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In vitro degradation of nanospheres from poly(D,L-lactides) of different molecular weights and polydispersities

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Abstract

The aim of this work was to study the stability of nanospheres from poly(D,L-lactide) in aqueous media. The influence of the poly(D,L-lactide) molecular weight and polydispersity, pH of the medium and temperature, was investigated. The degree of DL PLA degradation was estimated by monitoring the total acidity of the medium, the lactic acid production, the molecular weight of the polymer, the mean diameter of the nanospheres and the amount of polymer remaining insoluble. Poly(D,L-lactide) degradation rate depended on polymer molecular weight and polydispersity. The data suggested that it resulted from a hydrolysis which is much more catalysed at acidic and alkaline pH than at neutral pH. Two main hydrolysis mechanisms can be proposed: a random scission at acidic pH and a sequential cleavage from the chain end in alkaline medium. Higher temperature accelerated the degradation process. The best storage conditions for an aqueous dispersion of poly(D,L-lactide) nanospheres were deduced (temperature: 4°C, pH close to neutrality).

Keywords: Nanospheres; Poly(D,L-lactide); Degradation; Molecular weight; Lactic acid; pH

1. Introduction

The homo- and co-polymers of lactic and glycolic acid have the main advantage of being biocompatible and totally bioresorbable. When degrading in the body, their end-products are part of the Krebs cycle and thus are atoxic. The use of these polymers as suture material has progressively broadened to drug delivery systems with delayed release properties (Sanders et al., 1984; Heller, 1986; Holland et al., 1986; Schakenraad et al., 1988).

An important advantage of polymers of a hydroxy acids is the possibility of manufacturing systems with a rate delivery that can be modulated depending on duration of the desired effect. The modulation is obtained by changing the labil-

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ity of the polymeric material by various means: chemical composition (homo- or co-polymers of lactic and glycolic acids), tacticity (D- or L-isomers; D,L-racemic) (Makino et al., 1985; Makino et al., 1987) or physical properties (molecular weight, crystallinity, glass-transition temperature) (Reed and Gilding, 1981; Jamshidi et al., 1988). Furthermore, it has been shown that the rate of degradation of these polymers can be modulated by enclosing some molecules like certain amines (Cha and Pitt, 1989; Kishida et al., 1989) which can interfere in the degradation mechanism.

Several studies have been accomplished on the degradation of acid-derived polymers in vivo and in vitro using macroscopic systems (sutures (Schakenraad et al., 1988), films (Pitt et al., 1981; Pitt and Schindler, 1983), implants (Li et al., 1990; Pistner et al., 1994)), or microscopic devices (microspheres (Wada et al., 1990) and microcapsules (Makino et al., 1985)). These works studied the hydrolysis kinetics in relation to physico-chemical structure of the polymer and established the weak participation of enzymatic mechanisms in this hydrolysis (Williams, 1981).

The recent therapeutic use of these polymers as controlled release devices for peptides (Hutchinson and Furr, 1991) in the form of monolithic implant (for the parenteral way: Zoladex®) or microdispersed (microspheres for intra-muscular injection: Decapeptyl®) led to the development of manufacturing methods in an attempt to avoid the denaturation of unstable drugs.

An original method of manufacture of submicronic carriers which involves the nanoprecipitation of pre-formed polymer in aqueous media has been developed in our laboratory. The method requires a sole process of simple mixing and results in the formation of colloidal carriers of matricial type (nanospheres) (Fessi et al., 1987) or vesicular type (nanocapsules) (Fessi et al., 1988).

Given the huge specific area (in cm^2/g) of these nanoparticulate systems it is not possible to extrapolate the results of degradation of macrosopic and even microscopic systems to nanometric carriers. The aim of this work was to study the in vitro degradation of nanospheres of poly(D,L-lactide) obtained according to our original method of preparation. Two polymers of different molecular weights were used to determine the optimal stability conditions in aqueous media at different pH and temperatures.

2. Materials and methods

2.1. Materials

Poly (D,L-lactides) of inherent viscosities 0.30 (M_w = 25 000) and 0.85 (M_w = 95 000) were supplied by Boehringer Ingelheim Chimie (Le Vésinet, France) and Birmingham Polymer Inc. (Birmingham, AL, USA). These polymers are named DL PLA 0.30 and DL PLA 0.85 in the text. All the solvents used: acetone (Prolabo, Paris, France) and tetrahydrofuran (Carlo Erba, Milan, Italy) were of reagent grade. The sodium hydroxide, sulfuric acid and acids used in the buffer solutions were purchased from Prolabo and were of reagent grade.

2.2. Preparation of nanospheres

The nanospheres were prepared by nanoprecipitation of the polymer according to the method described by Fessi et al. (1987) and Fessi et al. (1988). An acetonic solution (1 volume) of DL PLA (0.5% w/v) was added to an aqueous solution (2 volumes) of sodium dodecyl sulphate 0.08% with moderate magnetic stirring (200 rpm). The desolvation of polymeric material occurred instantaneously in the form of colloidal particles. Acetone was then evaporated under reduced pressure.

2.3. Degradation of DL PLA nanospheres

The degree of degradation of DL PLA nanospheres in different conditions of pH and temperature was monitored by the determination of the following parameters: total acidity of the medium, lactic acid production, molecular weight of the polymer, mean diameter of nanospheres and the amount of polymer remaining insoluble in the form of nanospheres.

2.3.1. Effect of pH

Nanospheres dispersions were made up to 150 ml with distilled water then the volume was adjusted to 200 ml with buffer solution (acetic/orthoboric/orthophosphoric acids 0.04 N pH 2.2). The pH was adjusted to the required value (2.2–4.2–6.0–7.4–8.4–10.1) by addition of 5 N NaOH. Dispersions were kept in the dark at 37°C. At regular time intervals (15 days) the pH was re-adjusted to the starting value with a titrated solution of 0.01 N NaOH. The references were nanosphere dispersions at the same final concentration in distilled water.

2.3.2. Effect of temperature

Dispersions of nanospheres of DL PLA 0.30 were kept at − 18°C (255 K), + 4°C (277 K) and + 37°C (310 K) in buffer, pH 10.1.

2.4. Estimation of degradation

2.4.1. Total acidity

The total acidity was estimated from the volume of the titrated 0.01 N NaOH solution required to produce re-adjustments of the pH to the starting value.

2.4.2. Lactic acid

The amount of lactic acid generated by degradation and released in the dispersion medium was assayed by high performance liquid chromatography (Sawan and Barry, 1988; Lesh et al., 1993). The aliquot of the dispersing medium was obtained by ultracentrifugation of the nanospheres dispersions (Beckman Instruments, Palo Alto, CA, USA. Rotor type 70 ITI, 1 h at 4°C, 39 000 rpm, 150 000 × g) and the recovery of the supernatant.

2.4.3. Experimental conditions of HPLC

Carboxylic acids ion-exchange column (S.F.C.C., Neuilly sur Seine, France) thermostated at 40°C; detection by UV absorption at 210 nm (Waters 484 spectrophotometer, Waters, Saint Quentin en Yvelines, France). Mobile phase: sulphuric acid 0.01 N. Flow rate: 1 ml/mn. The standardization curve was a straight line and the minimum amount detectable was 1 μg/ml of lactic acid. The retention time of lactic acid was 10 min. The standard used was a lactic acid supplied by Sigma (Sigma Chimie, Saint-Quentin-Fallavier, France)

2.4.4. Molecular weight

The weight-averaged molecular weight of DL PLA was determined by Gel Permeation Chromatography (GPC). Aliquots of the nanosphere dispersions were lyophilised at definite time intervals and the lyophilisate dissolved in tetrahydrofuran. This solution was analysed through two Ultrastyragel columns serially set (Waters, Saint Quentin en Yvelines, France) having a mean porosity of 500 and 10 000 Å. The mobile phase was tetrahydrofuran. Polystyrene standards used for calibration had molecular weight between 1.8 and 354 kDa. The results are expressed as percentage of initial weight-averaged molecular weight of DL PLA, M_{w0}.

2.4.5. Size of nanospheres

The size of nanospheres was measured by Photon Correlation Spectroscopy with a Nanosizer™ N4MD (Coultronics, Margency, France).

2.4.6. Insoluble fraction

The weight loss of the nanospheres polymer at a given time of conservation, t, was calculated from the difference between the weight of the starting polymer (represented by the area of the GPC chromatogram) and the one of the polymer at time t. The $t_{w50\%}$, which refers to the time when the weight of residual DL PLA becomes half of the weight of the starting polymer, was then determined graphically.

3. Results

3.1. Evolution of the size of nanospheres

When measured just after preparation, the mean diameter of DL PLA 0.30 nanospheres was about 150 nm with an important dispersity (± 40 nm), whereas the nanospheres made with DL PLA 0.85 showed a mean diameter of 100 nm and a lower dispersity (± 20 nm). During conserva-

tion the mean diameters remained stable with time except for extreme pH (2.2 and 10.1 for DL PLA 0.30 and 10.1 for DL PLA 0.85) where they tend to increase. For example, at pH 10.1 at 37°C one notices at the early times (20 days) a flocculation which is followed after 2–3 months — depending on the DL PLA — by the disappearance of the nanospheres.

3.2. Degradation products

3.2.1. Evolution of molecular weight

Fig. 1 shows the influence of pH on mean molecular weight of the polymer forming the nanospheres of DL PLA 0.30 and 0.85 after conservation at 37°C for 24 and 102 days. At 24 days the degradation of the DL PLA 0.30 appeared to be directly related to the pH value, whereas the DL PLA 0.85 degradation was the highest at extreme pH values. In acidic medium (pH 2.2), however, the DL PLA 0.30 is more susceptible than DL PLA 0.85 and loses more than 75% of its initial molecular weight. Inversely, in basic medium (pH 10.1) the degradation of DL PLA 0.85 is much more pronounced (molecular weight loss of 40%) than that of DL PLA 0.30 which is negligible. After 102 days (Fig. 1b) the degradation of DL PLA 0.85 reached 50% in neutral media (pH 7.4), but was almost complete in very acidic (pH 2.2) and in very basic medium (pH

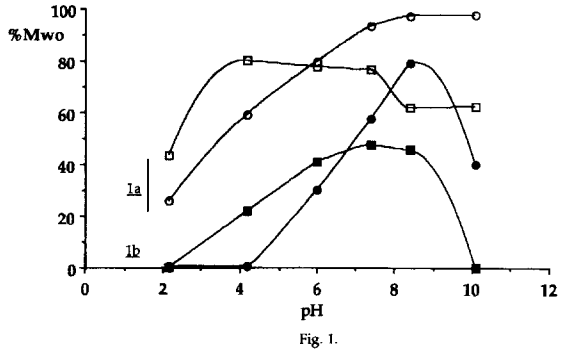

Fig. 1.

Fig. 1. Evolution of weight-averaged molecular weight of nanospheres expressed as a percentage of initial weight-averaged molecular weight at different conservation pH at 37°C. (a) DL PLA 0.30 (○) and DL PLA 0.85 (□) after 24 days conservation. (b) DL PLA 0.30 (●) and DL PLA 0.85 (■) after 100 days conservation.

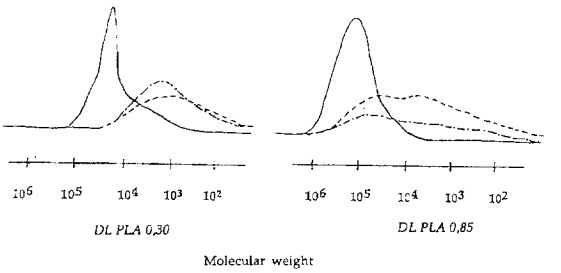

Fig. 2. Evolution of GPC chromatograms of DL PLA 0.30 and 0.85 nanospheres after one month conservation at pH 2.2 and 10.1 at 37°C. t_0 (——); pH 2.2 (- - -); pH 10.1 (– • –).

10.1). The DL PLA 0.30 was completely degraded at acidic media (pH 2.2 and 4.2) but retained 40% of its initial molecular weight at pH 10.1, and the best stability was observed between pH 7.4 and 8.4 where the degradation intensity remained lower than 50%.

The GPC chromatograms of DL PLA 0.30 and 0.85 nanospheres kept at pH 2.2 and 10.1 for one month (Fig. 2) show a decrease in area corresponding to a central peak at the two pH values and a spreading of the principal peak towards lower molecular weights especially at pH 2.2.

3.2.2. Half-life of nanospheres

The previous observations were confirmed by the data of Table 1 which give the half-life of DL PLA nanospheres (i.e., time required for reducing to half, the initial quantity of insoluble polymer) and the half-time of the polymer (required time for reducing by half the polymer mean molecular weight). The data are shown for extreme and neutral pH (pH 2.2, 7.4 and 10.1). At acidic pH (2.2), the half-life of DL PLA 0.30 is shorter than for DL PLA 0.85 but this order is inversed at basic pH (10.1).

3.2.3. Lactic acid production

Fig. 3 gives an example of production of lactic acid by DL PLA 0.30 and 0.85 nanospheres after being kept for 125 days at 37°C with the conservation pH. The trend of the curve is the same for both polymers.

Lactic acid production was intense at extreme pH, whereas at pH close to neutrality only a small amount of lactic acid was produced.

Table 1
Half-life of DL PLA 0.30 and 0.85 kept at 37°C in the form of nanospheres

Conservation pH	$t_{(Mw50\%)}$ (days)		$t_{(Weight50\%)}$ (days)	
	DL PLA 0.30	DL PLA 0.85	DL PLA 0.30	DL PLA 0.85
2.2	12	20	42	62
7.4	>150	93	64	>150
10.1	93	38	47	12

$t_{(Mw50\%)}$, time required for halving the initial weight-averaged molecular weight of the polymer. $t_{(Weight50\%)}$, time when the weight of the solid fraction in the form of nanospheres becomes half of the initial total weight of the starting polymer.

3.2.4. Total acidity

Total acidity corresponds to the whole terminal caboxylic functions produced by the cutting of polymer chains, i.e., the sum of liberated lactic acid molecules and water soluble oligomers. The total acidity production can be clearly assessed at experimental conditions corresponding to nanospheres in buffered basic media (pH 7.4–10.1), whereas it remains very low in buffered acid media (pH 2.2–6) and in the unbuffered samples used as reference. Furthermore, whatever the polymer and the starting pH, the nanosphere dispersion pH in unbuffered and unreadjusted media rapidly drops to values near the pK_a of lactic acid, i.e., 3.8 at 25°C (results not shown). Fig. 4 shows for example the evolution of total acidity generated by DL PLA 0.85 nanospheres at all studied pH values.

3.2.5. Influence of temperature

Fig. 5 shows the quantity of lactic acid released in the aqueous phase of the DL PLA 0.30 nanosphere dispersion after one month conservation at three temperatures: −18, +4 and +37°C. At pH 7.4 the degradation of the nanospheres is much faster at 37 than at +4°C and −18°C in terms of lactic acid production.

4. Discussion

The evolution of the mean size of nanospheres showed a flocculation at very basic pH. This flocculation can be related to the difference of adsorption of SDS which is very pH-dependent (Attwood and Florence, 1983). Indeed the surface of nanospheres being charged negatively by SDS,

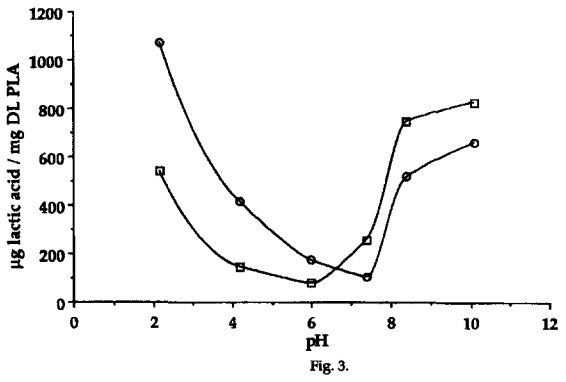

Fig. 3. Lactic acid production of the dispersions of DL PLA 0.30 (○) and 0.85 (□) nanospheres at different pH after 125 days conservation at 37°C.

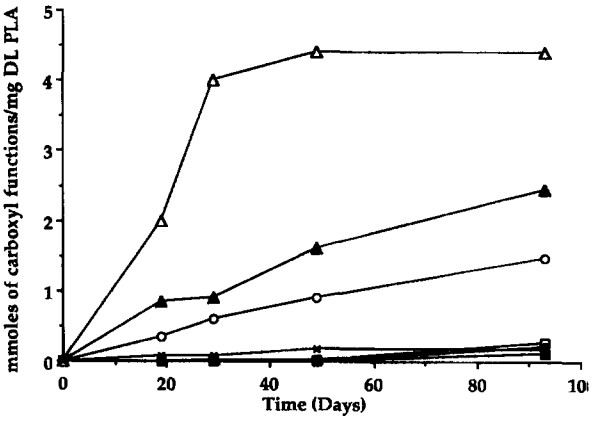

Fig. 4. Total acidity produced with elapsed time by DL PLA 0.85 nanospheres kept at 37°C. Conservation pH values were: 2.2 (■); 4.2 (□); 6.0 (●); 7.4 (○); 8.4 (▲); 10.1 (△); and reference (×).

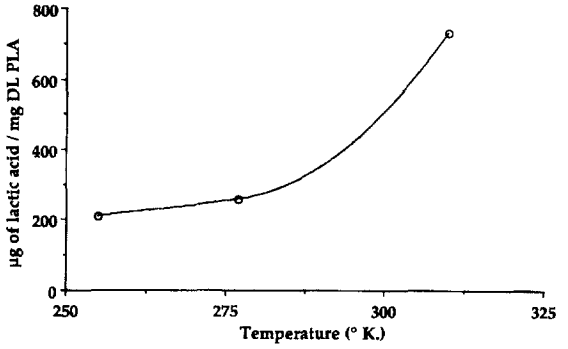

Fig. 5. Evolution of lactic acid production by DL PLA 0.30 nanospheres kept one month at pH 10.1 with conservation temperature (in K).

the adsorption will be more difficult at basic pH where the destabilisation of nanospheres occurred earlier.

The GPC chromatograms of nanospheres dispersed in acidic medium (pH 2.2) for one month showed an intense broadening of the distribution of polymer chain lengths with a tail covering the low molecular weight area. This phenomenon is caused by random attack of the polymeric backbone with H^+ resulting in a random cleavage leading to significant fall in molecular weight and mass loss.

At acidic pH the difference of behavior between the two DL PLA can be explained by their initial GPC chromatograms. Indeed the DL PLA 0.30 chromatogram shows a tail spreading towards very low molecular weights indicating the presence of more or less water-soluble oligomers which can take a significant part in the catalysis of the hydrolysis. The DL PLA 0.30 being more dispersed and having a mean molecular weight of a third of that of DL PLA 0.85, a given quantity of DL PLA 0.30 will contain more carboxylic end groups than DL PLA 0.85. This leads to a more accelerated catalysis in the case of DL PLA 0.30 in acidic medium. The fall of M_w and especially the faster mass loss of DL PLA 0.30 suggests that the polymer is degraded by chain cleavage. Moreover the oligomers of the starting DL PLA 0.30 gave a more acidic pH for the dispersion of nanospheres (pH 4.1 vs. 7.3 for DL PLA 0.85).

On the other hand, with a basic medium (pH 10.1), the degradation of DL PLA 0.30 is lower at early stages than in acidic medium. This can be explained by the adverse effect of the carboxylate ions which form a negatively charged screen hindering OH^- ions from penetrating into the polymer bulk. Thus the attack is concentrated at the surface at early stages which is in agreement with the moderate decrease of M_w and mass loss of the insoluble polymer.

Furthermore, some authors have shown that the addition of oligomeric PLA to a PLA of higher molecular weight results in a faster release of the drug from PLA microcapsules because of increase of polymeric matrix permeability (Bodmeier et al., 1989). This can lead to an important 'leaking' of the oligomers generating pores which permits the easier penetration of aqueous medium in the polymer bulk.

The degradation of aliphatic polyester devices in aqueous medium follows two main mechanisms: (i) a surface-erosion (the total mass decreases but the weight-averaged molecular weight remains nearly constant); (ii) an erosion in the bulk (the chain cleavage is a random process (every ester bond has the same probability to be cleaved)) leading to a rapid fall of M_w.

The degradation of DL PLA in aqueous medium is a bulk hydrolysis involving an attack of the whole polymeric matrix. In the first steps there is little degradation of the polymer and the aqueous medium can diffuse in the matrix. In the advanced stages there is formation of pores and invasion of the polymer by water resulting in an acceleration of the hydrolysis. The chains of lower molecular weight generated by scission of initial chains are considered to have a higher water solubility so they can 'leak' away in the aqueous surroundings generating channels through the matrix. Moreover this tendency to form pores leading to a significant mass loss before the final collapse of the polymer is more intense for the polymer of low molecular weight and especially of higher polydispersity (Heller, 1986; Hutchinson and Furr, 1991). The effect of specific area appeared difficult to quantify. Some

authors showed that the hydrolysis rate of DL PLA depends very much on the device size but concluded that it is faster for devices of greater thickness (Grizzi et al., 1995). Moreover crystallisation phenomena can occur during the degradation depending on experimental conditions (Vert et al., 1994).

The mechanism of degradation of DL PLA is a catalysed hydrolysis in acid and basic media (Makino et al., 1985; 1987). The mechanism of this catalysis is different depending on the predominating ion (H^+ or OH^-). Therefore the degradation of DL PLA 0.30 in basic medium is likely restricted to the surface, at least at early stages, which explains the moderate decrease of molecular weight at the beginning of the degradation.

The experimental findings by GPC and HPLC suggest that area of stability of DL PLA nanospheres is located around neutral pH (pH 5.5–7). The conservation of nanospheres at 4°C is imperative because at lower temperatures coalescence phenomena occurred (results not shown). The evolution of lactic acid production with temperature shows an exponential-type increasing curve. At lower temperatures (-18 and $+4$°C) the DL PLA which is well below its glass-transition temperature (T_g) is in a glassy state. So this evolution of the degradation can be explained by the fact that, as the DL PLA is a thermoplastic polymer with a low T_g, its conservation at a temperature near the T_g (e.g., 37°C) leads to a softening which is more pronounced for DL PLA of lower molecular weight. The T_g can also be lowered by the hydration of DL PLA during manufacture of nanospheres (Siemann, 1985). Additionally some authors established that at 37°C the sodium dodecyl sulphate adsorbed on PLA and led to a lowering of the T_g value bringing the polymer to a rubbery state (Coffin and McGinity, 1992). Indeed the curve of Fig. 5 which seems to cross two regions where the morphology of the polymer is different is not a straight line. This could be attributed to the fact that in the rubbery state the polymer is at a free energy level higher than in the glassy state so it will be more reactive in this state.

5. Conclusion

This study confirmed the main influence of the conservation temperature and the pH of the aqueous dispersion of nanospheres made from poly(D,L-lactide) on the chemical stability of the polymer. The best stability in aqueous medium was observed in a buffered solution with a pH corresponding to the physiological conditions and a temperature of 4°C. The conditions of this study did not permit investigation of the influence of the size of the carriers on the rate of degradation of their constitutive polymer.

The hydrolysis mechanism appeared to be highly pH-dependent. An acidic medium led preferably to a random scission along the polymeric chain resulting in the formation of mostly insoluble oligomers and low production of free carboxylic groups. On the contrary, an alkaline medium favours a non-random cleavage of the ester bonds at the end of the polymeric chains with high production of soluble derivatives including lactic acid.

Other parameters such as the presence of enzymes in the conservation medium, the ratio of glycolic acid in constitutive polyester of nanospheres and its permeability to water are under investigation.

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