Published in: Journal of controlled release (1996), vol. 38, iss. 2-3, pp. 109-122
Status: Postprint (Author's version)

Control of the biodegradation rate of poly(DL-lactide) microparticles intended as chemoembolization materials

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Abstract
There is an interest in polylactide microspheres that biodegrade within a few days, particularly for chemoembolization applications. For this purpose, two poly(DL-lactide) samples of a very different molecular weight have been combined. The basic concept relies upon the capability of the high molecular weight component (M_n: 65 000) to provide the microspheres with a high mechanical strength, whereas the low molecular weight component (M_n: 3500) should decrease the particle lifetime dramatically. It has been shown that changing the weight ratio of these two components is an efficient way to control the kinetics of the in vitro degradation of poly(DL-lactide) microspheres on the expected time scale. The microspheres have been prepared by the oil-in-water emulsion/evaporation process, and their final polymer content has been compared to the initial composition of the oil phase. They have also been analyzed by differential scanning calorimetry to know whether the two polymers form a monophase blend or not. Kinetics of the in vitro biodegradation has been estimated from the decrease in molecular weight of the constitutive poly(DL-lactide)s, the time-dependency of the microsphere weight and the observation of changes in the morphology by scanning electron microscopy. Progress in the hydrolysis of the ester groups has also been reckoned from the increasing acidity of the incubation medium and associated with the polymer.

Keywords*:* Poly(DL-lactide); Degradation; Chemoembolization; Microparticle; Microencapsulation; Solvent evaporation method

I. Introduction

Chemoembolization is an endovascular therapy, that was first proposed by Kato et al. [1], and that involves the selective arterial embolization of a tumor together with the simultaneous or subsequent local delivery of chemotherapeutic agents. This technique advantageously combines the beneficial effects of embolization and local chemotherapy, thus increasing the therapeutic efficiency of antimitotic drugs [2]. Spherical particles are ideal in achieving distal, homogeneous and effective occlusions [3]. Moreover, kinetics of drug release from the emboli and biodegradation rate of the drug carrier are of the utmost importance, since they determine the bioavailability of the drug within the pathological area. For a sequential treatment to be applied, microspheres must disappear before any foreign body reaction occurs. According to Madoule et al. [4], the optimum lifetime of the carrier should be close to 10 days.

Due to their well-known biocompatibility and bio-degradability, polylactide (PLA) and poly (lactide-co-glycolide) (PLGA) have been considered for chem-oembolization by several authors [2,5-8]. Although the biodegradation rate of these polyesters can be adjusted by changing the PLA configuration and the glycolide content, several weeks or months are needed to observe a significant weight loss of microspheres made of high molecular weight polyesters. For instance, microspheres of the more labile poly(lactide-co-glycolide) of a 50 wt% composition remain essentially unmodified after 1 month degradation at 37°C [3,9]. It should be stressed that relatively high molecular weight polymers (M_n>10 000) are recommended in order to prevent any undesirable burst in the drug release and the microsphere aggregation from occuring. A way to tackle this problem is to incorporate an acidic or a basic excipient into the polyester for speeding up the polymer hydrolysis [10,11]. Such an alternative may cause problems such as: toxic side effects of the additives, uneven distribution of the excipients within the material or worst its early release from the polymeric matrix.

This paper proposes to control the degradation rate of PLA microspheres, by blending a high molecular weight PLA with an oligomeric polylactide in various weight ratios. Although this strategy has already been reported by Bodmeier et al. [12] in order to promote the drug release from a poly(DL-lactide) matrix, the degradation kinetics has not been investigated by these authors.

This paper will analyse the in vitro degradation of the two poly(DL-lactide) components of the microspheres in relation to changes observed in particle morphology, particle weight, and polymer molecular weight distribution. These experimental data will be compared to the percentage of hydrolyzed ester groups estimated from the increasing acidity of the external buffer and the carboxylic acid groups attached to the polymer.

The microsphere size range, i.e. 100—160 μm, has been selected on the basis of a previous study [3,13] that has shown that this size allows a distal and homogeneous migration of the embolic material within the targeted

organ, whithout passing through the capillary filter. This particle size range is thus particularly suited for chemoembolization purposes, that requires a selective and homogeneous release of the antimitotic drug within the tumor.

2. Experimental

2.1. Materials

Poly(DL-lactide)s: Resomer 206 and Resomer 104 were purchased from Boehringer Ingelheim K.G. (Ingelheim, Germany). Mean number molecular weight, M., was measured by size exclusion chromatography (SEC) in THF as detailed further. The corresponding values were found to be 65 000 and 3500 for Resomer 206 and Resomer 104, respectively.
Polyvinyl alcohol, or PVA, (Mowiol VP 3-83) was provided by Hoechst. Methylene chloride, acetone and other reagents were of analytical grade and used without further purification.

2.2. Methods

Preparation of(DL)PLA microspheres
Poly (DL-lactide) microspheres were prepared by the oil-in-water emulsion evaporation technique, previously designed to produce particles of a size ranging from 100 to 160 μm [13]. Briefly, the two selected poly(DL-lactide)s were used in various weight ratios (Table 1) and dissolved in an acetone-methylene chloride (1:9 w/w) solvent mixture for 2 h at room temperature. This organic solution was then emulsified in 100 ml of a PVA solution (2.5 wt%) at 0°C. The two immiscible phases were mixed at 500 rpm with a four-pitched blade impeller (rod diameter, 6 mm; blades, 8 × 20 mm; pitched at 45°). The resulting oil-in-water emulsion was then stirred for 5 rain at 0°C, and for a further 4 h period at 30°C. Microspheres were washed four times with water, and sized mechanically on a series of six standard sieves (20 cm in diameter; Fritsch, Idar-Oberstein, Germany). The microsphere fraction in the 100—160 μm range was collected by filtration on a paper filter and vacuum dried at 30°C for at least 2 days.

Characterization of microspheres

Size and morphology of the particles

Size distribution of the particles was measured with a Coulter Multisizer (Coulter Electronics Inc. Hialeah, FL, USA) after redispersion in a saline medium (NaCl 1 % w / v). This analysis was carried out with a 560 μm aperture orifice tube and the results were reported as a number or volume size distribution.
The microparticle morphology was observed by scanning electron microscopy (JEOL, JSM-840A). Particles were previously coated with a ca. 30 nm thick Au-Pd film (Balzers, SCP-20).
Differential scanning calorimetry (DSC)
Aluminum pans were filled with microparticles (5-10 mg). Calorimetric measurements were performed with a Dupont Instrument (Model 910), over the temperature range from — 50 to 100°C at a heating rate of 10°C/min. The temperature scale was calibrated with high purity standards. The glass transition *(T_g)* was reported as the point of inflection of the specific heat increment.
Polymer molecular weight determination
Molecular weight distribution of PLA was analyzed by size exlusion chromatography in non stabilized tetrahydrofuran as the mobile phase (1 ml/min, 30°C, System Gold Beckman, France as the solvent delivery system). Polymer solutions (20 μl of a 1 wt% solution) were injected with an autosampler (WISP 710, Waters, Milford, MA USA) into a set of three μ-Styragel columns (Waters) with nominal pore sizes of 10^4, 10^3 and 500 .~. Polymer elution was traced with a UV detector at 231 nm (detector 166 Beckman). A universal calibration curve was made available by using polystyrene standards (Polymer Laboratories Ltd, Chruch Stretton, Shropshire, UK) and the Mark-Houwink viscometric relationship for (DL) PLA in THF at 30°C [14].

In vitro degradation
An incubation protocol was used such as to avoid any transfer of the microspheres and bacterial contamination. Filtration units allowed the microparticles to be washed without any loss, whilst performed in a glass vessel, part of them floats on the surface or adsorbs to the flask. Tween 80, a hydrophilic non-ionic surfactant, was added in order to facilitate water diffusion, as done in vivo by proteinaceous compounds.
100 mg microspheres, 100-160 *μm* fraction, were placed on previously weighed Ultrafree CL centrifu-gation units (Miltipore). They were sterilized at the surface by UV exposure in a laminar flow oven. Under asceptic conditions, the microparticles were resus-pended in prefiltered (0.22 μm sized) sodium phos-

phate buffer (2 ml, 0.2 M, pH 7.4) containing Tween 80 (0.1 w/v%) and NaN_3 (0.02% w/v). The latter compound was added to prevent any bacterial growth.
Microspheres were incubated at 37°C under slow tangential agitation in a water bath. A batch of 100 mg microspheres (duplicated experiment) was analyzed at regular intervals in order to follow the degradation kinetics process. These microspheres were separated from the medium by centrifugation at 2000 × *g*. pH of the medium was also measured. From the acidification of the incubation medium, the release of carboxylic acid moities was measured. These results were derived from the titration of the incubation medium with lactic acid *(pK_a,* 3.9) and bromacetic acid *(pK_a* 2.9), respectively. An average value was reported.
The acidity released in the medium (expressed in μeq/100 mg microparticles) was corrected for the carboxylic acid groups originally attached to the polymer (see below: end-group titration) and readily neutralized by the medium, and plotted versus time.
The recovered microspheres were washed twice with water, and weighed before and after drying in order to calculate the water uptake and the weight loss, respectively (variation coefficient: 5%). The partially degraded microspheres were kept in a dry atmosphere at - 20°C until the acid end-groups were titrated and SEC analysis was carried out.

End-group titration
Microparticles recovered on the filtration units were redissolved in THF and first analyzed by SEC.
The remaining part of the polymer was used to measure the protons associated with the polymer by acid-base titration (variation coefficient: 3%). Briefly, a known volume of the SEC sample was evaporated and the polymer (known amount) was dissolved in 4 ml of a toluene-methanol mixture (9:1, v/v). This solution was titrated with a sodium methylate solution (0.005M) in the same solvent mixture, with phenol-phthalein as an indicator. The sodium methylate solution was previously titrated with a standard benzoic acid solution.
The % of the hydrolyzed ester groups was expressed as the ratio of the protons released in the incubation medium and associated with the polymer and the number of the ester groups contained in the polymer sample (100 mg of microspheres/72= 1390 μeq. of ester groups).

3. Results and discussion

3.1. Preamble
As stressed earlier [15], degradation of biomaterials proceeds through to complex heterogeneous reactions, in particular due to variations in diffusion of water, catalyst and hydrolysis products with time and throughout the material. Composition of the surrounding fluid is also changing continuous during incubation. This explains why degradation of a polymer, such as a poly (α-hydroxy acid), is not random in the solid state,
in contrast to what happens in solution. Vert et al. [16] and Visscher et al. [9] have observed significant differences in the characteristics of the surface part and the core of microparticles under degradation.
Biomaterials dramatically change during degradation, sometimes in an unexpected manner. A recent paper by Li and Vert reported on the progressive formation of highly hydrolytic resistant crystalline oligomeric stereocomplexes, from an originally amorphous racemic poly(DL-lactide) matrix [17]. Due to this high complexity, it is very difficult to predict the degradation kinetics as well as in vitro and in vivo. In order to cope with this problem and to make available a polyester that degrades within a few days, (DL)PLA of two extreme molecular weights have been combined in various weight ratios and the blend has been analyzed as reported hereafter.

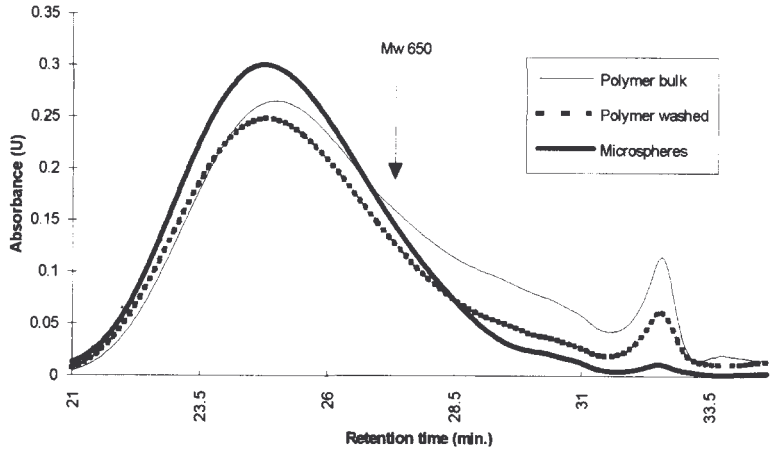

Fig. 1. SEC elution curves for the R104 oligomer, as received (polymer bulk), after extraction with water on an Ultrafree centrifugation unit and after microsphere formation.

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Table 1
Theoretical and experimental compositions of the microparticles

Polymer blends	Theoretical composition (wt%)	SEC area (a) (experimental)	SEC area (a) (calculated)	Experimental composition (wt%)
R206	100	0.57		
R104	100	1.25		
90-10				
-R206	63	0.5	0.36	77
-R104	37	0.33	0.46	23
75-25				
-R206	36	0.47	0.21	55
-R104	64	0.89	0.8	45
50-50				
-R206	15.8	0.22	0.09	28
-R104	84.2	1.2	1.05	72
25-75				
-R206	4.8	0.07	0.03	9.8
-R104	95.2	1.41	1.19	91.2

(a) = relative units.

Table 2
Glass transition temperature (°C) of two-component poly(DL-lactide) microspheres

	T_g (1 passage)	T_g (after 3 passages)	Theoretical T_g^a
Polymer R104	26	29	
Microspheres 25-79	28	29.5	29.7
Microspheres 50-50	30	31.5	32.2
Microspheres 75-25	27–38	37	36.1
Microspheres R206	38	45.2	
Polymer R206	49	50	

[a]Theoretical T_g has been calculated from the Fox equation and T_g of the bulk R104 (29.0°C) and R206 microspheres (45°C).

3.2. Characteristic features of the original microspheres

Composition

The original compositions of the (DL)PLA blends are listed in Table 1. Concentration of the (DL)PLA mixtures has been chosen so as to fix the solution viscosity in the range where microparticles are produced with a 100—160 μm size. Since it might be suspected that the (DL)PLA oligomer is not quantitatively incorporated into the microspheres, due to a partial solubility in water, composition of the microparticles has been controlled from the SEC peak area (see Fig. 1 and Table 1). The experimental SEC data in Table 1 have been referred to the same total amount of injected polymer and the related peak area have been expressed in relative units. From the knowledge of the peak area for the R206 and the R104 poly(DL-lactide)s, respectively, calculation of the expected peak area for these two components used in various weight ratios is straightforward (calculated values, in Table 1). Conversely, the actual weight composition of (DL)PLA blends can be extracted form the peak surface area of the size exclusion chromatogram. (Table 1). Data in Table 1 clearly show that part of the low molecular weight (DL)PLA originally dissolved in the oil phase of the emulsion has not been incorporated into the final microparticles. For instance, in the R206/R104 blend, designated as 75-25, only 45 wt% of R104 is part of the microparticles instead of the 64 wt% originally dissolved in the acetone/methylene chloride mixture. The lowest molecular weight species of the R104 (DL) PLA oligomer are thus partly extracted by the water phase during the emulsification process. This phenomenon is confirmed by the comparison of the SEC profiles for the R104 oligomer as received from Boehringer, as isolated after washing with water on the ultrafree cen-trifugation unit, and as incorporated in microspheres (Fig. 1). The same conclusion has been drawn by Bod-meier et al. from DSC analysis [12]. It is for the same reason that Wichert and Rohdewald [18] have recommended to add isopropanol in the aqueous phase of the dispersion, so as to increase the precipitation rate of the polylactide oligomers.

From the comparison of the SEC profiles of R104 as received from Boehringer and after washing (Fig. 1), the critical molecular weight of PLA, below which it becomes significantly hydrosoluble, has been approximated.

Provided that the Mark-Houwink coefficients are valid in this low molecular weight range, this molecular weight would be equal to ~ 650, thus corresponding to nine lactic acid units per chain.

Homogeneity of the (DL)PLA blends

Although two polymers that are just different in molecular weight are expected to form homogeneous blends, a partial phase separation cannot be ruled out depending on the way they are precipitated from a common solution. In the case under consideration, the R104 oligomer has a higher affinity for the oil/water interface and even for the water phase than the R206 component, so that it could migrate preferentially to the microsphere surface. Moreover, the larger R206 macromolecules might precipitate earlier within the oil droplets due to a lower solubility. In order to clarify this point, microspheres have been analyzed by DSC. Table 2 shows that only one T_g is observed for the 25-75 and 50-50 blends during the first heating run (see for example blend 25-75 in Fig. 2a. The experimental glass transition temperature is intermediate to the value characteristic of the two polymers, in agreement with the Fox equation, thus attesting to the homogeneity of the blends.

However, in the case of the 75-25 composition, where R206 is the major component (Table 1), the first DSC run shows two distinct T_g*s,* 26.8 and 38.4°C, which correspond to T_g of the constitutive (DL)PLA (Fig. 2b). After quenching of the sample, a second DSC run shows a unique T_g, that corresponds to a homogeneous blend. The final conclusion is that a phase separation of the two polylactides might occur during the microsphere formation, which is not necessarily detectable by DSC in the whole composition range. This result is in an apparent disagreement with data previously reported by Bodmeier et al. [12] who observed only one T_g whatever the composition of their blends. This discrepancy might result from a difference in the microsphere preparation technique or from a possible influence of the drug added into the oil phase.

It should also be mentioned that the R104 oligomer shows an endotherm peak close to the glass transition, the intensity of which decreases as the DSC runs are repeated. This endotherm has been attributed by others [19] to an enthalpy relaxation effect in relation to the thermal history of the sample.

It is also worth mentioning that T_g of the R206 microspheres is significantly smaller than T_g of the original polymer. No difference in molecular weight for the two samples cannot however be detected by SEC analysis. The origin for this plasticization effect could be found in the presence of water or residual solvents in the microspheres. In this respect, 0.6 and 0.08 wt%. of methylene chloride and acetone, respectively, are left in the microspheres which have been dried at 30°C for 2 days as reported in the sectiorf 2 (solvent residues have been analyzed by headspace GC-MS).

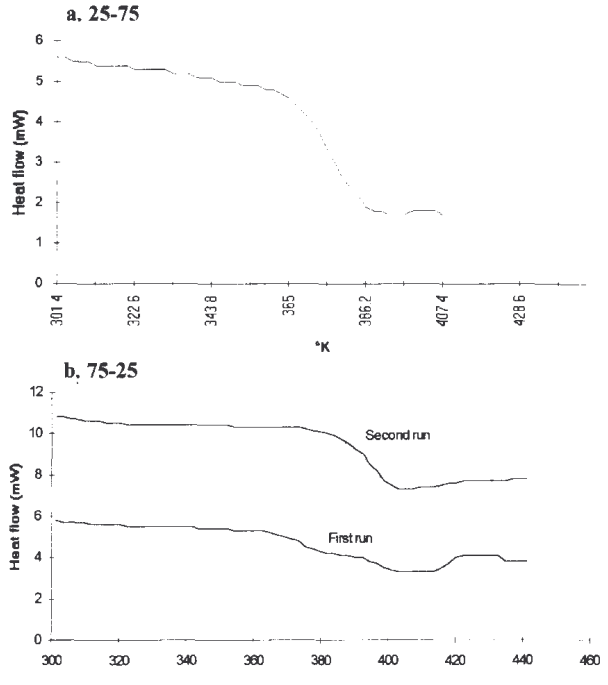

Fig. 2. DSC thermograms for 25-75 (a) and 75-25 (b) (see Table 1).

Size distribution
Size distribution of the microparticles has been measured with a Coulter Multisizer. This equipment allows the particle size distribution to be established in volume and/or number. Altough the volume distribution only shows microspheres of a 100—160 μm size (Fig. 3b), the number distribution is bimodal (Fig. 3a) after standard sieve procedure performed in a wet mode. It shows that significantly small particles, in the 1—50 μm range, amount to ca. 50% of the total number of particles. This information is of a critical importance when chemoembolization is concerned.
Adsorption of small particles onto larger ones has been reported by others [20] in case of particles protected by an adsorbed layer of a water-soluble polymer, such as poly (vinyl alcohol-co-vinyl acetate). This observation has been accounted for by the adsorption isotherm of the smaller particles onto the larger ones. However, Fig. 3c shows that sizing of the original poly-lactide particles under ultra-sound irradiation in the wet mode is efficient, eliminating more than 90% (in number) of the smallest particles. This efficiency would be due to the deaggregation of the particles by the energy released by the collapse of cavitation bubbles within the liquid.
For the sake of comparison, the number size distribution of a commercially available non degradable embolic material (Ivalon®, Unipoint, High Point, SC) of a size advertised in the 150 and 300 μm range is shown in Fig. 3d. The poor adequacy of this product for (chemo)embolization purposes is obvious.

3.3. Biodegradation study

Particle weight loss
Fig. 4a shows that the R104 oligomer significantly increases the water uptake which is close to be negligible for the R206 microparticles. When 45 wt% R104 are combined with R206, 100 mg of water are absorbed per 100 mg of microparticles (75-25) after 24 h of incubation. This amount does not change further. It increases up to 150 mg of water per 100 mg of 50-50 and 25-75 microparticles, i.e. close to the value reported for pure R104 (DL)PLA after 24 h. These kinetics data suggest that continuous patches of the R104 component are available for water diffusion
throughout the 50-50 and 25-75 blends. Approximately six water molecules per ester group are absorbed in these microparticles. Compared to a water content of 22 mg for 100 mg microparticles in case of a 1:1 water-ester group [21], the experimental data are consistent with microscopic aqueous pores throughout the (DL)PLA matrix. This high hydration is expected to favor more homogeneous hydrolysis associated with a rapid clearance of hydrosoluble oligomers and fragmentation of the parent microparticles, as it will be shown later.
In addition to water uptake, the weight loss of the microspheres has been measured (Fig. 4b). The two series of data run in a parallel way. Except for the microspheres made of the high molecular weight (DL) PLA, the weight of the microparticles decreases shortly after the onset of incubation. The first part of the degradation profiles differs from the published data, which show a lag time in the time dependence of the weight loss [21,22]. This induction period would correspond to the time required for decreasing the poly-lactide molecular weight to make it soluble in the aqueous phase. The faster degradation that has been reported for the core of the microparticles compared to the surface [9,16,22] could contribute to the observed lag time.
In contrast, the addition of the R104 oligomer to the R206 PLA is responsible for a rapid and homogeneous degradation. The observed weight loss is, however, not linear with time. An initial loss that corresponds to 10—12% first occurs until 72 h. The weight loss then slows down, more likely due to the aggregation of the micro-particles that ultimately form a polymer film. These macroscopic morphological changes result from a decrease in T_g of shorter PLA chains and lead to decreasing surface/volume ratios and thus diffusion rates of the oligomers.
It is also worth noting that the weight loss is very similar for blends containing 85 wt% and more of R104, at least after the initial step (ca. 50h) (Fig. 4b). This observation supports that a sufficient amount of oligomer (at least 85 wt%) enhances the degradation rate of the high molecular weight component. This conclusion will be supported by the analysis of the molecular weight distribution of PLA as discussed later.

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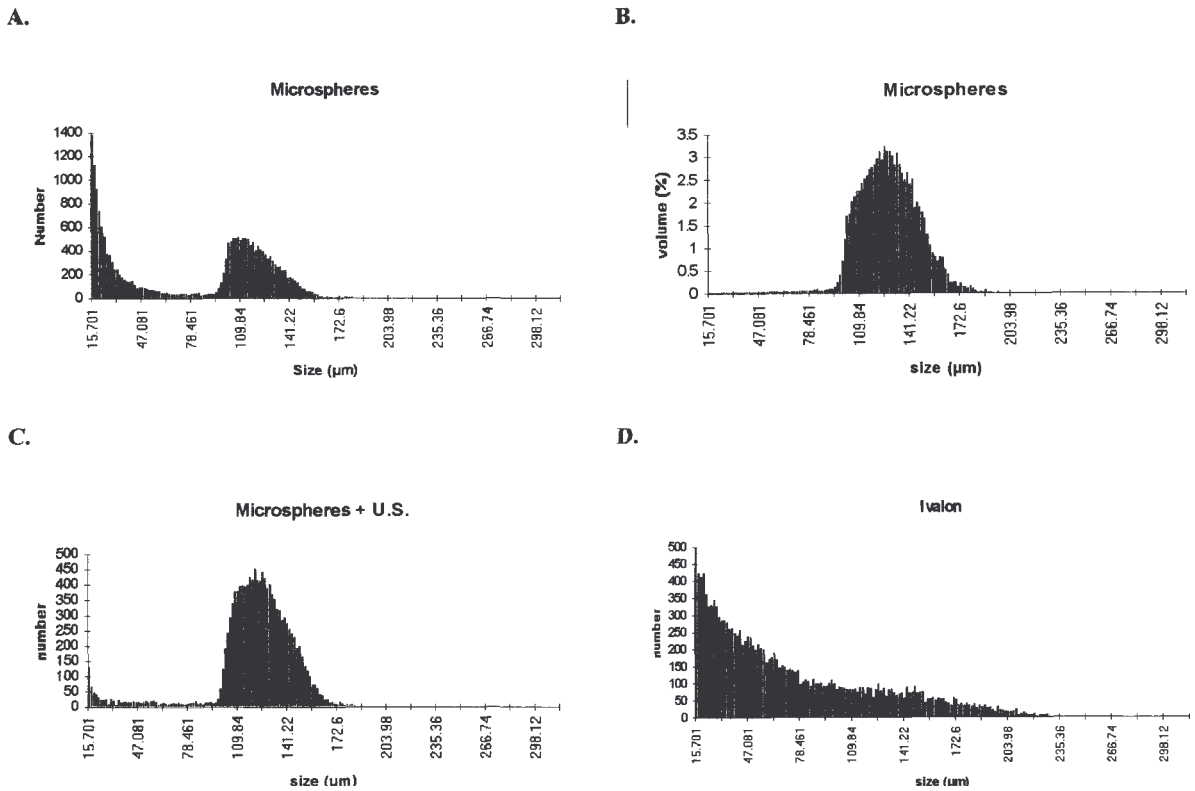

Fig. 3. Size distribution of microparticles, as measured with a Coulter Multisizer II equipment. Panels A and B are the number and volume distribution of the polylactide microspheres after a conventional sizing procedure. Panel C reports the number size distribution of the same 100-160 µm fraction, but after sizing under ultra-sound irradiation. Panel D shows to the number size distribution of an Ivalon suspension (Ivalon®, Unipoint, High Point, SC).

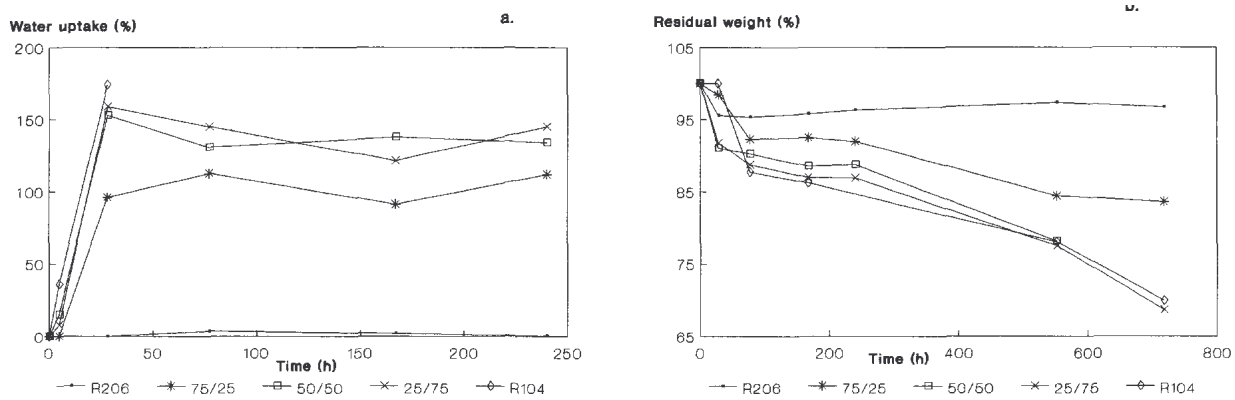

Fig. 4. Water uptake (a) and weight loss (b) for various microspheres during in vitro degradation at 37°C. The actual compositions of the blends denoted 75/25, 50/50 and 25/75 are respectively: 55/45, 28/72 and 9.8/91.2.

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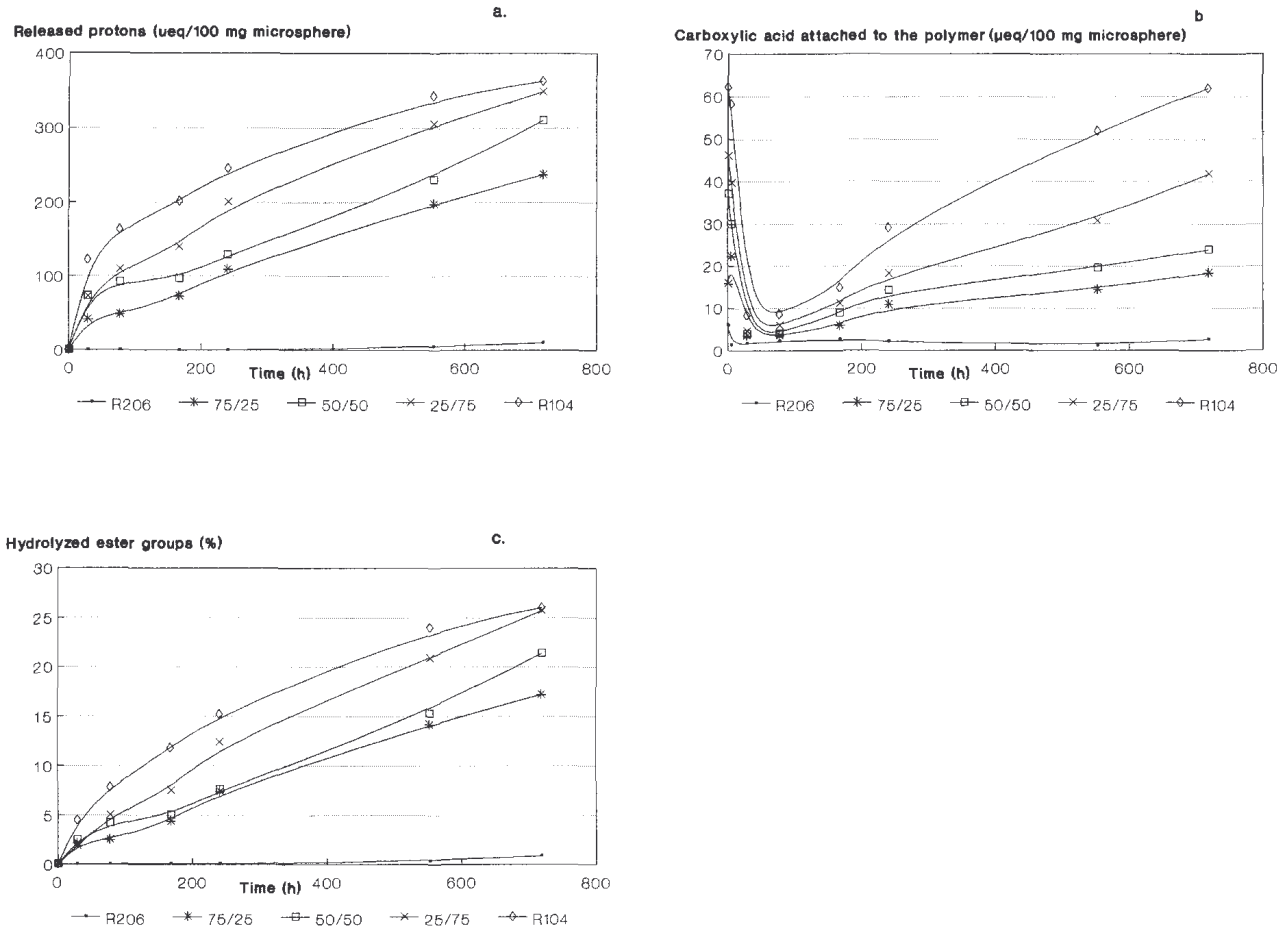

Fig. 5. Polyester hydrolysis as estimated from (a) protons released in the external buffer; (b) carboxylic acid groups attached to the polymer; (c) cleavage of the ester groups of the polymer. The actual compositions of the blends referred to as 75/25, 50/50 and 25/75 are respectively: 55/45, 28/72 and 9.8/91.2.

Table 3
R206 and R104 molecular weight evolution during in vitro degradation as analyzed by SEC

Time (h)	R206 $10^{-3} M_n$	R206 $10^{-3} M_w$	R206 $10^{-3} M_p$	75-25 $10^{-3} M_n$	75-25 $10^{-3} M_w$	75-25 $10^{-3} M_p$	50-50 $10^{-3} M_n$	50-50 $10^{-3} M_w$	50-50 $10^{-3} M_p$	25-75 $10^{-3} M_n$	25-75 $10^{-3} M_w$	25-75 $10^{-3} M_p$
0	64.50	109.59	64.13	86.76	181.64	72.50	92.04	179.73	77.07	81.57	125.46	77.07
24	63.33	112.07	62.81	82.54	176.12	71.03	77.21	123.50	73.97	78.84	121.69	52.18
72	60.48	110.57	59.09	77.21	158.69	68.16	69.85	105.17	66.77	78.17	120.04	53.24
168	56.61	98.22	54.43	74.34	149.33	66.77	63.91	96.28	61.57	70.29	109.37	46.16
240	–	–	–	63.84	129.54	57.85	57.05	85.72	54.43	–	–	–
504	54.21	93.45	56.69	44.88	105.69	39.64	40.79	55.81	29.96	35.36	44.67	24.99
720	50.01	85.05	51.17	39.43	102.10	35.21	25.74	33.22	27.07	NR	NR	NR
Time (h)	R104 $10^{-3} M_n$	R104 $10^{-3} M_w$	R104 $10^{-3} M_p$	75-25 $10^{-3} M_n$	75-25 $10^{-3} M_w$	75-25 $10^{-3} M_p$	50-50 $10^{-3} M_n$	50-50 $10^{-3} M_w$	50-50 $10^{-3} M_p$	25-75 $10^{-3} M_n$	25-75 $10^{-3} M_w$	25-75 $10^{-3} M_p$
0	3.46	5.66	3.25	4.00	6.51	3.42	3.75	6.09	3.30	3.59	5.75	3.30
24	3.44	5.16	3.25	4.08	6.52	3.45	3.72	6.08	3.36	3.81	5.97	3.39
72	3.37	5.24	3.19	3.96	6.46	3.42	3.63	5.87	3.28	3.80	6.10	3.39
168	3.29	5.35	3.11	4.07	6.41	3.48	3.51	5.59	3.19	3.71	6.08	3.30
240	3.03	5.00	2.91	3.79	5.75	3.28	3.44	5.77	3.03	–	–	–
504	1.87	2.78	1.59	3.54	5.26	3.03	3.52	6.40	2.69	3.02	5.07	2.16
720	1.37	1.97	1.06	3.37	4.77	2.93	2.97	4.92	2.01	2.93	8.92	1.83

M_n, M_w and M_p have their conventional meanings and are absolute values.

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Rate of the ester group hydrolysis

It is interesting to compare the experimental weight loss with the hydrolysis rate of the ester groups. Since this hydrolysis results in carboxylic acid formation, the change in pH of the incubation medium has been measured together with the amount of carboxylic acid groups attached to the polymer. Of course, the buffer of the incubation medium will modulate the change in the external pH and the carboxylic acid content of the polymer. In this respect, Fig. 5a shows that the carboxylic acid end-groups of the original polylactide are rapidly neutralized, in a possible relation to the rapid water uptake. After ca. 72 h, the content of the carboxylic acid groups associated with the polymer increases as rapidly as the relative amount of RI04 in the polymer blend. In spite of the low pK_a of the lactoyl lactic group (3.0), at least part of them remains protonated, although pH of the external medium does not fall below
6.3 for the 75-25 microspheres. This observation can be accounted for by the negative zeta-potential of the microparticle surface. According to Makino et al. [23], protons and hydroxyl ions are redistributed within the microparticles in a way that depends on the surface potential in agreement with Eqs. 1 and 2:

$$n_{H}^{m} = n_{H}^{0} e^{(-F\varsigma/RT)} \quad (1)$$

$$n_{OH}^{m} = n_{OH}^{0} e^{(F\varsigma/RT)} \quad (2)$$

where n_H and n_{OH} are the concentrations of H^+ and OH^- ions, respectively, the superscripts m and o refer to the concentrations within the matrix and the external phase, F is the Faraday constant, R the gas constant, T the absolute temperature and ζ is the Donnan potential across the microparticle membrane.
Persistance of unneutralized carboxylic acid groups thus results from a local acidic microenvironment. This might explain why Kenley et al. [24] have observed that the degradation rate constant of polylactide was independent of pH of the reaction medium.
From the acidification of the incubation medium, the amount of the released carboxylic acids has been calculated, while using a pK_a of 3.45 which is an intermediate value between the lactic acid (3.9) and the lactoyl lactic acid. Data plotted on Fig. 4b are the dif-
ference between the total carboxylic acid content and the acid groups originally associated with the polylac-tide chains and rapidly neutralized as shown in Fig. 5a. Consistently to the microparticle weight loss, there are two distinct steps in the proton release. A rapid release of lactic acid and/or polylactide oligomers occurs for the first 24 h of incubation. Then the proton release is
slown down and the rate becomes apparently independent of the microparticle composition.
The percentage of the hydrolyzed ester groups has been calculated from the protons released in the external medium and the carboxylic acid groups attached to the polymer. Fig. 5c shows an initially fast hydrolysis, that depends however on the microparticle composition. For instance, an average rate of 0.45 and 1.40 μmol of ester groups hydrolyzed per h has been calculated for the 75-25 blend and the R104 oligomer, respectively. For R206 microparticles, the titration method is not sensitive enough to follow the progress of chain degradation.
In qualitative agreement with Fig. 5a and b, hydrolysis (Fig. 5c) becomes slower after ca. 72 h and shows a linear dependence on time, the slope of which does not change significantly with the R104 content. An average rate of 0.40 μmol of ester group hydrolyzed per h is then observed. In the particular case of R206, the acidification of the external medium is only detectable after ca. 170h.
In addition to the percentage of the hydrolyzed ester groups, the mean number of ester groups cleaved per original molecule, (x), has been approximated and compared to the value estimated from the decrease in the number average molecular weight (M_n) (see Table 3), in agreement with Eq. 3. This equation has been derived in the case of a random chain scission [24].

$$M_n^t / M_n^0 = 1/(1+x) \quad (3)$$

where the superscripts t and 0 refer to M_n at time t and 0, respectively.
For pure R104 microparticles, M_n decreased from 3450 to 1360 for the whole experiment (720 h), so that x is approx. 1.55. This value is much slower than that estimated for the percentage of hydrolyzed esters (12.5). This apparent disagreement is due to the fact that the decrease in molecular weight refers to the insoluble polymer, although the acidity of the medium is essentially affected by the extracted oligomers. This contribution is particularly important for pure R104. On the assumption that the 9-mer oligomer is the higher product still soluble in water, on can easily calculate that 38% of the total number of ester bonds in a 48-mer chain should generate hydrosoluble products (18 ruptures/47).

Published in: Journal of controlled release (1996), vol. 38, iss. 2-3, pp. 109-122
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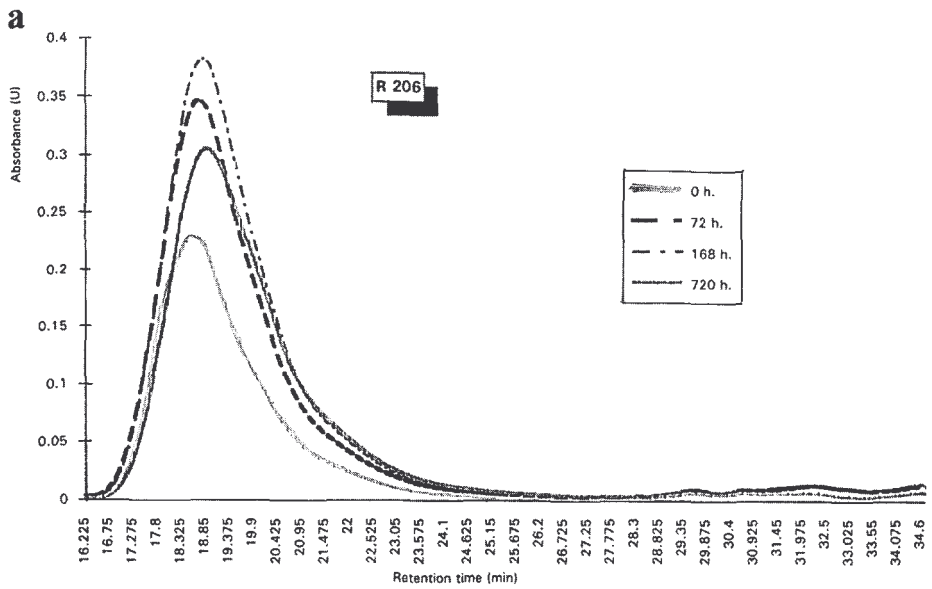

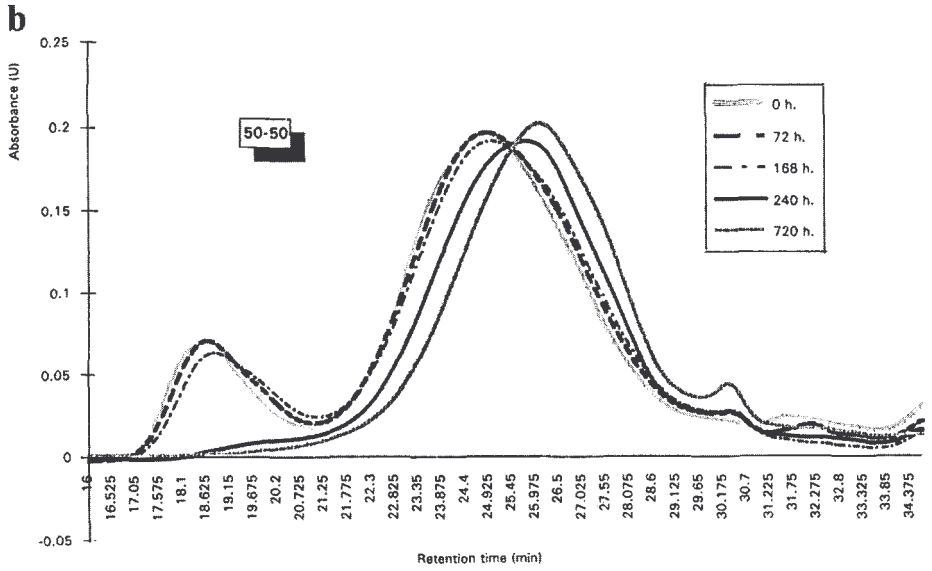

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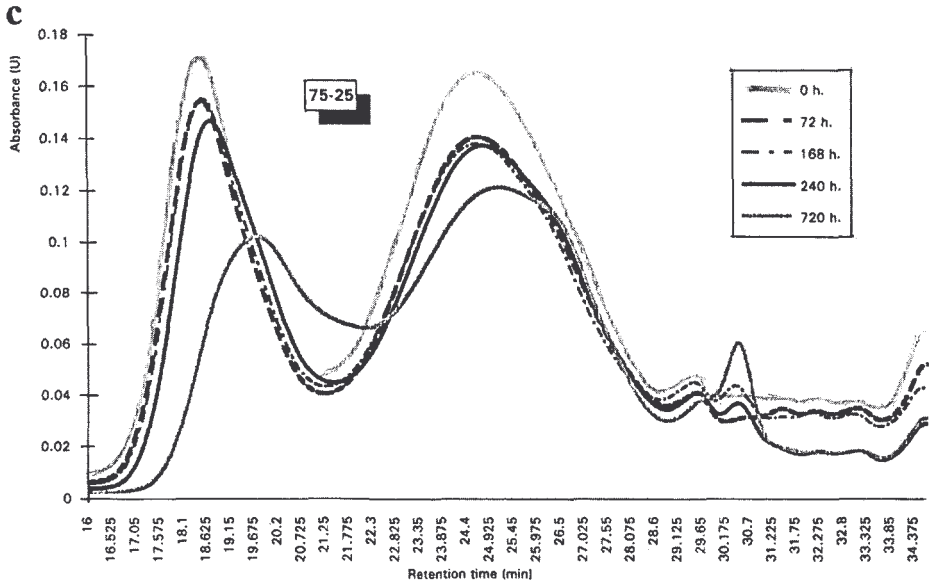

Fig. 6. SEC analysis of microspheres at increasing degradation times. The actual compostions of the blends 75/25 and 50/50 are respectively: 55/45 and 28/72.

Molecular weight distribution

Data reported in the previous section do not allow to appreciate properly the effect of the R104 oligomer on the degradation rate of the high molecular weight poly-lactide (R206). It is the reason why, molecular weight distribution of polyblends has been measured by size exclusion chromatography at various degradation times. SEC elution profiles are reported in Fig. 6 and the related molecular characteristic features are listed in Table 3. The high molecular weight polylactide (R206), whithout any added oligomer, is essentially stable over 720 h. M. is only decreased by 21% although the poly-dispersity does not change significantly (1.70). Fig. 6 shows that the addition of the R104 oligomer increases the degradation rate of R206. In the 75-25 polyblend, M_n of R206 has decreased from 86 800 to 39 400 after 720 h. For the 50-50 and 25-75 polyblends, degradation of the high molecular weight component is not linear with time: the chain rupture proceeds firstly very slowly (until 168 h), and then it accelerates to be complete after 240 h.

From these results, it is obvious that the degradation rate of high molecular weight polylactide is enhanced by the addition of oligomers. This effect more likely results from the acidity within the polymer matrix which is favorable to the polyester hydrolysis. A parallel increase in water uptake and byproduct release has also to be taken into account.

Microsphere morphology evolution

Morphology of microspheres has been investigated by scanning electron microscopy. Fig. 7 compares SEM of microspheres of the 75-25 and 50-50 composition after 10 days of in vitro incubation. Microparti-cles of the 50-50 composition are completely disrupted into highly porous pieces that merge into a continuous film. The 75-25 microspheres preserve their spherical shape after the same degradation time, although they are slightly deformed and become more porous.

It is worth pointing out that the time requested for the microsphere disruption as observed by SEM is in a good agreement with SEC observation for the disappearance of the R206 species. This supports that the high molecular weight component basically contributes to the mechanical strength of the miccroparticles. Consistently, Fig. 7 shows microspheres consisting of a polylactide blend of an intermediate composition compared to the two previous ones. These microparticles fall in pieces after 15 days.

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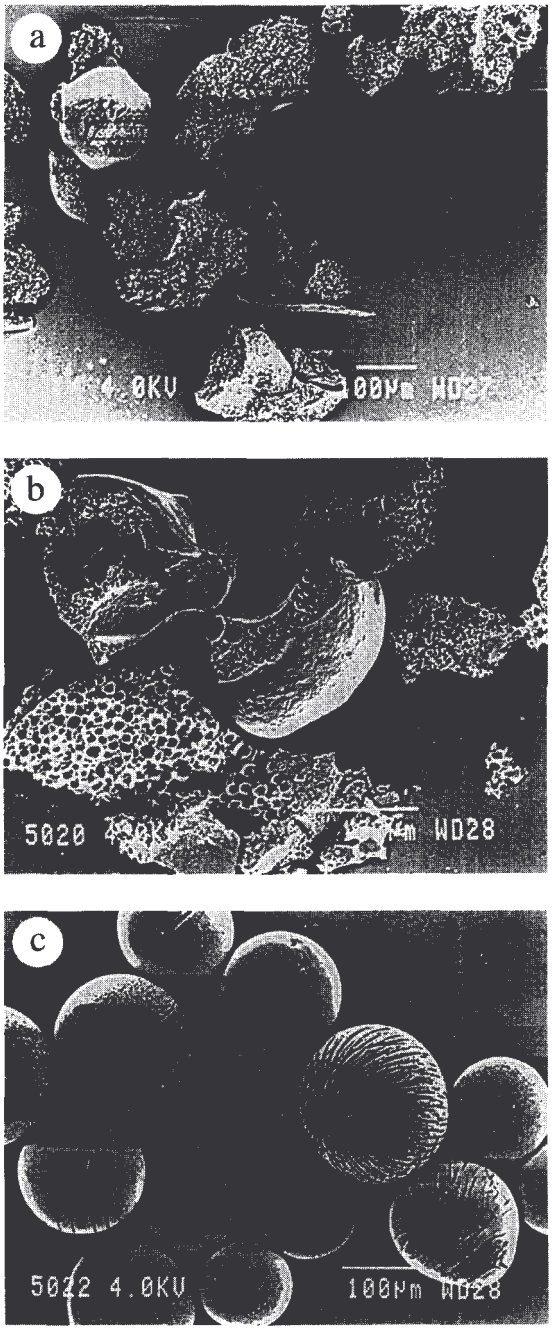

Fig. 7. Morphology of the 50-50 (a) and 75-25 (c) microspheres as observed by SEM after 10 days of degradation. Micropheres of an intermediate composition compared to (a) and (c) are shown in (b) after 15 days. The actual compositions of the blends called: 75/25 and 50/50 are respectively: 55/45 and 28/72.

4. Conclusions

The degradation rate of polylactides is known to depend on the polymer microstructure that may be of the L, D or DL type. These modifications have an effect on the propensity of the polyester to crystallize and thus to be ultimately hydrolyzed. Degradation can also be enhanced by copolymerization with glycolide, so that the polyester lifetime can be controlled in a range of a few months. Nevertheless, the commercially available poly(lactide-co-glycolide) which is more labile (50-
50 composition) is not degraded fast enough to be suitable for repetitive chemoembolization. Combination of two poly(DL-lactide)s, one of them being of a low molecular weight, is a potential way for the production of microspheres biodegradable within a few days.
It has been shown in this paper that changing the weight composition of the polyblends allows the in vitro

degradation rate to be precisely controlled. The two-component microspheres have been characterized in terms of blend homogeneity and particle size distribution. A fast water diffusion and a high water content at equilibrium within the material account for the rapid bulk hydrolysis and mass fragmentation of the polylac-tide microspheres. They are observed to fall in pieces, when most of the high molecular weight component is degraded into oligomers. The basic role of the low molecular weight component is to increase the local acidity and, accordingly, to accelerate the hydrolysis of the high molecular weight species. Nevertheless, 70 wt% of the original polymer are retained after 720 h of in vitro incubation. Merger of the microparticles into a film during the in vitro degradation might be unfavourable to the polyester hydrolysis compared to the in vivo situation where individual microparticles are expected to be homogenously distributed throughout biological tissues. It is the reason why a forthcoming paper will focus on the in vivo biodegradation of these polylactide microparticles.

Acknowledgements
The authors thank the 'Fonds de la Recherche Scientifique M2dicale' (FRSM) for their financial support (grant No. 3.4553.91) and the 'Services Fédéraux des Affaires Scientifiques et Culturelles' for support in the frame of the 'Pôles d'Attraction Interuniversitaires: Polymères. The technical assistance of Miss M. Deje-neffe is also appreciated.

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