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In vitro degradation behavior of poly(lactide)–poly(ethylene glycol) block copolymer micelles in aqueous solution

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ABSTRACT

Self-assembling micelles were prepared from polylactide–poly(ethylene glycol) (PLA–PEG) block copolymer by using two different methods: direct dissolution and dialysis. The in vitro degradation properties of the micelles were investigated at 37 °C and monitored by using various techniques. During the investigated degradation time, the size of micelles by dialysis remains stable, while that of micelles by direct dissolution first increases, followed by a collapse of micellar structure. The composition of PLA–PEG copolymers greatly affects the degradation of micelles. Micelles with longer hydrophobic PLA blocks exhibit less size changes due to more compact structure. On the other hand, the structural integrity of L/D mixed micelles is preserved for longer time than that of single micelles, in agreement with the stereocomplexation effect between L-PLA and D-PLA blocks. As degradation proceeds, the average molar mass of copolymer decreases and the distribution becomes wider, especially for micelles by dialysis and L/D mixed micelles with a more compact structure. Additionally, the PEG content in the copolymer chains increases during degradation, leading to a decrease of glass transition and crystallization temperature of the copolymers. However, the residual LA fragments produced by degradation disfavors the crystallization of PEG blocks, thus resulting in the decrease of melting temperature and melting enthalpy.

1. Introduction

Bioresorbable amphiphilic block copolymers, such as poly(lactide)–poly(ethylene glycol) (PLA–PEG), poly(ε-caprolactone)–poly(ethylene glycol) (PCL–PEG) and poly(lactide-co-glycolide)–poly(ethylene glycol) (PLGA–PEG), have been widely investigated in the past decades as carriers in drug delivery systems (Zhang et al., 1996; Jeong et al., 1999; Hu et al., 2003; Nair and Laurencin, 2007). They can self-assemble in aqueous media to form micelles with a core–shell structure, with an inner core composed of hydrophobic polyesters and an outer shell of hydrophilic polyethers (Kwon and Kataoka, 1995; Burt et al., 1999; Riley et al., 2003). With good degradability and biocompatibility, aliphatic polyesters can be degraded into small molecules through hydrolytic or enzymatic degradation, which are then excreted from the human body by renal clearance. The degradation rate of polyesters is enhanced when they are combined with flexible hydrophilic polyethers such as PEG (Wang et al., 2001; Li et al., 2002a).

Recently, micelles prepared from amphiphilic PLA–PEG block copolymers have attracted increasing attention as hydrophobic drug carriers (Hagan et al., 1996; Liu et al., 2001; Pierri and Avgoustakis, 2005). The hydrophobic core of the micelles consists of PLA segments, which is able to incorporate hydrophobic drugs with improved solubility; while the hydrophilic PEG shell can stabilize the micelles by limiting the opsonification and subsequent non-specific uptake by the reticuloendothelial system (RES) after intravenous administration, thus the drug circulation time in plasma could be consequently prolonged (Jones and Leroux, 1999; Zambaux et al., 1999; Gref et al., 2000). Additionally, the size of these micelles is normally in the range of 10–200 nm, which is small enough to avoid filtration by the lung and spleen (Moghimi et al., 2001). In our previous work (Yang et al., 2007, 2009), micelles were prepared in aqueous solutions through self-assembly of PLA–PEG block copolymers. The micellization properties and drug encapsulation abilities of the micelles were investigated, using paclitaxel as model drug. In particular, the mixed micelles derived from equal molar L-PLA/PEG and D-PLA/PEG block copolymers were investigated to examine the stereocomplexation effect between L-PLA and D-PLA blocks.

The degradation behavior of degradable polymers is of major importance for biomedical applications. Li et al. reported on the hydrolytic degradation of PLA–PEG triblock copolymers with short

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and long PLA chains (Li et al., 1996; Rashkov et al., 1996). The authors found that ester bond cleavage proceeded at random along the PLA blocks. Compositional and morphological changes were observed during degradation of triblock copolymers (Li et al., 1998). Stefani et al. (2006) studied the in vitro degradation of PLA–PEG diblock copolymer-based nanoparticles. They found that degradation generated PEG chains bearing short PLA segments through erosion mechanism, the molar mass remaining relatively constant during degradation. Venkatraman et al. comparatively investigated the degradation profiles of PLA–PEG nanoparticles and films (Jie et al., 2005; Venkatraman et al., 2005). Degradation of the nanoparticles appeared faster than the films. Li et al. (2002b) also studied the degradation of physically crosslinked hydrogels prepared from PLA–PEG triblock copolymers. Two stages were observed: degradation was initially very fast and then slowed down, in agreement with initial release of PEG-rich segments.

Previous efforts in PLA–PEG degradation studies mainly focused on films, particles or hydrogels which can be considered as bulk materials. To the best of our knowledge, there has been no report referring the degradation properties of PLA–PEG micelles. This situation might be related to the fact that investigations become much more complex in such a fluid system with dynamic stability. Traditional measurements such as weight loss and water uptake, which are usually employed in monitoring the degradation of bulk materials, cannot be applied in the case of micelle systems. In this paper, a series of PLA–PEG diblock and triblock copolymer micelles were prepared through direct dissolution or dialysis method, and their hydrolytic degradation properties were investigated under in vitro conditions. The influence of copolymer composition, stereocomplexation between L-PLA and D-PLA blocks, and fabrication methods on the degradation of micelles was discussed in detail.

2. Materials and methods

2.1. Materials

L-Lactide and D-lactide were obtained from Purac and recrystallized from ethyl acetate. Monomethoxy poly(ethylene glycol) (mPEG) with molar masses of 2000, 5000 and dihydroxyl PEG with molar mass of 8000 were supplied by Fluka. Zinc lactate was purchased from Sigma. All organic solvents were of analytic grade and used without further purification.

2.2. Polymerization

PLA–PEG diblock and triblock copolymers were synthesized by ring-opening polymerization of L- or D-lactide in the presence of mPEG or dihydroxyl PEG using low toxic zinc lactate as catalyst, as described previously (Li and Vert, 2003). Briefly, PEG and L-lactide or D-lactide with preset ethylene oxide to lactate molar ratio (EO/LA) were introduced into a polymerization tube. Zinc lactate (0.1 wt%) was then added. After degassing, the tube was sealed under vacuum, and polymerization was allowed to proceed at 140 °C. After 3 days, the product was recovered by dissolution in dichloromethane and precipitation in diethyl ether. Finally, the product was dried under vacuum to constant weight.

2.3. Preparation of PLA–PEG micelles

PLA–PEG diblock and triblock copolymer micelles were prepared by two different methods, direct dissolution and dialysis. As previously described (Yang et al., 2009), the direct dissolution method involves dissolving PLA–PEG copolymers in distilled water under stirring. While in dialysis method, PLA–PEG copolymers were first dissolved in 1-methyl-2-pyrrolidone, and then the solution was transferred into a pre-swollen dialysis membrane (MWCO = 3500) and dialyzed during 24 h against distilled water which was regularly renewed with fresh water.

2.4. Methods

The molar mass and molar mass distribution of block copolymers were measured by gel permeation chromatography (GPC) which was performed on a Shimadzu LC-20AT apparatus equipped with an RI detector (RID-10A). Tetrahydrofuran (THF) was used as the mobile phase at a flow rate of 1.0 ml/min. 20 μl of 1.0% (w/v) polymer solution were injected for each analysis. Calibration was accomplished with polystyrene standards with molar masses ranging from 500 to 700,000 g/mol.

The composition of the copolymers was determined by proton nuclear magnetic resonance (^{1}H NMR), which was recorded at room temperature with a Bruker spectrometer operating at 250 MHz by using DMSO as solvent. Chemical shifts (δ) were given in ppm using tetramethylsilane as an internal reference.

Differential scanning calorimetry (DSC) thermograms were registered with a PerkinElmer DSC 6 instrument, the heating rate being 10 °C/min. 10 mg of product were used for each analysis.

The size and size distribution of micelles were measured by using dynamic light scattering (DLS), which was carried out using a commercial laser light scattering spectrometer (Malvern Autosizer 4700, Malvern Instrument, Worcs, UK) equipped with a digital time correlator (Malvern PCS7132) and Compass 315 M-100 Diode-Pumped Laser as the light source (output power ≥100 mW, CW at λ_0 = 633 nm, Coherent Laser division, Santa Clara, CA). All the DLS measurements were made at 25.0 ± 0.1 °C with a 90° scattering angle. The samples were filtered through a 0.45 μm filter (Millipore) before measurements. The autocorrelation functions from DLS were analyzed by using the constrained regularized CONTIN method to obtain the diameter distribution. All analyses were run in triplicate and the results are reported as the average values.

The morphology of micelles was observed by using Hitachi H-600 transmission electron microscopy (TEM), operating at an accelerating voltage of 75 kV. One drop of micelle solution was placed on a copper grid covered with nitrocellulose membrane and dried in air before measurement.

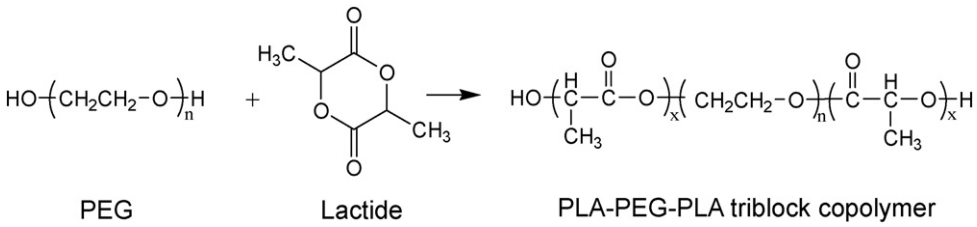
2.5. Degradation of PLA–PEG micelles

10 ml micellar solution with concentration of 3 mg/ml were placed into vials. 0.01% (w/v) sodium azide was added to inhibit the growth of bacteria. The vials were then stored at 37 °C. At predetermined intervals, samples were withdrawn for DLS and TEM analyses. Afterwards, the samples were lyophilized and analyzed using GPC, NMR and DSC measurements.

3. Results and discussion

3.1. Synthesis and characterization

A series of PLA–PEG diblock and triblock copolymers were synthesized by ring-opening polymerization of L- or D-lactide in the presence of PEG by using nontoxic zinc lactate as catalyst, as shown in Scheme 1. ^{1}H NMR measurements allowed to determine the composition of various PLA–PEG copolymers from the integrations of NMR resonances belonging to the methylene protons of the oxyethylene unit of PEG at 3.6 ppm and to the methine proton of the lactate unit of PLA at 5.2 ppm, as previously described in literature (Li and Vert, 2003). The molar mass of PLA–PEG copolymer measured by NMR was also calculated according to the following



Scheme 1. Ring-opening polymerization of L(D)-lactide in the presence of dihydroxyl PEG using zinc lactate as catalyst.

Table 1
Molecular characteristics of PLA–PEG block copolymers.

Copolymer	M_{nPEG}	EO/LA (feed)[a]	EO/LA (product)[b]	DP_{PEG}[c]	DP_{PLA}[d]	M_n[e]
L-mPEG2K3	2000	3	3.7	45	12	2860
D-mPEG2K3	2000	3	4.2	45	11	2790
L-mPEG5K3	5000	3	4.3	113	26	6870
D-mPEG5K3	5000	3	4.3	113	26	6870
L-mPEG5K4	5000	4	6.6	113	17	6220
D-mPEG5K4	5000	4	6.3	113	18	6300
L-PEG8K-L3	8000	3	3.8	182	48	11,460
D-PEG8K-D3	8000	3	3.5	182	52	11,740

[a] Molar ratio in feed.
[b] Molar ration in the synthesized copolymer calculated from the integration of NMR bands belonging to PEG blocks at 3.6 ppm and to PLA blocks at 5.2 ppm.
[c] $DP_{PEG} = M_{nPEG}/44$.
[d] $DP_{PLA} = DP_{PEG}/(EO/LA)$.
[e] $M_n = M_{nPEG} + DP_{PLA} \cdot 72$.

equation:

$$M_n = M_{nPEG} + DP_{PLA} \cdot 72 \quad (1)$$

where $DP_{PLA} = (M_{nPEG}/44)/(EO/LA)$.

The physicochemical properties of the resulting PLA–PEG diblock and triblock copolymers are summarized in Table 1. In the acronyms, L or D represents L- or D-lactide, 2, 5 or 8 K represents the molar mass of PEG, and the last number represents the EO/LA ratio in the feed. It appears that the EO/LA ratios of the copolymers were higher than the initial ratios, which can be assigned to the fact that the conversion of lactide was not complete, unreacted lactide being eliminated by the purification procedure.

3.2. Size and morphology changes of PLA–PEG micelles during degradation

The PLA–PEG micelles prepared by two different methods for hydrolytic degradation studies are presented in Table 2. It is well known that due to the larger surface area, the degradation characteristics of PLA–PEG micelles can be quite different from those of the bulk materials. Figs. 1–3 show the average size changes of various PLA–PEG micelles as a function of degradation time in aqueous solutions. It appears that the preparation method strongly influences both initial micelle size and size changes. The diameters of micelles prepared by direct dissolution methods (Figs. 1 and 2) appeared around 100 nm, while micelles by dialysis method (Fig. 3) were much larger with diameters higher than 200 nm. This could be ascribed to the different structures of these two kinds of micelles. In dialysis method, micelles are formed by continuous exchange between organic solvent inside and distilled water outside through osmosis effect of dialysis membrane. However, in direct dissolution method, micelles are formed just by hydrophobic interactions between PLA blocks, which are much weaker than the driven force of micelle formation in dialysis process. Thus much more molecular chains are incorporated into micelles by dialysis, leading to larger micelle size. Similar observations have been reported in literature (Peng et al., 2006; Slomkowski et al., 2006).

Table 2
PLA–PEG micelles prepared by direct dissolution and dialysis methods for hydrolytic degradation.

Sample	Copolymer	Method
M1-LD2K3	L/D-mPEG2K3[a]	Direct dissolution
M1-LD5K3	L/D-mPEG5K3	Direct dissolution
M1-L5K4	L-mPEG5K4	Direct dissolution
M1-LD5K4	L/D-mPEG5K4	Direct dissolution
M2-LD5K3	L/D-mPEG5K3	Dialysis
M2-L8K3	L-PEG8K-L3	Dialysis
M2-LD8K3	L/D-PEG8K3	Dialysis

[a] L/D-, Mixed micelles which were prepared from equal molar PLLA–PEG and PDLA–PEG block copolymers.

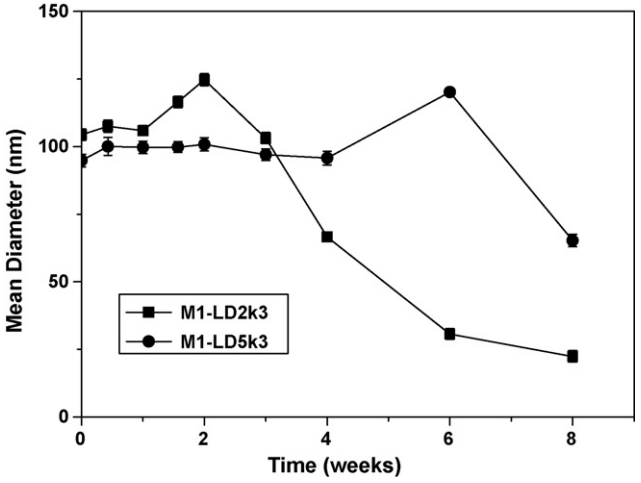


Fig. 1. Average size changes of M1-LD2K3 and M1-LD5K3 micelles by direct dissolution method as a function of degradation time in aqueous solution.

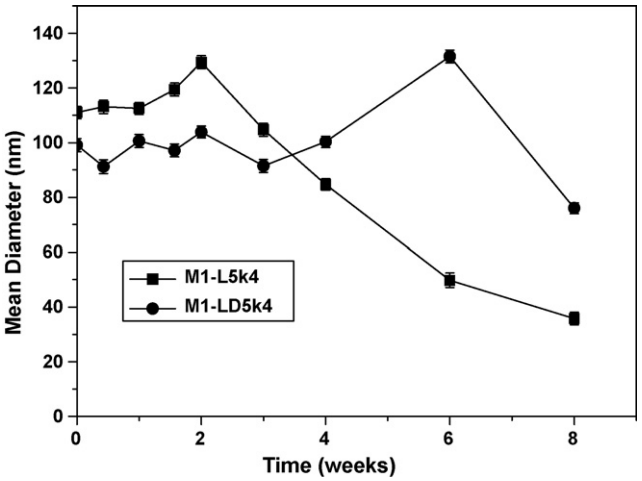


Fig. 2. Average size changes of M1-L5K4 and M1-LD5K4 micelles by direct dissolution method as a function of degradation time in aqueous solution.

Comparison of Figs. 1–3 shows that the size of micelles prepared by direct dissolution method decreased after 8 weeks degradation, while the size of micelles by dialysis remained almost unchanged. This is because the micellar structure of the former micelles is less stable than that of the micelles by dialysis method, in which a compact solid inner core is progressively organized as discussed above. According to literature (Hu et al., 2004), the hydrolytic degradation of this kind of micelles can be divided in two stages. In the initial degradation stage, the hydrolytic cleavage of ester bonds along polymer chains first takes place at the interface between PEG shell and PLA core. The thus produced LA oligomers with different block lengths cannot easily diffuse out of the micellar core due to its compact structure. Therefore, the size of micelles is hardly affected. As degradation proceeds, more and more water can penetrate the inner core through the channels formed by chain breakage during degradation, leading to the size increase. Thereafter, the degradation of PLA core is accelerated and the size of micelles eventually decreases. The degradation behavior of micelles by dialysis is supposed to belong to the first stage. However, in direct dissolution method, the micelles exist in a dynamic system with permanent exchanges between micelle-forming molecules and free molecules in solution, continuously breaking and reforming (Savic et al., 2006). The micellar core thus constitutes a loose

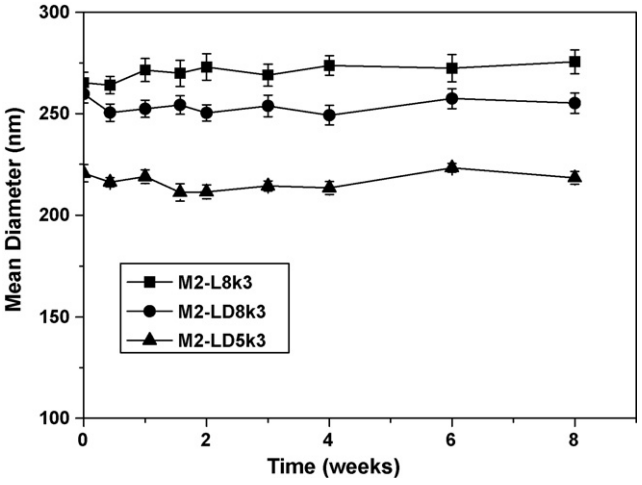


Fig. 3. Average size changes of M2-L8K3, M2-LD8K3 and M2-LD5K3 micelles by dialysis method as a function of degradation time in aqueous solution.

structure in a more or less swollen environment. Therefore, the hydrolytic cleavage of ester bonds occurs both in the core–shell interface and in the PLA core, leading to a quite faster size decrease than the micelles by dialysis.

The composition of PLA–PEG block copolymers also influences the micelle degradation property. Fig. 1 shows that the diameter of M1-LD2K3 micelles remained stable at 110 nm during the 1st week, slightly increased to 125 nm after 2 weeks, and then continuously decreased down to 25 nm. In contrast, the size of M1-LD5K3 micelles remained stable in the first 4 weeks, followed by an increase at 6 weeks and finally collapsed. This is because L-mPEG5K3 and D-mPEG5K3 have much longer hydrophobic block length, resulting in a more compact micelle structure due to stronger hydrophobic interactions. It is also of interest to compare M1-LD5K3 in Fig. 1 with M1-LD5K4 in Fig. 2. The size of M1-LD5K4 exhibited much fluctuation in the first stage, which could be attributed to the looser micellar structure due to shorter PLA block length in PLA–mPEG5K4. In addition, as shown in Fig. 2, M1-L5K4 single micelles began to lose the structural integrity beyond 2 weeks, while M1-LD5K4 mixed micelles kept stable structure until 6 weeks degradation. This finding can be ascribed to the stereocomplexation effect between L-PLA and D-PLA blocks, as previously described (Yang et al., 2007, 2009).

The changes of micellar structure during degradation were observed by TEM. As shown in Fig. 4, M1-LD5K3 micelles by direct dissolution method initially appeared as discrete dark spots. After 8 weeks degradation in aqueous solution at 37 °C, micelles can be barely distinguished due to the breakdown of the micellar structure. However, M2-LD5K3 micelles by dialysis method preserved micellar integrity after 8 weeks degradation although the number of micelles decreased, as shown in Fig. 5. This result confirms that micelles prepared by dialysis method exhibit more compact and stable structure than those by direct dissolution method. On the other hand, the micellar structure of M2-L8K3 by dialysis remained almost unchanged after 8 weeks (Fig. 6). This could be assigned to the dialysis method, and to the different micellar structure of triblocks as compared to that of diblocks.

3.3. Effects of hydrolysis on PLA–PEG copolymers

The micellar solutions were lyophilized to yield solid samples in order to investigate the influence of hydrolytic degradation on the PLA–PEG copolymers. GPC measurements were realized in THF which allowed to destroy the micellar structure, and to elucidate molar mass changes of PLA–PEG copolymers during degradation of the micelles. Figs. 7 and 8 show the GPC curves of the two kinds of PLA–mPEG5K3 micelles after different degradation time periods. Obviously, as degradation proceeds, the peak of GPC curve shifts to longer elution time with broader molar mass distribution due to hydrolytic cleavage of ester bonds. It is of interest to note that, after the same degradation time, the GPC curves of micelles by dialysis method (Fig. 8) are much broader with longer elution time than those by dissolution method (Fig. 7). The GPC results of different micelle samples during degradation are shown in Table 3. Obviously, micelles by dialysis method exhibit larger M_n and M_w/M_n changes. This may be attributed to the different micellar structures formed by the two different methods. As mentioned above, for micelles by dialysis method with a compact inner core, the short segments such as LA oligomers formed by degradation cannot diffuse out at the initial stage of degradation. Thus the concentration of carboxylic endgroups becomes much higher inside the PLA core, which further accelerates the internal hydrolysis of the remaining ester bonds and leads to more oligomers and broader molar mass distribution. In addition, comparing L/D mixed micelles (M1-LD5K4 and M2-LD8K3) with single micelles (M1-L5K4 and M2-L8K3), the former copolymers present lower molar mass and broader dis-

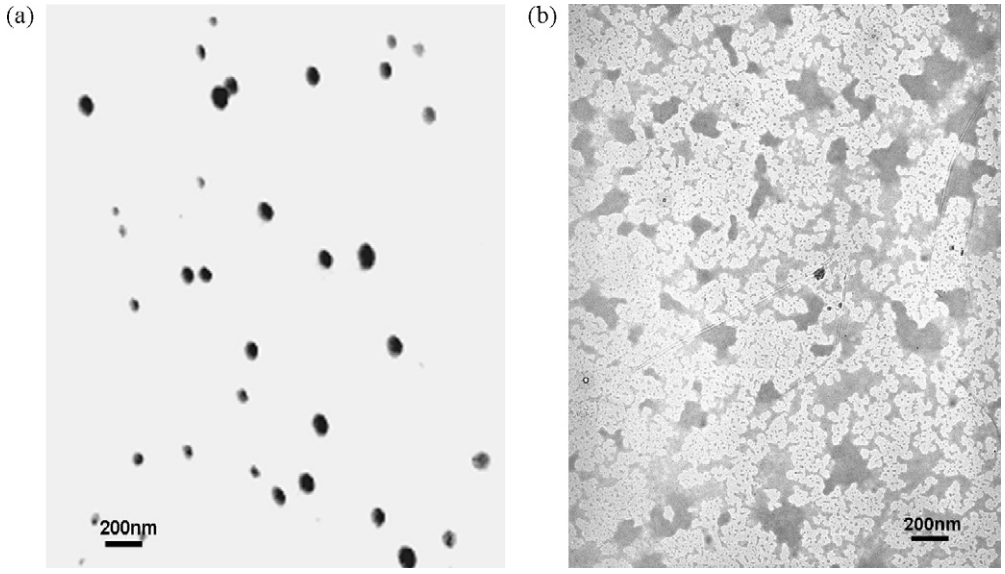


Fig. 4. Transmission electron micrographs of M1-LD5K3 micelles after (a) 0, and (b) 8 weeks' degradation in aqueous solution.

tribution. This finding confirms that micelles with more compact structure are subjected to accelerated internal degradation. However, the acceleration effect on cleavage of ester bonds is far much weaker than the "autocatalysis effect" in the case of bulk materials (Li and Vert, 1999).

^{1}H NMR was used to detect the changes of the chemical composition of PLA–PEG copolymers during the degradation of micelles. Herein we used DMSO-d_6 as the solvent, because it has been reported that the spectral resolution can be considerably improved by using DMSO-d_6 instead of $CDCl_3$ (Espartero et al., 1996; Li et al., 1996; Rashkov et al., 1996). Fig. 9 presents the NMR spectra of M1-LD5K3 micelles after different degradation time periods. It is known that the CH protons of hydroxylated lactyl end units appear in the 4.3–4.1 ppm range, and methyl protons of these end units appear in the 1.3–1.2 ppm range, as shown at position a and a' in Fig. 9 (Rashkov et al., 1996). While resonances in the 5.2–5.0 ppm range (CH) and in the 1.5–1.4 ppm range (CH_3) belong to PLA blocks, including both PEG-connecting and main chain units, as shown at position b and b'. In addition, the signals detected at 4.03 ppm and 1.23 ppm can be respectively assigned to CH and CH_3 from lactic acid monomer (unit c and c') (Espartero et al., 1996). Comparison of the NMR spectra in Fig. 9 shows that the content of terminal lactyl protons ($a+c$ and $a'+c'$) increases as degradation proceeds. This indicates that the hydrolytic degradation of PLA–PEG micelles takes place by hydrolysis of ester bonds in the PLA chains (Buwalda et al., 2010). Table 3 presents the EO/LA ratio changes of various PLA–PEG copolymers during degradation. In all cases, the EO/LA ratio increases with degradation time, confirming that the hydrolysis leads to the loss of LA segments in the copolymer chains, especially for micelles by dialysis method and L/D mixed micelles which possess a more compact structure, as mentioned above.

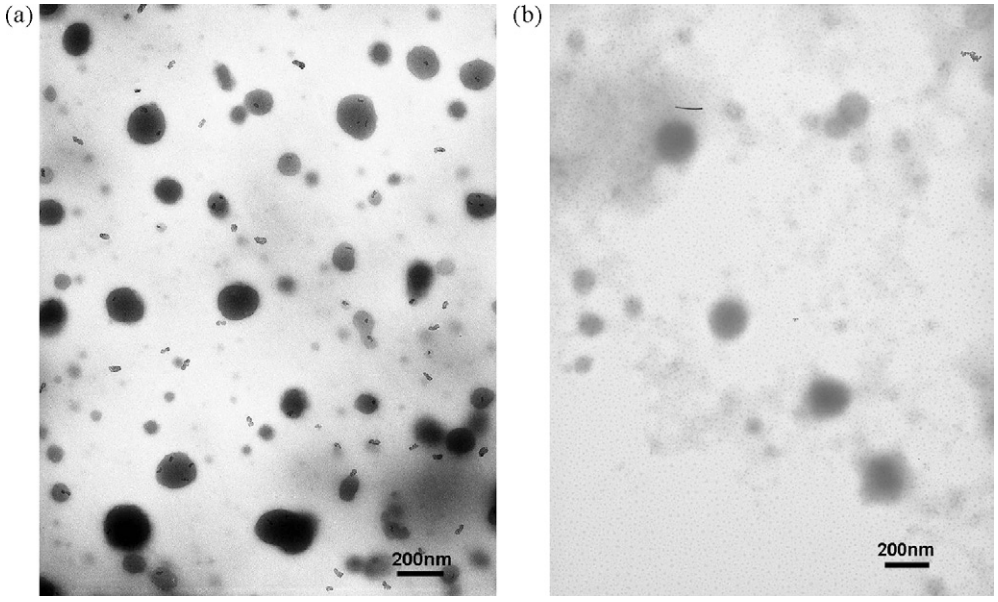


Fig. 5. Transmission electron micrographs of M2-LD5K3 micelles after (a) 0, and (b) 8 weeks' degradation in aqueous solution.

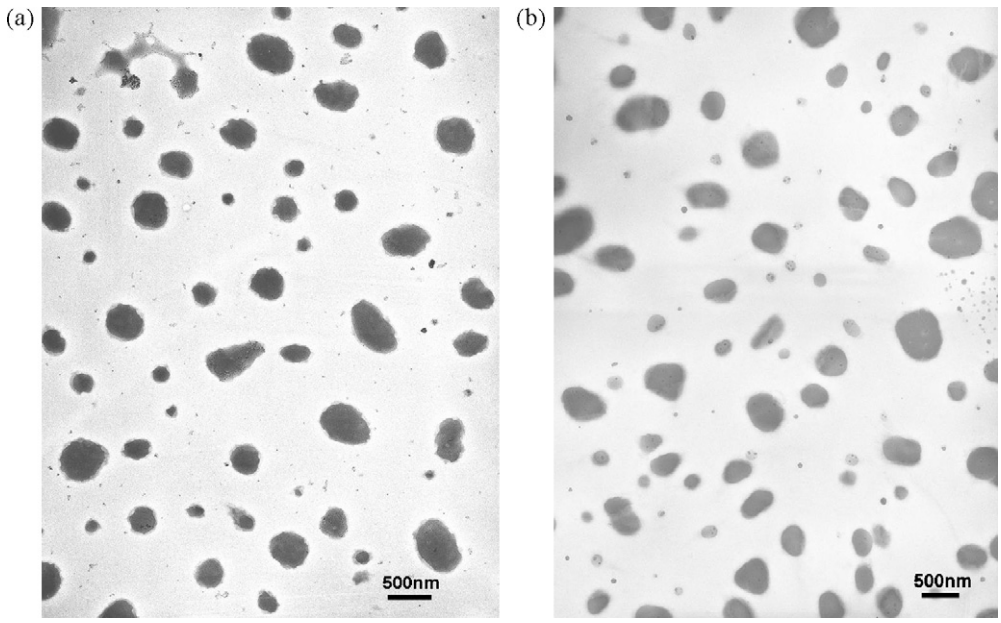


Fig. 6. Transmission electron micrographs of M2-L8K3 micelles after (a) 0, and (b) 8 weeks' degradation in aqueous solution.

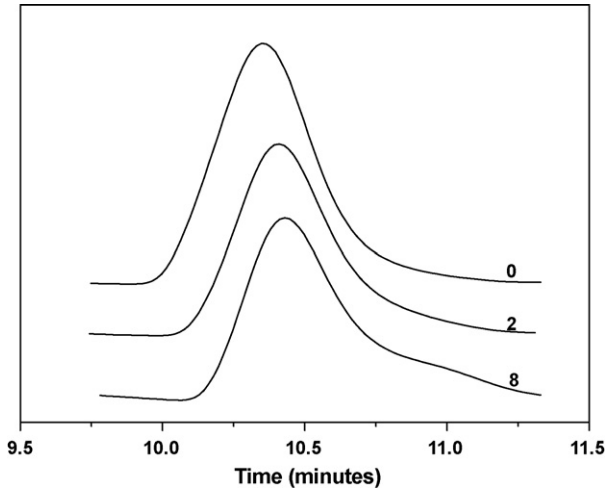


Fig. 7. GPC curves of PLA–PEG copolymers obtained by lyophilizing M1-LD5K3 micelles after 0, 2 and 8 weeks' degradation.

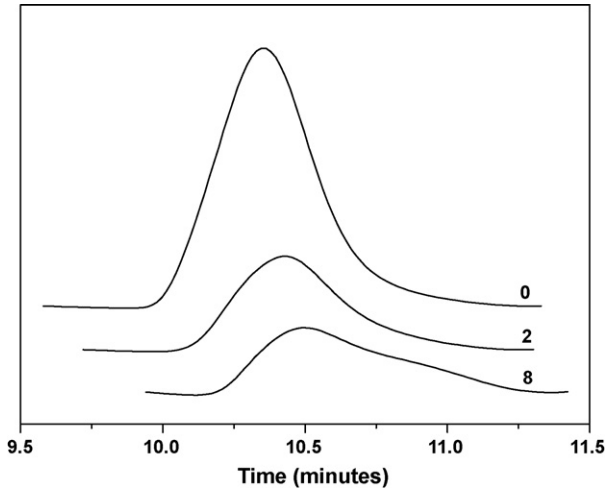


Fig. 8. GPC curves of PLA–PEG copolymers obtained by lyophilizing M2-LD5K3 micelles after 0, 2 and 8 weeks' degradation.

Fig. 10 presents the DSC thermograms of M1-LD5K3 micelles after different degradation time intervals. After the end of the first run, the molten sample was rapidly cooled by immersion in liquid nitrogen. The quenched sample was then scanned in a second run in order to observe the glass transition (T_g) and crystallization (T_c). As degradation proceeds, T_g and T_c of the copolymers decreased. So did the endothermal melting transition (T_m) and melting enthalpy (ΔH_m). Similar trends were found for other micelles (Table 4). It is well known that the T_g of PEG is about −65 °C and that of PLA about 60 °C. The T_g of PLA–PEG micelles appeared between these two values and closer to that of PEG. After hydrolytic degradation, PEG content in the block copolymers increased as shown above, thus leading to a decrease of T_g. In addition, higher PEG content also led to easier crystallization and lower T_c of PEG blocks. On the other hand, the residual PLA short segments remaining in the lyophilized samples disfavored the crystallization of PLA–PEG copolymers, resulting in the decrease of T_m and ΔH_m.

Table 3
Changes of molecular characteristics of PLA–PEG copolymers during degradation.

Sample	M_n[a]	M_w[a]	M_w/M_n[a]	EO/LA[b]
L-mPEG2K3	3020	3280	1.09	3.70
M1-LD2K3-2w[c]	2720	2960	1.09	4.66
M1-LD2K3-8w	2350	2590	1.10	4.68
L-mPEG5K3	5950	6670	1.12	4.27
M1-LD5K3-2w	5050	5690	1.13	4.92
M1-LD5K3-8w	4330	5050	1.17	5.73
M2-LD5K3-2w	4730	5470	1.16	5.22
M2-LD5K3-8w	3470	4210	1.21	5.96
L-mPEG5K4	5870	6470	1.10	6.63
M1-L5K4-2w	5030	5570	1.11	7.68
M1-L5K4-8w	4270	4840	1.13	9.90
M1-LD5K4-2w	4860	5560	1.14	7.89
M1-LD5K4-8w	3880	4620	1.19	10.78
L-PEG8K-L3	9700	11,110	1.15	3.77
M2-L8K3-2w	7260	8690	1.20	4.22
M2-L8K3-8w	4000	4910	1.23	4.75
M2-LD8K3-2w	6390	7880	1.23	4.60
M2-LD8K3-8w	3680	4600	1.25	5.66

[a] Determined by GPC.
[b] Determined by NMR.
[c] Obtained after 2 weeks (2w) degradation.

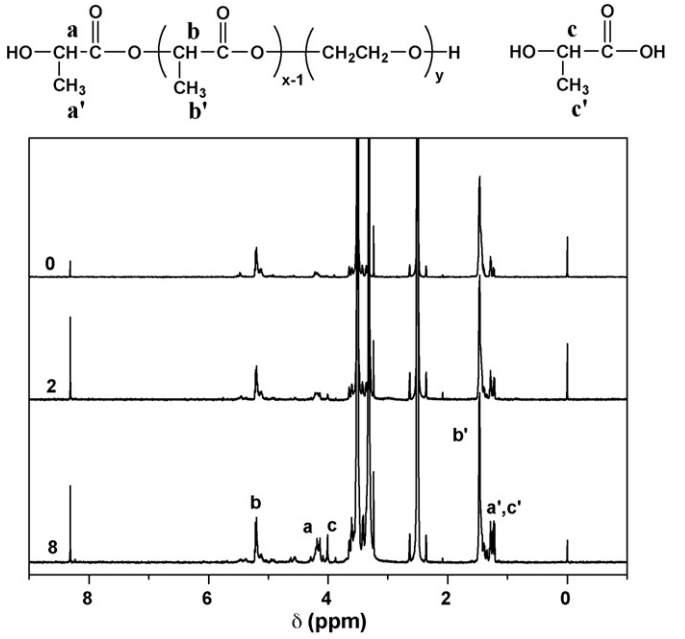


Fig. 9. ^{1}H NMR spectra of PLA–PEG copolymers obtained by lyophilizing M1-LD5K3 micelles after 0, 2 and 8 weeks' degradation.

Based on the above results and discussion, the following degradation mechanisms are proposed for the PLA–PEG micelles prepared by the two different methods. For micelles by dialysis method, in the early degradation stage, the ester bonds in the molecular chains are hydrolyzed to produce various LA oligomers with different molar masses. These short fragments cannot easily diffuse out due to the compact micellar structure, thus the size of the micelles remains stable. While for micelles by direct dissolution method, the structure is much looser and the short segments produced by hydrolysis of ester bonds can easily diffuse out. Afterwards, more and more water could penetrate into the inner core to induce an increase of micelle size, followed by a collapse of the micellar structure thereafter. On the other hand, both micelles by dialysis or derived from L/D mixture possess a more compact structure, and the short LA fragments produced by degradation cannot easily diffuse out. In consequence, carboxyl endgroups are concentrated inside, which could accelerate the cleavage of remaining ester bonds.

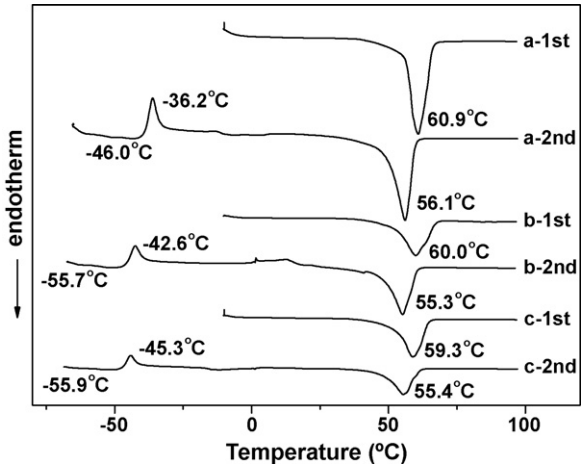


Fig. 10. DSC thermograms of PLA–PEG copolymers obtained by lyophilizing M1-LD5K3 micelles after (a) 0, (b) 2 and (c) 8 weeks' degradation (1st-first heating, 2nd-second heating after quenching).

Table 4
Thermal property changes of PLA–PEG copolymers during degradation.

Sample	T_m (°C)[a]	ΔH_m (J/g)[a]	T_g (°C)[b]	T_c (°C)[b]
L-mPEG2K3	53.3	101.2	−45.5	−33.1
M1-LD2K3-2w[c]	53.2	98.0	−56.6	−44.5
M1-LD2K3-8w	52.1	75.0	−58.5	−49.3
L-mPEG5K-3	60.9	112.5	−46.0	−36.2
M1-LD5K3-2w	60.0	93.1	−55.7	−42.6
M1-LD5K3-8w	59.3	89.0	−55.9	−45.3
M2-LD5K3-2w	59.4	78.6	−56.1	−45.3
M2-LD5K3-8w	56.1	70.5	−56.9	−46.7
L-mPEG5K4	61.2	132.3	−54.5	−43.2
M1-L5K4-2w	58.9	127.3	−55.7	−44.7
M1-L5K4-8w	57.1	102.2	−56.2	−45.4
M1-LD5K4-2w	58.0	123.6	−55.9	−45.9
M1-LD5K4-8w	55.5	94.7	−56.4	−46.4
L-PEG8K-L3	58.5	88.3	−48.4	−33.6
M2-L8K3-2w	55.1	78.6	−48.9	−36.3
M2-L8K3-8w	54.8	74.9	−55.6	−40.3
M2-LD8K3-2w	55.0	75.7	−57.4	−49.4
M2-LD8K3-8w	54.1	71.9	−59.5	−50.3

[a] Determined by the first scanning.
[b] Determined by the second scanning.
[c] Micelles after 2 weeks (2w) degradation.

4. Conclusions

In this work, a series of PLA–PEG block copolymer micelles were prepared by two different methods: direct dissolution and dialysis. The hydrolytic degradation properties of these micelles were investigated by DLS, TEM, GPC, ^{1}H NMR and DSC. During the investigated degradation time (8 weeks), the size of micelles by dialysis remained stable, while that by direct dissolution first increased and followed by a collapse of the structure. This is because the former micelles possess a more compact structure due to the different micellization methods. The composition of PLA–PEG copolymers has much influence on the degradation of micelles. It was found that micelles with longer hydrophobic PLA blocks have a more compact structure which shows less size change during the same degradation time. The structural integrity of L/D mixed micelles could be preserved for longer time than that of single micelles, which is ascribed to the stereocomplexation effect between L-PLA and D-PLA blocks. As degradation proceeds, the average molar mass of copolymers decreases and the distribution becomes wider, especially for micelles by dialysis and L/D mixed micelles which possess more compact structure. This can be attributed to the fact that LA oligomers produced by degradation could not diffuse out easily, thus resulting in concentrated carboxyl endgroups and an acceleration of cleavage of residual ester bonds. As shown by NMR, the PEG content in the copolymer chains increases during degradation, leading to a decrease of T_g and T_c of the copolymers. However, the remaining LA oligomers greatly disfavors the crystallization of PEG blocks, leading to the decrease of T_m and ΔH_m. Due to the constant exchange between copolymer unimers and micelles, PLA–PEG copolymers in a micellar system degrade faster than bulk materials such as nanoparticles or films. In conclusion, this work elucidated the hydrolytic degradation mechanisms of PLA–PEG micelles prepared by two different methods, and the effects of copolymer composition and stereocomplexation on the degradation properties, which are of major importance for the understanding of in vivo release properties of PLA–PEG micelles as drug carriers.

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