_n \pm 600$, $\bar{M}_W \pm 600$, PI ± 0.02.

of stability of the microspheres PLA > PLGA 75/25 > PLGA 50/50.

The kinetic behaviour of the degradative process of polymeric matrices can be studied using the degradation index (DI), which represents the proportion of links broken at each time regarding to the initial links:

$$\mathrm{DI} = \left(\frac{\bar{M}_n^0}{\bar{M}_n^t}\right) - 1 \tag{3}$$

The evolution of the degradation index of the polymers of the microspheres (Fig. 3) indicated that for PLGA 50/50 and PLGA 75/25 microspheres there was an initial phase of induction or lag period, in which breaks of links scarcely took place. The lag period was then following by a second phase of quicker degradation, in which the breaks of links was increased.

Fig. 4 shows the logarithmic plots of ratio (molecular weight at time *t*/molecular weight at initial time) versus time for PLGA 50/50, PLGA 75/25 and PLA microspheres incubated in phosphate buffer (pH 7.4) at 37 °C for 5 months. The phases of the degradation process and their duration were shown, and the degradation rate constants (k) and the half-life times ($t_{1/2}$) of the polymers (Table 3) were obtained:

$$k = \left(\frac{\ln(\bar{M}_n^t/\bar{M}_n^0)}{t}\right); \qquad t_{1/2} = \frac{\ln 2}{k}$$

Thus, the largest decrease in molecular weight took place in the second phase of degradation, which agrees with a first order kinetic. This second phase began the day 22 of incubation of PLGA 50/50 microspheres and the day 77 of incubation of PLGA 75/25 microspheres. Microparticles of PLGA 50/50 prepared by an oil in water emulsion solvent evaporation method (Dunne et al., 2000) showed a quicker decrease in $\bar{M}_n$ and $\bar{M}_W$ during the first phase than during the second phase of degradation. This fact could be related to the preparation method of microspheres. Microsphere preparation by oil in water emulsion solvent evaporation (Dunne et al., 2000) causes a decrease in $\bar{M}_n$

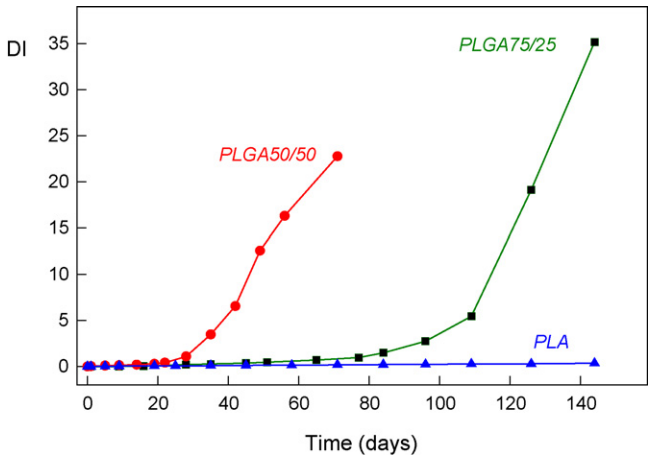

Fig. 3. Degradation index (DI) at different times of the degradation process of the PLGA 50/50, PLGA 75/25 and PLA microspheres.

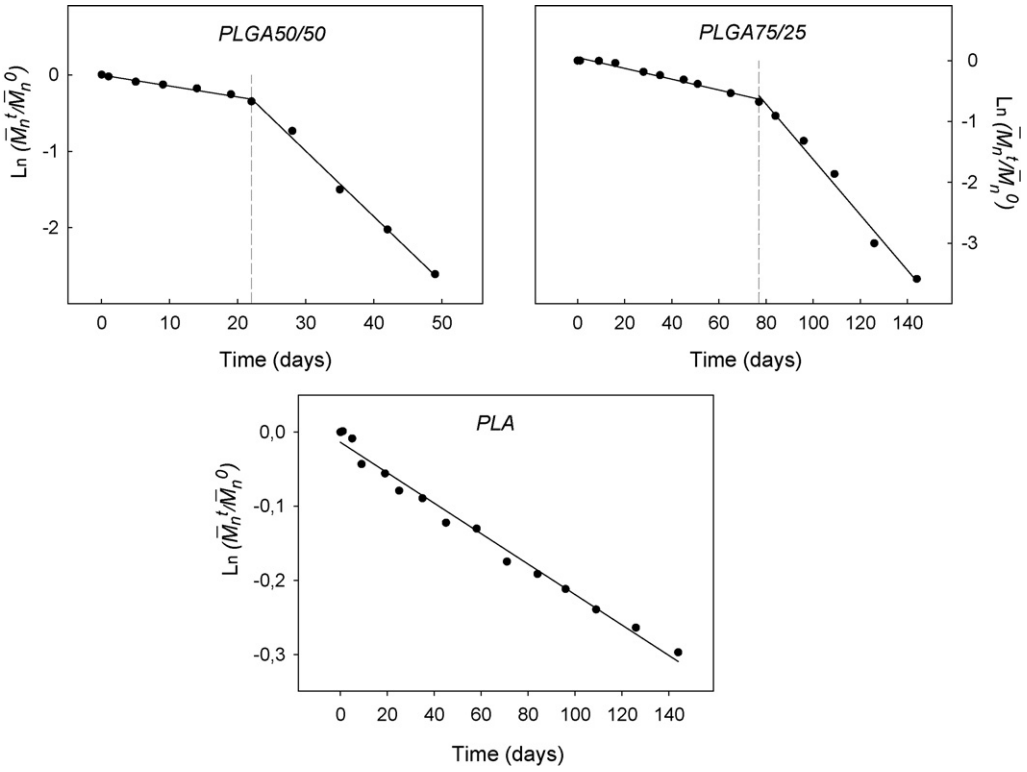

Fig. 4. Logarithmic plots of the ratio (number-average molecular weight at time *t*/number-average molecular weight at initial time) [ln ($\bar{M}_n^t/\bar{M}_n^0$)] vs. degradation time of the PLGA 50/50, PLGA 75/25 and PLA microspheres.

Table 3
Degradation rate constants (*k*) derived from the slopes of logarithmic plots of number-average molecular weight at time *t*/initial number-average molecular weight of the polymeric microspheres vs. time, regression coefficient (r^2), and half-life times ($t_{1/2}$)

Type of microspheres	First phase				Second phase			
	Time (day)	k (day^{-1})	$t_{1/2}$ (day)	r^2	Time (day)	k (day^{-1})	$t_{1/2}$ (day)	r^2
PLGA 50/50	22	14.3×10^{-3}	48.4	0.98	27	85.4×10^{-3}	8.1	0.99
PLGA 75/25	77	9.2×10^{-3}	75.5	0.98	67	45.5×10^{-3}	15.2	0.99
PLA	>144	2.1×10^{-3}	338.1	0.99	–	–		

and $\bar{M}_W$ of the polymer of the microspheres with regard to those of the raw polymer, probably as a consequence of the cleavage of long polymer chains during processing. However, preparation of PLGA and PLA microspheres by the spray-drying method caused the opposite effect, there is an increase in $\bar{M}_n$ and $\bar{M}_W$ of the polymer of the microspheres with regard to those of the raw polymers (Blanco et al., 2005). The chains of lower molecular weight are swept away and eliminated with the solvent by the air stream of the aspirator during the spray-drying process, and as a consequence they do not form a part of microspheres.

In the case of PLA microspheres, after 144 days of incubation the second phase of degradation was not detected. However, the variation of the degradation rate constant of the first phase of the process as a function of the polymeric composition of the microspheres (Fig. 5) was in accordance with a first order plot ($r^2 = 0.991$), which seemed to indicate that the degradation patron of PLA microspheres was not very different to that of the copolymeric microspheres, although the second phase of the degradation process was not detected in 5 months of incubation.

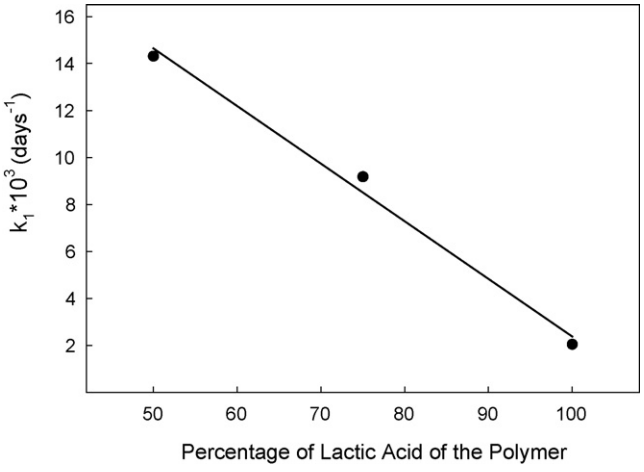

Fig. 5. Variation of the degradation rate constant of the first stage of decrease in molecular weight of microspheres as a function of the polymeric composition of microspheres, expressed as percentage of lactic acid of the (co)polymer.

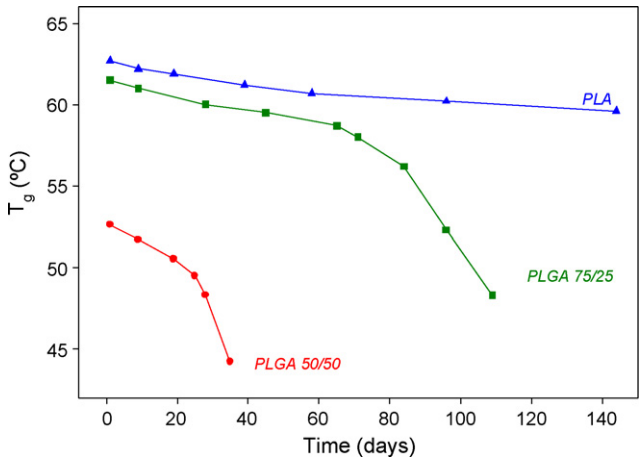

Fig. 6. Glass transition temperature (T_g) at different times of the degradation process of the PLGA 50/50, PLGA 75/25 and PLA microspheres.

The degradation of these polyesters is due to the hydrolysis of backbone ester groups, which is autocatalysed by carboxylic end groups, however differences in hydrophobicity determine the rate of *in vitro* degradation (Lemoine et al., 1996). Thus, as larger the percentage of glycolic acid in the copolymer as higher the hydrophilicity of the microspheres and, as a consequence, a larger water uptake and quicker hydrolysis of the ester bonds takes place. Degradation products trapped within the microsphere have the potential to catalyse the degradation of the remaining polymer material. This effect was low significant in PLA microspheres since this polymer is more hydrophobic, and it can explain that the second phase of degradation was not observed in 5 months.

The degradation process of the microspheres began with a decrease of molecular weight as a consequence of the hydrolysis of ester bonds of the polymers, which originated smaller polymer chains. When the size of the polymer fragments were small enough and the microsphere became more porous, degradation products could escape from the matrix and solved in the incubation medium. Thus, the mass loss of the microspheres began to be observed. The mass loss of the microspheres began from a critical value of number-average molecular weight ($\bar{M}_n$), which was 5400 for PLGA 50/50 and 6200 for PLGA 75/25. As a consequence, the beginning of mass loss took place 10 and 20 days later than the beginning of molecular weight decrease of PLGA 50/50 and PLGA 75/25 microspheres, respectively. In degradation studies of PLGA films (Cai et al., 2003) the mass loss also occurred when the $\bar{M}_W$ decreased to about below

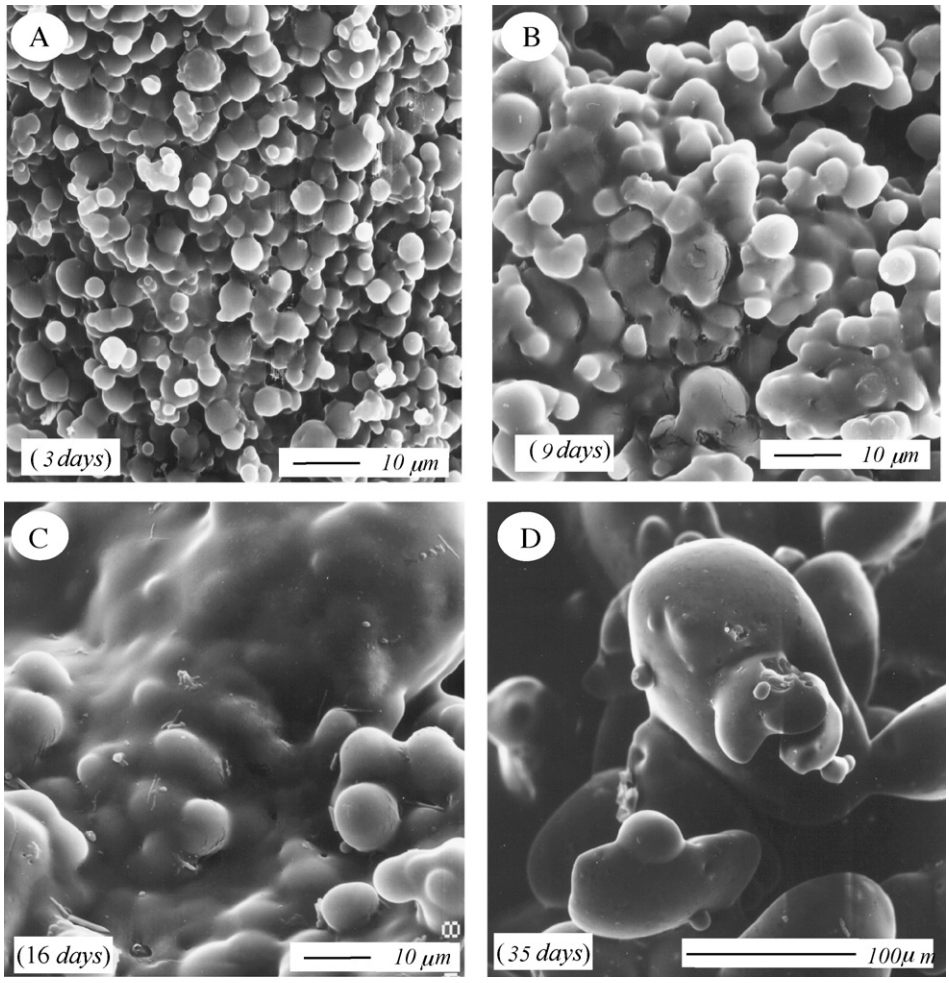

Fig. 7. Scanning electron (SEM) micrographs of PLGA 50/50 microspheres at different times of the degradation process in phosphate buffer pH 7.4 at 37 °C.

10,000. This was consistent with the common hydrolysis rule of aliphatic polyesters that the mass loss would not take place until the molecular weight of samples had decreased to a critical value able to dissolve in water (Park, 1995).

Glass transition temperature (T_g) of the microspheres decreased during the degradation process (Fig. 6). For PLGA 50/50 microspheres, the last value of T_g that could be determined was that of the sample incubated during 35 days ($T_g = 42.8\,^{\circ}C$), and for PLGA 75/25 microspheres that of the sample incubated during 109 days ($T_g = 48.3\,^{\circ}C$). T_g of PLGA 50/50 microspheres incubated 40 days and of PLGA 75/25 incubated 126 days have to be very low since the heating scans were performed from 15 °C. There was a sharp decrease of T_g values in the last stage of the degradative process. T_g of PLA microspheres only decreased 3.1 °C during 144 days of incubation, whereas T_g decrease of PLGA75/25 was 13.2 °C in 109 days, and that of PLGA 50/50 microspheres was 8.4 °C in 35 days of incubation. Stability studies of PLA and PLGA 75/25 nanoparticles prepared by oil and water emulsion and stored in water at room temperature during 12 months indicated a decrease of of T_g of 2.5 and 6.5 °C, respectively (Lemoine et al., 1996).

The sharp decrease of T_g was accompanied by a physical change of the samples, and microspheres were transformed in a solid elastic mass when the T_g value was lower than the incubation temperature. The new physical state made easier the degradation process, and from this moment the degradative process was quicker, which caused the strong diminution of T_g. At this point, the beginning of the mass loss of the microspheres was observed (Fig. 1). Morphological changes of the microspheres were similar, although a time-dependent behaviour was observed as a function of their copolymeric composition; in fact the degradation stages were earlier for microspheres obtained from copolymers with larger amount of glycolic acid. The first morphological changes were the loss of the spherical form of the microspheres and the fusion of some of them to form a lattice in which deformed microspheres could be observed. These changes were observed in PLGA 50/50 and PLGA 75/25 microspheres after the first 3 days and 56 days of incubation in phosphate buffer at 37 °C, respectively (Figs. 7 and 8). The fusion of the particles increased with the time of incubation to form large particles with smooth surface, in which individual microspheres were not observed in the last stages of the degradation

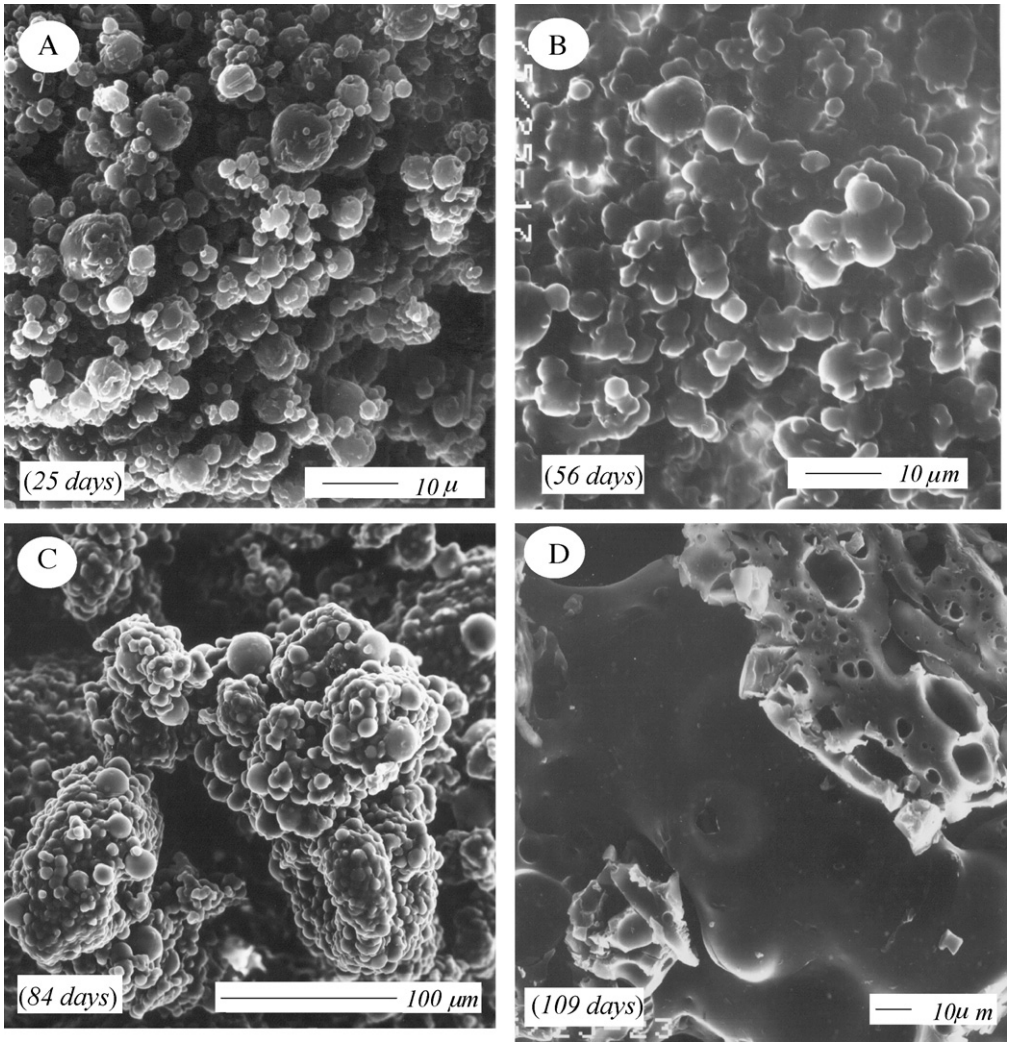

Fig. 8. Scanning electron (SEM) micrographs of PLGA 75/25 microspheres at different times of the degradation process in phosphate buffer pH 7.4 at 37 °C.

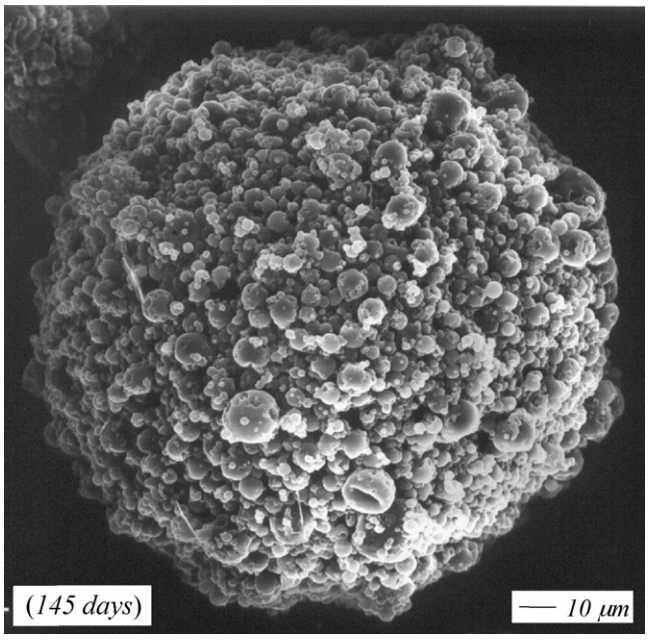

Fig. 9. Scanning electron (SEM) micrograph of PLA microspheres after 145 days of degradation in phosphate buffer pH 7.4 at 37 °C.

process. This was observed after the first 35 days and 109 days of incubation for PLGA 50/50 and PLGA 75/25 microspheres, respectively. The formation of these large particles coincides with the sharp decrease of the T_g. From this time, the fusion of the large particles took place quickly and a unique mass of polymer was formed. Regarding PLA microspheres, the degradation process was very much slower, and after 5 months of incubation only groups of slightly deformed or broken microspheres were observed (Fig. 9).

The fusion of microspheres caused a decrease of the surface area of the systems, particles of large size were formed and, as a consequence, an easier degradation took place (Grizzi et al., 1995; Dunne et al., 2000). The quick degradation, due to the increase of autocatalysis, caused the loss of molecular weight of the polymer material. The smaller polymeric chains were soluble in the solvent medium, which caused the mass loss of the polymeric material and, as a consequence, the decrease of the T_g value. When the T_g value was under the incubation temperature, the polymeric material behaves as an elastic solid.

4. Conclusion

The degradation process of PLGA microspheres prepared by spray-drying methodology took place in two stages. For PLA microspheres only the first stage was observed during the 5 months of the study. For PLGA microspheres, during the first stage of degradation mass loss was not observed, and the decrease in molecular weight took place with a slower constant rate than the constant rate of the second stage, phase at which mass loss occurred from number-average molecular weight values about 6000. This critical value of number-average molecular weight was earlier reached for microspheres prepared with polymers with larger content of glycolidyl units since they showed the largest rate constants of decrease in molecular weight at both degradation stages, as well as the largest rate constant of mass loss. A linear relationship between the rate constant of the first stage of decrease in molecular weight and the percentage of lactic acid of the polymer was observed. Thus, the slow rate of degradation of PLGA and PLA microspheres prepared by spray-dying methodology during the first stage of the degradation process, whose value depends on the percentage of lactic acid of the polymer, can be significant in the application of these microspheres in drug release mainly in *in vivo* systems.

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