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In vitro degradation of nanoparticles prepared from polymers based on DL-lactide, glycolide and poly(ethylene oxide)

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Abstract

Nanoparticles of poly(DL-lactic acid) (PDLLA), poly(DL-lactic-co-glycolic acid) (PLGA) and poly(ethylene oxide)–PLGA diblock copolymer (PEO–PLGA) were prepared by the salting-out method. The in vitro degradation of PDLLA, PLGA and PEO–PLGA nanoparticles in PBS (pH 7.4) at 37 °C was studied. The particle size, molecular weight of the polymers and the amount of lactic and glycolic acids formed were followed in time. PDLLA nanoparticles gradually degraded over a period of 2 years and retain their size during that period. A faster degradation was observed for PLGA nanoparticles, which was nearly complete after 10 weeks. PLGA nanoparticles retained their size during that period. In PEO–PLGA nanoparticles, the ester bond connecting the PEO and the PLGA segments was preferentially cleaved, which led to a relatively fast decrease in molecular weight and to (partial) aggregation, as multimodal size distributions were observed. PEO–PLGA nanoparticles were almost completely degraded within 8 weeks.

Keywords: Biodegradable nanoparticles; Salting-out method; In vitro degradation; Polyesters; PEO–PLGA block copolymers

1. Introduction

Block copolymers based on poly(ethylene oxide) (PEO) and poly(DL-lactic-co-glycolic acid) (PLGA) have been applied for the preparation of nanoparticles for drug delivery. The use of poly(ethylene oxide)–PLGA (PEO–PLGA) block copolymers enables the preparation of nonaggregating particles without the need of an additional stabilizer such as poly(vinyl alcohol) (PVA) [1].

PEO is an uncharged, highly flexible polymer that is known to decrease protein adsorption and cell interactions when present at the surface [2]. PEO has outstanding physiochemical and biological properties, including solubility in water and in organic solvents [3]. Furthermore, it is nontoxic, nonantigenic and nonimmunogenic [3]. It has been shown that PEO with a molecular weight less than $6 \cdot 10^3$ g/mol is passively excreted by the kidney [4].

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PLGA copolymers are known for their biodegradability and biocompatibility [5–9]. The final degradation products are lactic and glycolic acids, which are either excreted by the kidneys or enter the Krebs cycle to be eventually eliminated as carbon dioxide and water [10].

The term degradation is used to describe the chain scission of the polymer, whereas erosion is used to describe mass loss [11]. Depending on relative rates of water diffusion into the polymer matrix and degradation of the polymer, two degradation processes can be distinguished. When polymer degradation is faster than diffusion of water into the matrix, degradation and erosion become a surface phenomenon. In the opposite case, where diffusion of water into the matrix is faster than polymer degradation, the whole matrix is affected by degradation and subsequent erosion [11].

PLGA is known to undergo bulk degradation [12], as water diffusion into the matrix is faster than polymer degradation [11]. This process is characterized by random hydrolytic scission of the polyester backbone. The rate of degradation increases with increasing glycolide content in the polymer [13,14] due to a higher amount of bound reactive water [15], being highest for copolymers with 70 mol% of glycolide [5]. The degradation rate of PEO–PLGA copolymers is higher than of PLGA copolymers [16] due to increased hydrophilicity [17], as characterized by an increased water uptake [18]. Oligomers of PLGA with a molecular weight less than approximately $1 \cdot 10^3$ g/mol are soluble in water [19]. Oligomers with more monomeric units can still be solubilized when they are connected to PEO [20]. Consequently, earlier mass loss will be encountered for the degradation of PEO–PLGA as compared to PLGA for similar PLGA composition and molecular weight.

Numerous investigations have dealt with the in vitro degradation of PLGA. However, the results often differ because the samples used for the degradation studies vary in size and shape. It has been reported that the degradation rate increases with increasing thickness of the sample [21]. The main reason for this phenomenon is the occurrence of autocatalysis caused by the carboxylic end groups formed by chain cleavage [22]. Autocatalysis is more pronounced for thicker samples because of the longer pathway for diffusion of oligomers to the surface [21] and for hydroxide ions into the matrix [23]. This results in accumulation of oligomers in the bulk and therefore in an increased concentration of carboxylic end groups which cannot be neutralized by buffer ions, leading to a faster degradation rate in the inside than the outside of the sample. This will result in a bimodal molecular weight distribution in time [21]. It has been observed that thick films or plates develop a skin of approximately 200 μm in which degradation is much slower than in the bulk [21]. On the basis of these results, it can be expected that when the thickness of the samples is decreased, the contribution of faster bulk degradation to the degradation process of the whole sample will be diminished [21]. This corresponds with the fact that microparticles degrade slower than millimeter-sized beads [21], and that an even slower degradation was found for nanoparticles [24].

There is abundant information on the degradation behavior of poly(DL-lactic acid) (PDLLA) and PLGA microparticles [6,14,16,21,24–37], but only a few studies on the degradation of nanoparticles prepared from PDLLA [38,39], PLGA [19,24,40] or PEO–PLGA [41] are available. The influence of temperature [38] and pH [39] on the degradation of PDLLA nanoparticles has been studied. At neutral pH at 37 °C, the molecular weight half-life of PDLLA nanoparticles was more than 150 days in both studies [38,39]. However, in one study, this conclusion was drawn based on only two time points [39]. In one paper, the in vitro degradation of PLGA nanoparticles (530±300 nm) was studied. The particles were totally degraded after 150 days, and it was concluded that autocatalysis occurred [24]. In a degradation study of drug-loaded PLGA nanoparticles, no substantial decrease in molecular weight was observed. However, the molecular weight was only monitored for 60 h [40]. In the degradation study of PEO–PLGA nanoparticles, copolymers with constant PEO length ($\bar{M}_n$=$5 \cdot 10^3$ g/mol) and various PLGA lengths ($\bar{M}_n$=$7-68 \cdot 10^3$ g/mol) were used [41]. PEO was preferentially cleaved, and it was stated that these drug-loaded particles were degraded by surface erosion. This conclusion was based on the occurrence of mass loss without a decrease in molecular weight. However, the molecular weight was only measured for 7 days [41]. Comparison of these studies is hampered due to differences in particle preparation conditions and molecular weights of the polymers.

To avoid these problems, nanoparticles of PDLLA, PLGA and PEO–PLGA of similar (relatively low) molecular weights were prepared, and their degradation in PBS at pH 7.4 and 37 °C was studied.

2. Materials and methods

2.1. Materials

DL-lactide and glycolide were purchased from Purac Biochem (Gorinchem, The Netherlands). Stannous octoate, L-lactic acid and sodium azide (NaN_3) were purchased from Sigma (St. Louis, USA) and used as received. Hexanol (Merck, Darmstadt, Germany) was distilled from calcium hydride (Acros Organics, NJ, USA) prior to use. Monomethoxy poly(ethylene glycol) (MPEG) ($\bar{M}_n$=3.0·10^3 g/mol) was purchased from Shearwater Polymers (Huntsville, USA). Deuterated chloroform ($CDCl_3$), magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$) and glycolic acid were purchased from Merck, and poly(vinyl alcohol) [PVA; $\bar{M}_n$=9−10·10^3 g/mol; 80% hydrolyzed from poly(vinyl acetate)] was purchased from Aldrich (Milwaukee, USA) and used as received. Phosphate buffered saline (PBS; pH 7.4; NPBI, Emmer Compascuum, The Netherlands) was used as received. All solvents used were of analytical grade (Biosolve, Valkenswaard, The Netherlands).

2.2. Polymer syntheses

A mixture of DL-lactide (10.1 g; 69.8 mmol) and glycolide (5.45 g; 47.0 mmol) with stannous octoate in pentane (5.0 ml; 1.89 g/l) and an appropriate amount of initiator (318 μl hexanol or 7.59 g MPEG; 2.53 mmol) were transferred to an ampoule. After removal of the pentane by applying vacuum, the ampoule was evacuated, vacuum-sealed and subsequently transferred to an oil bath at 130 °C. After 24 h of reaction, the crude product was dissolved in chloroform, precipitated into a tenfold volume of methanol, filtered and dried in vacuo at 40 °C for 3 days.

The synthesized (co)polymers are denoted as PDLLA for the homopolymer of DL-lactide, as PLGA for the copolymer of DL-lactide and glycolide and as PEO–PLGA for the block copolymer of poly(ethylene oxide) and PLGA.

The number average molecular weight ($\bar{M}_n$) and the composition of the (co)polymers were determined by proton nuclear magnetic resonance (^{1}H-NMR), with $CDCl_3$ as the solvent. The sequence lengths of monomeric units in PLGA were determined by ^{13}C-NMR from copolymer solutions in $CDCl_3$ (100 mg/ml). Average sequence lengths were calculated from the dyad splitting of the carbonyl signals [42]. NMR spectra were obtained using a Varian Inova (Varian, Palo Alto, USA) operating at 300 MHz. The $\bar{M}_n$ and molecular weight distribution of the (co)polymers were determined by gel permeation chromatography (GPC) at 25 °C, using chloroform as an eluent at a flow rate of 1.5 ml/min. The GPC system consisted of a Waters Model 510 pump, an HP Ti-Series 1050 autosampler, a Waters Model 410 Differential Refractometer and a Viscotek H502 Viscometer Detector with HR0.5, HR2 and HR4 Waters Ultra-Styragel columns (Waters, Milford, USA) placed in series. Polystyrene standards with narrow molecular weight distributions (PSS, Mainz, Germany) were used for calibration.

2.3. Nanoparticle preparation

Nanoparticles were prepared using the salting-out method [1,43] in which acetone was chosen as the water-miscible organic solvent because of its pharmaceutical acceptance with regard to toxicity [44]. The method consists of the addition of a water-soluble PVA in a highly concentrated salt solution in water (aqueous phase) to a polymer solution in acetone (organic phase). Although acetone is miscible with pure water in all ratios, the high salt concentration of the aqueous phase prevents mixing of the phases. After emulsification, the addition of pure water in a sufficient quantity causes acetone to diffuse into the aqueous phase, resulting in the formation of nanoparticles.

Typically, an acetone solution (5.0 g) containing 2 wt.% (co)polymer was emulsified under mechanical stirring (20,500 rpm; 40 s; T25 Ultraturrax equipped with a S25 dispersing tool, Ika-Labortechnik, Staufen, Germany) in an aqueous phase (7.5 g) containing 60 wt.% $MgCl_2 \cdot 6H_2O$ as the salting-out agent and 2 wt.% PVA as a stabilizer (in a glass beaker; 3.5-cm diameter; 6.6-cm height). After the fast addition (5 s) of pure water (7.5 g) under mechanical stirring

(20,500 rpm) causing acetone to diffuse into the water phase, nanoparticles were formed, and stirring was continued for 20 s at 20,500 rpm. PEO–PLGA nanoparticles were prepared in the same manner, but no PVA was used [1].

The nanoparticles were purified by rinsing with water. First, the nanoparticles were separated by ultracentrifugation (65,000 $\times g$ for 30 min; Centrikon T-2180, Kontron Instruments, Watford, UK), and the supernatant was removed. The nanoparticles were redispersed in water, centrifuged, and the supernatant was removed. This procedure was repeated three times.

2.4. In vitro degradation of nanoparticles

PDLLA, PLGA and PEO–PLGA nanoparticles were redispersed in PBS containing 0.02% (w/v) NaN_3 at a known concentration of approximately 4 mg/ml. Subsequently, the dispersions were transferred to ultracentrifugation tubes, closed and placed in an oven at 37 °C. At different time points, the particle size was determined. At the same time, nanoparticles were separated from the medium by ultracentrifugation (65,000 $\times g$ for 40 min). The supernatant was analyzed by HPLC to determine the amount of lactic and glycolic acids, and the pH of the supernatant was measured at 25 °C. The sediment was lyophilized and subsequently analyzed with respect to the molar composition and $\bar{M}_n$ of the polymer.

2.5. Particle analyses

The nanoparticle size was determined by dynamic light scattering (DLS; Zetasizer 4000, Malvern Instruments, Malvern, UK) at 25 °C at an angle of 90°, taking the average of three measurements. The particle dispersion was diluted with PBS to such a degree that the desired number of counts was obtained. The desired number of counts is the number of counts that is high enough to obtain a good signal to noise ratio yet small enough to prevent multiple scattering to occur.

First, the polydispersity index (PI) is determined by the cumulants method. The PI is a dimensionless number indicating the width of the size distribution and has a value between 0 and 1, being 0 for monodisperse particles. If the PI is small enough (<0.08), the particle size can be determined by the cumulants method, and the size distribution obtained is based on a log normal distribution characterized by mean and width. For polydispersity indices higher than 0.08, the CONTIN-method is used to determine the particle size. The CONTIN-method developed by Provencher et al. [45] describes bimodal and smooth distributions without the need for information such as an initial estimate for the particle size.

The thermal properties of the nanoparticles in PBS were evaluated by differential scanning calorimetry (DSC) using a DSC 7 (Perkin-Elmer, Shelton, USA). A heating rate of 10 °C/min was applied, and stainless steel pans (Perkin-Elmer) were used. PBS (50 μl) was added to nanoparticle samples (10–15 mg) obtained after ultracentrifugation and removal of the supernatant. The samples were heated from −10 to 70 °C. The samples were then cooled (300 °C/min) to −10 °C, and after 5 min, a second scan was recorded. The data presented are from the second scan. The glass transition temperatures (T_g) were taken as the midpoint of the heat capacity change. Indium and gallium were used as standards for temperature calibration.

2.6. Determination of lactic and glycolic acid concentration in the supernatant

The supernatant of the samples after ultracentrifugation was analyzed by high-performance liquid chromatography (HPLC) to determine the amount of lactic and glycolic acids formed. The supernatant (50 μl) was injected (Injector 50 μl loop Valco) on an Inertsil ODS-3 column (250×4.6 mm; 5 μm; Chrompack, Bergen op Zoom, The Netherlands). UV-treated 0.1 M $NH_4H_2PO_4$ (pH 2.5; Merck) was used as an eluent at a flow rate of 1 ml/min (Varian HPLC pump 2510). Detection of lactic and glycolic acids was performed at 210 nm, using a Varian variable λ detector 2550 and compared to a calibration curve for lactic and glycolic acids. Glycolic acid eluted after 3.7 min and lactic acid after 6.1 min at the used conditions.

3. Results and discussion

After polymerization, the crude product was analyzed by ^{1}H-NMR to determine the actual compo-

sition and the number average molecular weight ($\bar{M}_n$). The $\bar{M}_n$ and polydispersity index (PDI) were determined by GPC. The results are presented in Table 1.

The polymer composition is close to the monomer ratio in the feed, with slightly more glycolide than DL-lactide incorporated. The higher reactivity of glycolide in comparison with DL-lactide, as previously reported [46], accounts for the larger fraction of glycolide in the copolymer than in the monomer feed. From ^{1}H-NMR, it becomes clear that molecular weights close to the theoretical molecular weight have been obtained. The polydispersity indices range from 1.24 to 1.95. Polydispersity indices close to 2 are typical for stannous octoate catalyzed ring-opening polymerizations of lactide and glycolide [27,47,48] and are indicative of transesterification reactions [49]. The molecular weights of the three polymers as determined by ^{1}H-NMR are comparable. The $\bar{M}_n$ values obtained from GPC measurements are slightly different. The lactyl to glycolyl ratio is similar for the PLGA and PEO–PLGA polymers.

The average sequence lengths of lactyl and glycolyl units in the PLGA block were determined by ^{13}C-NMR [42]. The integrals of the carbonyl peaks of lactyl next to glycolyl (I_{LG}; δ=171.67 ppm) and of lactyl next to lactyl (I_{LL}; δ=171.42 ppm) were used to calculate the average sequence length of lactyl units ($\bar{L}_L$; Eq. (1)).

$$\bar{L}_L = \frac{I_{LL}}{I_{LG} + I_{LL}} \quad (1)$$

The integrals of the carbonyl peaks of glycolyl next to lactyl (I_{GL}; δ=168.63 ppm) and of glycolyl next to glycolyl (I_{GG}; δ=168.64 ppm) were used to calculate the average sequence length of glycolyl units ($\bar{L}_G$; Eq. (2)).

$$\bar{L}_G = \frac{I_{GG}}{I_{GL} + I_{GG}} \quad (2)$$

The average sequence lengths calculated from Eqs. (1) and (2) are given in Table 1. The fact that the lactyl and glycolyl sequence lengths are similar suggests that, in the PEO–PLGA and PLGA copolymers, the monomers are equally distributed.

Aqueous dispersions of PDLLA, PLGA and PEO–PLGA nanoparticles were prepared and characterized with respect to T_g (aqueous suspension) and particle size (Table 2). The T_g of the PDLLA nanoparticles is higher than 37 °C, which indicates that these particles initially may be in a glassy state in an aqueous environment at body temperature. This is in contrast to the PLGA and PEO–PLGA nanoparticles, which are in the rubbery state under the same conditions. This might have consequences for the rate of degradation, as above the T_g, the mobility of the polymer chains is higher, and therefore, water diffusion proceeds faster. As water lowers the T_g due to a plasticizing effect [50], the T_g (aqueous

Table 2
The T_g, average size and polydispersity index (PI) of the nanoparticles in PBS before in vitro degradation

Nanoparticle	T_g (°C)	Average particle size (nm)	PI (−)
PDLLA	39	248	0.04
PLGA	35	230	0.09
PEO–PLGA	2	139	0.19

Table 1
The molar composition, number average molecular weight ($\bar{M}_n$), polydispersity index (PDI) and average sequence lengths of the synthesized (co)polymers

Polymer	Composition x:y[a]		$\bar{M}_n$ (kg/mol)			PDI[b]	$\bar{L}_L$[c]	$\bar{L}_G$[c]
	Feed	Polymer	Theoretical[d]	Experimental[e]	Experimental[b]			
PDLLA	100:0	100:0	10.1	11.5	14.5	1.42		
PLGA	60:40	57:43	10.1	11.4	12.8	1.95	3.1	2.1
PEO–PLGA[f]	60:40	52:48	11.9	11.2	9.9	1.24	2.4	1.8

[a] x:y denotes the ratio of lactyl/glycolyl units of the PLGA block, determined by ^{1}H-NMR.
[b] Determined by GPC.
[c] The average sequence length of lactyl ($\bar{L}_L$) and glycolyl ($\bar{L}_G$) units of the PLGA block, determined by ^{13}C-NMR and calculated from Eqs. (1) and (2), respectively.
[d] The theoretical $\bar{M}_n$ is calculated from the [Monomer]/[Initiator] ratio.
[e] Determined by ^{1}H-NMR.
[f] $\bar{M}_{n,PEO}=3 \cdot 10^3$ g/mol.

suspension) is lower than the T_g (polymer) [1,43]. However, it should be noticed that it is possible that the T_g as determined in the DSC experiment does not adequately represent the T_g of particles that are equilibrated in an aqueous environment.

A further explanation for the difference between PDLLA/PLGA and PEO–PLGA particles is that PDLLA and PLGA nanoparticles have approximately the same size, whereas the PEO–PLGA nanoparticles are smaller (Table 2). The smaller the particles, the more autocatalysis is expected to be diminished [21]. However, inasmuch as water uptake is high [1], diffusion of buffer ions into and of water-soluble oligomers out of the particles is probably rapid, and autocatalysis is not likely to occur for any of the nanoparticles. This means that the difference in size probably will not affect the degradation rate.

PDLLA nanoparticles had a size of approximately 250 nm at the start of the degradation and a PI of 0.04. The $\bar{M}_n$ of the PDLLA decreases in time, and the particle size increases slightly (Fig. 1A). No solid material was visible anymore at week 104. The increase in size can be explained by a higher swelling of the particles due to the formation of carboxylic acid and hydroxyl groups. The PI remains below 0.1 until 60 weeks and does not exceed 0.2 even up to 98 weeks, which demonstrates that the particles do not aggregate upon degradation. This can be explained by the presence of PVA. It has previously been shown that after several purification steps, up to 10% of PVA can remain present in or on the particles [44]. During degradation, PVA might remain present at the surface to stabilize the particles. Moreover, the increasing number of end groups might contribute to the stabilization of the particles.

The small decrease in $\bar{M}_n$ during the first 5 weeks coincides with negligible lactic acid formation (Fig. 1B) and no change in pH. After week 5, a linear increase of lactic acid formation in time is observed, resulting in a gradual decrease in the pH. The relative amount of lactic acid determined in the medium at week 104 is approximately 15% of the total lactic acid units initially present. The pH was 6.4 at week 104, which is clearly higher than the pK_a of lactic acid (3.8 at 25 °C [39]). The fact that only 15% of total lactic acid units available in the polymer were converted to free lactic acid indicates that large amounts of water-soluble oligomers are present and are not detected. The onset of lactic acid formation for 0.3-mm thick films or microparticles (125–250 μm) is reported to be 10 weeks [21]. This indicates that diffusion of lactic acid from the nanoparticles is faster than from films or microparticles, and that the penetration of buffer in nanoparticles is more rapid than in films of microparticles. This implies that accumulation of oligomers containing carboxylic end groups that could catalyze the hydrolysis is less likely to occur in the case of nanoparticles when compared to films or microparticles. Correspondingly, the time needed to completely degrade PDLLA nanoparticles (approximately

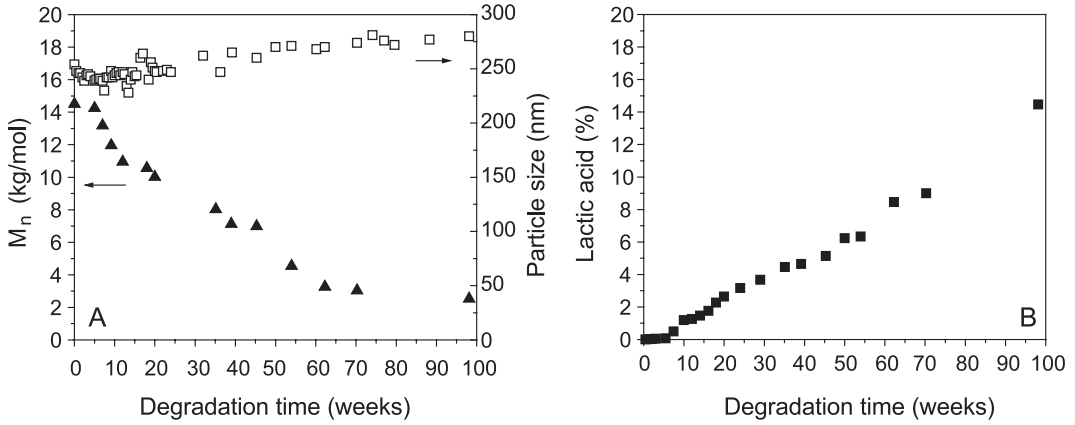

Fig. 1. (A) The $\bar{M}_n$ (▲) and the particle size (□) of PDLLA nanoparticles as a function of the degradation time at 37 °C in PBS (pH 7.4). (B) Relative amount of lactic acid in the degradation medium as a function of the degradation time for PDLLA nanoparticles at 37 °C in PBS (pH 7.4), presented as a percentage of the total amount of lactic acid units initially present in the nanoparticles.

100 weeks) is longer than the time needed to completely degrade PDLLA films or PDLLA microparticles (approximately 50 weeks) [21] and corresponds to results on the degradation of PDLLA nanoparticles of others [38,39].

In Fig. 2, the $\bar{M}_n$, size and relative amount of lactic and glycolic acids formed as a function of degradation time of PLGA nanoparticles are depicted. The $\bar{M}_n$ gradually decreases, reaching 20% of its initial value after 8 weeks. As in the case of PDLLA particles, the particle size increases slightly in time with a PI lower than 0.2. This indicates that the PLGA nanoparticles do not aggregate upon degradation, which can be explained by the presence of PVA or the increase in number of end groups, as was the case for PDLLA nanoparticles. The degradation of PLGA nanoparticles clearly proceeds more rapidly than the degradation of PDLLA nanoparticles and is complete in 18 weeks.

During degradation of the copolymer, water-soluble oligomers and their corresponding monomeric units, lactic and glycolic acids, are formed. From the first day on, both acids can be detected in the degradation medium (Fig. 2B). The initial formation of glycolic acid is faster than the formation of lactic acid, as reported before by others [11]. The reason for this is that the glycolic ester bond is more susceptible to hydrolysis [15]. Therefore, there is preferential cleavage at the glycolic–glycolic and glycolic–lactic bonds, resulting in faster release of glycolic acid. This is confirmed by a shift in the copolymer composition towards higher lactide contents increasing from 57 mol% initially to 68 mol% at week 7 as determined by ^{1}H-NMR. The pH gradually decreased in time, being 6.2 after 18 weeks. After 18 weeks, a plateau in the level of acids is reached, and the relative amount of acids corresponds to the percentage of the monomeric units initially present in the nanoparticles (Fig. 2B). This indicates that PLGA nanoparticles were completely degraded into lactic and glycolic acids after 18 weeks, as also observed by Dunne et al. [24]. The fact that lactic and glycolic acids released from the PLGA nanoparticles is seen from day 1 on indicates that rapid degradation takes place and that degradation products rapidly diffuse into the medium. This also implies that accumulation of oligomers containing carboxylic end groups that could catalyze the hydrolysis is minimal or even absent.

The results of the degradation of PEO–PLGA nanoparticles that do not contain PVA are presented in Fig. 3. The $\bar{M}_n$ decreases during the first two weeks, was stable during the next few weeks and then further decreases (Fig. 3A). The decrease in the first two weeks is caused by a rapid hydrolysis of ester bonds connecting PEO, as can be concluded from the decrease of the molar content of PEO in the resulting polymer, as determined by ^{1}H-NMR (32 mol% initially while 12 mol% at week 2), which is in correspondence to the results of Avgoustakis et al. [41]. After 4 weeks, the $\bar{M}_n$ decreases further and

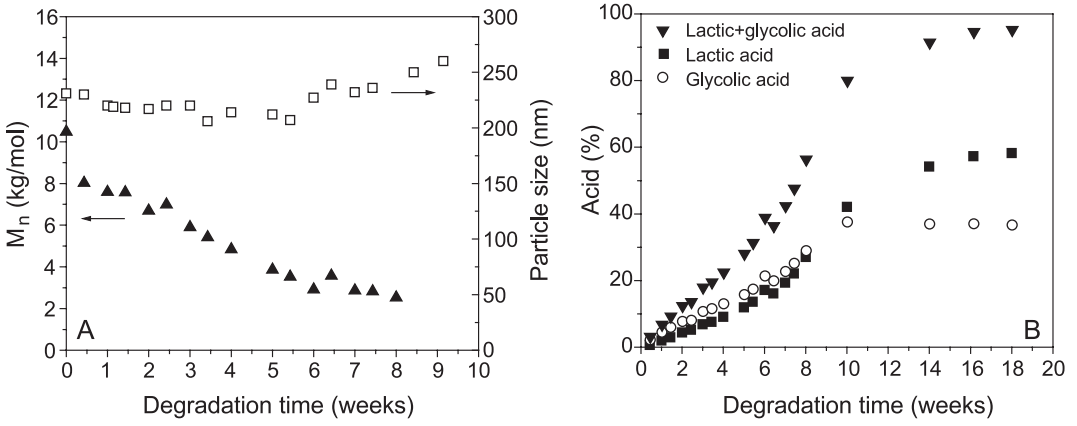

Fig. 2. (A) The $\bar{M}_n$ (▲) and the particle size (□) of PLGA nanoparticles as a function of the degradation time at 37 °C in PBS (pH 7.4). (B) Relative amount of lactic and glycolic acid in the degradation medium as a function of the degradation time for PLGA nanoparticles at 37 °C in PBS (pH 7.4), presented as a percentage of the total units initially present in the nanoparticles.

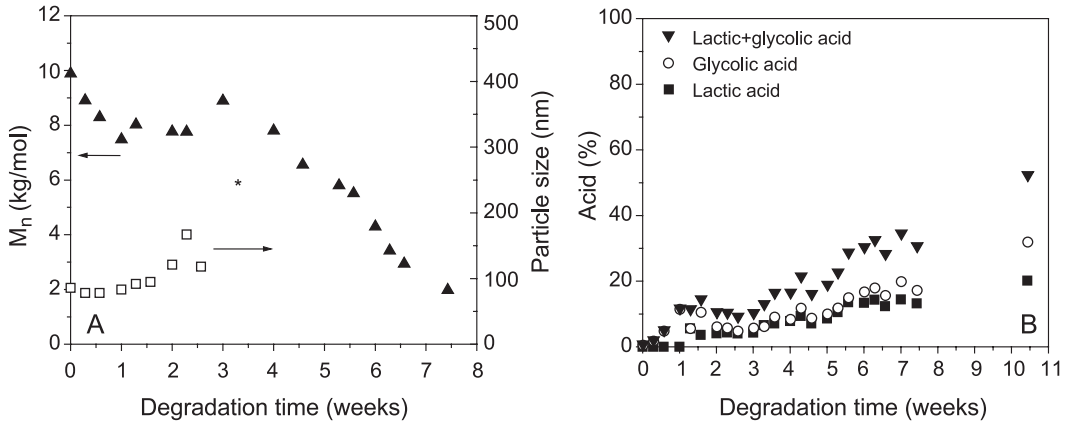

Fig. 3. (A) The $\bar{M}_n$ (▲) and the particle size (□) of PEO–PLGA nanoparticles as a function of the degradation time at 37 °C in PBS (pH 7.4). The * indicates the time point from which multiple size distributions were observed. (B) Relative amount of lactic and glycolic acid in the degradation medium as a function of the degradation time for PEO–PLGA nanoparticles at 37 °C in PBS (pH 7.4), presented as a percentage of the total units initially present in the nanoparticles.

reaches a value below $2 \cdot 10^3$ g/mol after 7 weeks. However, in contrast to the PDLLA and PLGA nanoparticles, the particle size and the PI increase rapidly in time. The PI even reaches a value of 1 after three weeks due to aggregation as concluded from multimodal size distributions detected with DLS. This aggregation is caused by the release of PEO upon hydrolysis of PLGA. As observed for PLGA nanoparticles, lactic and glycolic acids are detected in the medium from day one on, and more glycolic acid than lactic acid is formed (Fig. 3B). This is confirmed by a shift in the copolymer composition towards higher lactide contents as determined by ^{1}H-NMR: the molar ratio of lactyl/glycolyl increases from 52:48 initially to 68:32 at week 8. The pH gradually decreased in time, reaching a value of 6.4 after 10 weeks.

After the fast release of PEO, the particles are mainly composed of PLGA. Still, the decrease in $\bar{M}_n$ is faster than observed for PLGA solely. The reason for this is that the amount of PEO still present (8 mol% as determined by ^{1}H-NMR) results in a higher water uptake, resulting in higher hydrolysis rates [18].

The in vitro degradation of PDLLA and related aliphatic polyesters involves the generation of carboxylic end groups that are able to catalyze the hydrolysis [51]. However, the fact that fast acid formation is observed indicates that degradation products rapidly diffuse into the medium. This implies that accumulation of oligomers containing carboxylic end groups that could catalyze the hydrolysis is not likely to occur. This is confirmed by GPC, inasmuch as no bimodal molecular weight distributions were observed at any time point, for any of the different nanoparticles.

4. Conclusions

The in vitro degradation rate of PLGA-based nanoparticles is dependent on the composition of the copolymer. PDLLA nanoparticles gradually degrade over a period of 2 years. A faster degradation was observed for PLGA, which was nearly complete after 10 weeks. Both PDLLA and PLGA nanoparticles maintained their size until they were totally degraded without noticeable aggregation. In PEO–PLGA nanoparticles, the ester bond connecting the PEO and the PLGA segments was preferentially cleaved, which led to a relatively fast decrease in molecular weight and to (partial) aggregation, as multimodal size distributions were observed. The overall degradation rate of PEO–PLGA particles was slightly higher than of PLGA particles. In contrast to the heterogeneous in vitro degradation of devices based on copolymers of lactide and glycolide, as described in literature, PDLLA, PLGA and PEO–PLGA nanoparticles appear to degrade homogeneously in time without autocatalysis.

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