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Aggregation behavior of self-assembling polylactide/poly(ethylene glycol) micelles for sustained drug delivery

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ABSTRACT

A series of diblock copolymers were synthesized by ring-opening polymerization of L- or D-lactide in the presence of monomethoxy poly(ethylene glycol) (mPEG) with molar masses of 2000 and 5000. The aggregation behavior of the resulting water soluble PLA/PEG diblock copolymers in aqueous medium was studied with dynamic and static light scattering (DLS and SLS), in combination with aqueous gel permeation chromatography (GPC). The average hydrodynamic radius (R_h) of L-PLA/PEG and D-PLA/PEG mixed micelles is lower than that of L-PLA/PEG single micelles due to the stereocomplexation effect between L-PLA and D-PLA blocks. It is also confirmed that the micelle size increases with increasing temperature and hydrophobic block length, but decreases after salt addition. Aqueous GPC and SLS were used to evaluate the molecular weight (M_w) and aggregation number (N_{agg}) of the micelles. Mixed micelles present lower N_{agg} than single copolymer micelles due to stereocomplexation. N_{agg} decreases with increasing hydrophobic block length, and decreases upon addition of NaCl, in agreement with a more compact structure. In contrast, N_{agg} increases with elevating temperature. The average radius of gyration (R_g) and R_g/R_h ratio data show that both increase with increasing temperature, suggesting that micelles exhibit a compact hard-sphere structure at 15 °C and a swollen structure at 35 °C. As the temperature increases from 15 °C to 35 °C, the second virial coefficient (A_2) of PLA/PEG copolymers turns from negative to positive, which means that water changes from poor solvent to good solvent. The fact that the average density (ρ) of PLA/PEG micelles decreases with increasing temperature confirms that micelles exhibit a looser structure at higher temperatures due to water swelling effect. The higher ρ value of mixed micelles as compared to single micelles also confirms a more compact structure of the former. In addition, due to the much higher N_{agg} and lower R_h, PLA/PEG2000 micelles present higher ρ value than PLA/PEG5000 ones.

1. Introduction

Bioresorbable polylactide/poly(ethylene glycol) (PLA/PEG) block copolymers have been widely investigated as drug delivery carriers in the form of hydrogels, micro- or nanoparticles, and micelles (Burt et al., 1999; Matsumoto et al., 1999; Li and Vert, 2003; Ruan and Feng, 2003). Micelles are very attractive because of the core-shell structure formed through self-assembly of PLA/PEG in aqueous media: bioresorbable PLA blocks aggregate to form an inner core which is able to encapsulate hydrophobic drugs with improved solubility; hydrophilic PEG blocks constitute the hydrated outer shell able to stabilize the micelles in aqueous solution. PEG blocks can prevent the opsonification and subsequent non-specific uptake by the reticuloendothelial system (RES), thus leading to prolonged drug circulation in plasma. The nano-scale size range with a narrow distribution enables the micelles to achieve higher accumulation at the target site through an enhanced permeation retention (EPR) effect (Shuai et al., 2004; Hamaguchi et al., 2005). In addition, polymeric micelles as novel drug vehicles present numerous advantages, including reduced side effects of anticancer drugs, selective targeting, stable storage, etc (Liu et al., 2001; Shuai et al., 2004).

In the past decade, most research has focused on the drug encapsulation and release behavior (Kim et al., 2001; Liggins and Burt, 2002; Jie et al., 2005; Pierri and Avgoustakis, 2005). In contrast, little attention has been paid to the basic micellization properties of PLA/PEG micelles. It is well known that polymeric micelles with a core-shell structure coexist in aqueous solutions with unimers. A dynamic system is formed with permanent exchanges between micelle-forming molecules and free molecules (Hagan et al., 1996; Savic et al., 2006). Thus the aggregation number (N_{agg}) becomes a key parameter for determining the stability and aggregation behavior of polymeric micelles. In literature, dynamic light scattering (DLS) and static light scattering (SLS) are widely used to investi-

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gate the aggregation phenomena of polymers in solution (Forster et al., 1996; Riley et al., 2001; Liu et al., 2004; Nuopponen et al., 2004; Garstens et al., 2005; Garnier and Laschewsky, 2006; Paillet et al., 2009), including the micelle size and size distribution, the hydrodynamic radius (R_h), the average radius of gyration (R_g), the weight-average molecular weight ($M_w^{micelle}$), and the second virial coefficient (A_2). The structure of polymeric micelles, the polymer–solvent interactions, and intra- and interpolymer interactions in dilute solution can be thus determined (Mya et al., 2005; Li et al., 2006; Yao et al., 2008).

In our previous work, self-assembling PLA/PEG micelles were prepared by direct dissolution method without using any organic solvents. The critical micellar concentration (CMC), the structure and morphology of micelles, and the thermodynamic parameters of micellization including enthalpy (ΔH), entropy (ΔS), and free energy (ΔG) were determined (Yang et al., 2007). The negative ΔG and positive ΔS indicate that the micellization process is spontaneous and driven by entropy changes. Paclitaxel was used as a prototype drug to investigate the drug encapsulation and *in vitro* release properties of PLA/PEG micelles. The *in vivo* biodistribution and antitumor efficacy of paclitaxel-loaded micelles were also determined (Yang et al., 2009). All these results showed that PLA/PEG micelles present great potential as hydrophobic drug carriers, especially the L-PLA/PEG and D-PLA/PEG mixed micelles which present higher stability and higher drug loading efficacy due to the stereocomplexation effect. However, the aggregation properties of PLA/PEG diblock copolymers have not been investigated in detail, so far.

In this work, a series of PLA/PEG diblock copolymers were synthesized and characterized. The aggregation properties of the copolymers were investigated by using aqueous gel permeation chromatography (GPC) and light scattering measurements, taking into account the influence of copolymer composition, temperature and electrolyte addition. The results are reported herein in comparison with literature data.

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 and 5000 were supplied by Fluka. Zinc lactate was purchased from Sigma. All organic solvents were of analytic grade and used without further purification.

2.2. Synthesis of PLA/PEG diblock copolymers

PLA/PEG diblock copolymers were synthesized by ring-opening polymerization of L- or D-lactide in the presence of mPEG using low toxic zinc lactate as catalyst, as described previously (Yang et al., 2007, 2009). Briefly, predetermined amounts of PEG and L-lactide or D-lactide were introduced into a polymerization tube, the initial molar ratio of ethylene oxide (EO) to lactate (LA) repeat units (EO/LA) being 3–4. 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

Predetermined amounts of L-PLA/PEG, D-PLA/PEG or their equal molar mixtures were dissolved in distilled water under stirring at room temperature. Homogeneous micellar solutions were obtained with different concentrations.

2.4. Molecular characterization

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 $CDCl_3$ as solvent. Chemical shifts (δ) were given in ppm using tetramethylsilane as an internal reference.

GPC measurements in organic solvent were performed on a Waters 410 apparatus equipped with a RI detector. THF was used as the mobile phase at a flow rate of 1.0 ml/min. A 1.0% (w/v) solution (20 μl) was injected for each analysis. Calibration was accomplished with polystyrene standards (Polysciences, Warrington, PA). GPC measurements in aqueous medium were carried out with a Waters Ultrahydrogel 250 column, Millipore Corporation, connected with a UV detector. Water/methanol (90:10, v/v) containing 0.1 M sodium nitrate was used as the eluent at a flow rate of 1.0 ml/min at room temperature. PEG was used as the calibration standard sample.

2.5. Dynamic and static light scattering

Dynamic and static light scattering (DLS and SLS) measurements were performed with a Brookhaven BI-200 goniometer with vertically polarized incident light of wavelength λ = 488 nm supplied by an argon laser operating at 200 mW. Prior to both DLS and SLS measurements, all the solutions were filtered through 0.22 μm millipore membrane filters.

In DLS measurements, the intensity correlation function with a maximum number of 256 channels was measured by using a BI-9000AT digital autocorrelator. Measurements were made at different temperatures and at an angle of 90°. The non-negatively least-squares (NNLS) algorithm was used to obtain the best-fit values of the parameters. The diffusion coefficient (D) of the micelles was calculated from $\Gamma = Dq^2$, where Γ is the relaxation rate ($\Gamma = 1/\tau$, τ is the relaxation time) and q is the scattering vector determined by the scattering angle θ ($q = 4\pi n/\lambda \sin(\theta/2)$, where n is the refractive index of the liquid medium and λ is the wavelength of the laser). The hydrodynamic radius (R_h) was then evaluated from D by using Stoke–Einstein relationship (Hussain et al., 2008a):

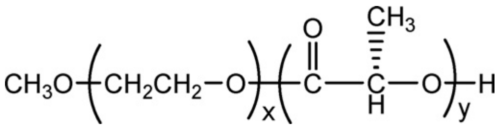
$$R_h = \frac{k_B T}{6\pi\eta_s D} \tag{1}$$

where k_B, T, and η_s are the Boltzmann constant, the absolute temperature, and the viscosity of the solvent, respectively.

SLS measurements were carried out at different scattering angles (50–130°) and different concentrations (0.5–1 g/l) to determine the weight-average molecular weight (M_w), the average radius of gyration (R_g), and the second virial coefficient (A_2) of PLA/PEG copolymer micelles. To obtain the absolute scattering light intensity, toluene was used as a standard. The various parameters can be determined by the angle (θ) and concentration (c) dependence of $Kc/\Delta R_\theta$ according to Zimm plot formulation (Rijcken et al., 2005; Dimitrov et al., 2007; Hussain et al., 2008b; Giacomelli et al., 2009):

$$\frac{Kc}{\Delta R_\theta} = \frac{1}{M_w}\left[1 + \frac{16\pi n^2}{3\lambda^2} R_g^2 \sin^2\left(\frac{\theta}{2}\right)\right] + 2A_2 c \tag{2}$$

where $K = 4\pi^2 n^2 (dn/dc)^2 / N_A \lambda^4$ is the optical parameter with n being the refractive index of the liquid medium, N_A is Avogadro's constant, λ is the wavelength of the laser (488 nm), and ΔR_θ is the excess Rayleigh ratio [$\Delta R_\theta = R_\theta(\text{solution}) - R_\theta(\text{solvent})$], respectively. The value of M_w is estimated by extrapolation of c and θ to



Scheme 1. Chemical structure of PLA/PEG diblock copolymer.

zero, while R_g and A_2 were obtained from the slope of the angle and concentration dependence of Zimm plots, respectively.

3. Results and discussion

3.1. Characterization of PLA/PEG diblock copolymers

PLA/PEG diblock copolymers with PEG molar masses of 2000 and 5000 were synthesized, and characterized by using GPC and ^{1}H NMR. The chemical structure of the copolymers is shown in Scheme 1. Table 1 presents the molar mass, molar mass distribution, and copolymer composition (EO/LA ratios and block lengths). The EO/LA ratios of the copolymers were found to be higher than the feed ratios. This may be assigned to the fact that the conversion of lactide was not complete, and unreacted lactide was eliminated by the purification procedure. All the copolymers are water soluble, the average degree of polymerization DP_{PEG} being much higher than DP_{PLA}. The polydispersity index is inferior to 1.3 in all cases, in agreement with the narrow molar mass distribution.

3.2. DLS measurements

It is well known that polymeric micelles can be formed only when the block copolymer concentration is higher than the critical micellar concentration (CMC) (Kwon and Okano, 1996; Jones and Leroux, 1999). In our previous work, the CMC values of PLA/PEG micelles were obtained from surface tension measurements (Yang et al., 2007). The CMC was found to be 0.05 g/l for copolymers derived from PEG2000 and 0.1 g/l for those derived from PEG5000, respectively, which indicates that the CMC of PLA/PEG diblock copolymers increases with increasing hydrophilic PEG block length. Herein, the DLS and SLS measurements of PLA/PEG copolymer micelles were carried out at concentrations well above their CMC.

Table 2 shows the hydrodynamic radius of PLA/PEG diblock copolymer micelles obtained by DLS measurements in aqueous solutions at a concentration of 1.0 g/l. The distribution diagram of a monomodal peak with relative narrow size distribution indicates that only one size of aggregation was found in solution, which is identified as the polymeric micelles (data not shown). The composition of the copolymers greatly influences the micelle size (Table 2). L-PLA/PEG and D-PLA/PEG mixed micelles present lower radius than single copolymer micelles as reported before (Yang et al., 2007). This finding has been assigned to the more compact structure of mixed micelles due to the stereocomplexation effect between L-PLA and D-PLA blocks. Although with similar EO/LA ratios, micelles formed by PLA/PEG5K-3 are larger than that of PLA/PEG2K-3 copolymers, in which 5K and 2K representing mPEG5000 and mPEG2000, while 3 representing the EO/LA ratio in feed. This indicates that the micelle size increases with increasing hydrophilic PEG block length, which is consistent with literature data (Li et al., 2006). On the other hand, comparison between PLA/PEG5K-3 and PLA/PEG5K-4 micelles shows that copolymers with the same PEG but longer PLA will form micelles with larger size. Riley et al. (2001) have confirmed the relationship between radius and hydrophobic block length. The authors found out that the hydrodynamic radius of the PLA–PEG 2:5–30:5 assemblies scale linearly when plotted against $N_{PLA}^{1/3}$, where N_{PLA} is the number of lactyl units per PLA block. Power laws were used to express the dependence of hydrodynamic radius (R_h) on the number of monomeric units in the core-forming (N_A) and shell-forming (N_B) blocks. It was predicted that when $N_A \gg N_B$, $R_h \propto N_A^{2/3}$; while when $N_B \gg N_A$, $R_h \propto N_A^{4/25} N_B^{3/5}$. The same trend was observed although the compositions of the PLA/PEG copolymers used in our work are not in that given range.

The effect of temperature on the micelle size was determined by DLS measurements from 15 °C to 35 °C. Table 2 shows that the radius increases with increasing temperature in all cases. This finding could be assigned to the fact that at higher temperature, the copolymer molecules exhibit higher mobility with more water

Table 1
Molecular characteristics of PLA/PEG diblock copolymers.

Copolymer[a]	M_{nPEG}	EO/LA[b]	DP_{PEG}[c]	DP_{PLA}[d]	$M_{n(NMR)}$[e]	$M_{n(GPC)}$[f]	$M_{w(GPC)}$[f]	M_w/M_n[f]
L-PLA/PEG2K-3	2000	3.7 (3)	45	12	2860	2370	2840	1.2
D-PLA/PEG2K-3	2000	4.2 (3)	45	11	2790	2310	2770	1.2
L-PLA/PEG5K-3	5000	4.5 (3)	113	25	6800	5050	6490	1.3
D-PLA/PEG5K-3	5000	4.3 (3)	113	26	6870	5110	6630	1.3
L-PLA/PEG5K-4	5000	6.3 (4)	113	18	6300	4890	6080	1.2
D-PLA/PEG5K-4	5000	6.3 (4)	113	18	6300	4860	6150	1.3

[a] The numbers at the end represent the EO/LA ratios in feed.

[b] 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] $M_n = M_{nPEG} + DP_{PLA} \cdot 72$.

[f] Determined by GPC.

Table 2
Hydrodynamic radius (R_h) and polydispersity (PD) of PLA/PEG diblock copolymer micelles.

Sample	15 °C R_h (nm)	PD	25 °C R_h (nm)	PD	35 °C R_h (nm)	PD
L-PLA/PEG2K-3	58.3 ± 2.1[b]	0.17	62.9 ± 1.5	0.15	72.1 ± 2.8	0.18
L/D-PLA/PEG2K-3[a]	49.2 ± 1.4	0.16	58.5 ± 2.3	0.18	65.6 ± 1.1	0.13
L-PLA/PEG5K-3	65.4 ± 2.9	0.13	72.8 ± 2.5	0.15	88.6 ± 3.7	0.17
L/D-PLA/PEG5K-3	56.8 ± 2.4	0.13	64.4 ± 3.2	0.20	78.8 ± 3.1	0.16
L-PLA/PEG5K-4	64.8 ± 2.1	0.12	69.4 ± 3.6	0.21	83.4 ± 3.9	0.19
L/D-PLA/PEG5K-4	53.0 ± 2.4	0.13	60.6 ± 2.9	0.13	69.6 ± 2.4	0.12

[a] L/D-Mixed micelles prepared by dissolving equal molar L- and D-PLA/PEG copolymers in water.

[b] Data represent mean value ± S.D., $n = 3$.

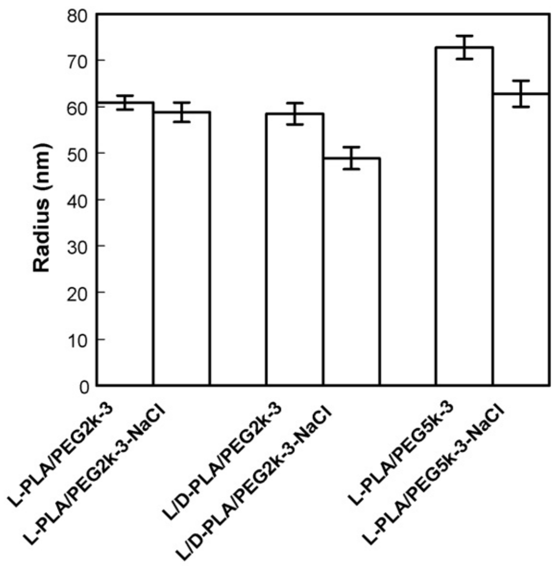


Fig. 1. Electrolyte addition effect on the size of PLA/PEG micelles, as evaluated from DLS measurements of micelle aqueous solutions containing 0.15 M NaCl.

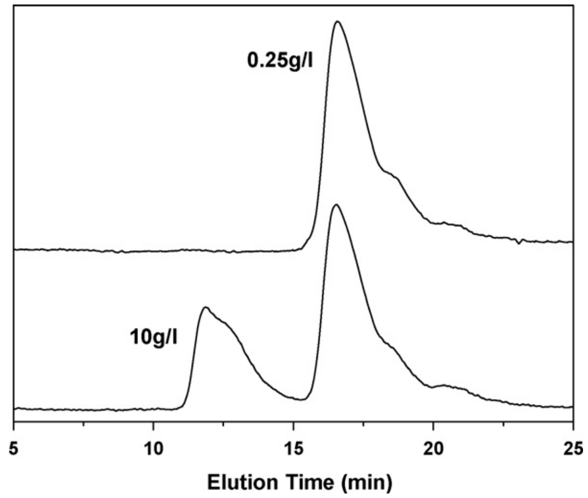


Fig. 2. Aqueous GPC graphs of l-PLA/PEG5K-3 with concentrations of 0.25 g/l and 10 g/l. Only one peak corresponding to the copolymer is observed at 0.25 g/l. In contrast, two peaks are detected at 10g/l, corresponding to the micelles and unimers, respectively.

engaged in the hydrodynamic micelle structure, thus leading to enlarged micelle size. The influence of electrolytes on the size of micelles was evaluated by addition of 0.15 M NaCl in the aqueous micelle solutions. As shown in Fig. 1, the micelle size decreases after salt addition, in agreement with a more compact structure of micelles due to the electrolyte effect (Hagan et al., 1996; Colombani et al., 2007). However, this effect is rather limited due to the non-ionic nature of the copolymers. In fact, there is little difference between the CMC values of the copolymers in pure water and in NaCl solutions (Yang et al., 2007).

3.3. Aggregation number of PLA/PEG micelles

Aqueous GPC was used to evaluate the average molecular weight of the micelles (La et al., 1996; Inoue et al., 1998; Kim et al., 1998; Iijima et al., 1999; Schilli et al., 2004). Each aqueous solution of PLA/PEG copolymers was injected with two concentrations: 0.25 g/l, which would become lower than the CMC after dilution in the eluent; and 10 g/l, which could be high enough to remain above the CMC even after dilution. Fig. 2 shows the aqueous GPC graphs of L-PLA/PEG5K-3. At 0.25 g/l, only one peak at M_w of 7680 is observed, corresponding to the PLA/PEG copolymer. In contrast, two peaks are detected at 10 g/l, namely one in the high molecular weight region with M_w of 292,000 and one at the same elution time as the peak observed at 0.25 g/l. It is believed that the peak with high molecular weight at 10 g/l corresponds to the micelles formed by PLA/PEG copolymers. Then the aggregation number (N_{agg}) of the micelles can be obtained by using the following equation:

$$N_{agg} = \frac{M_w^{micelle}}{M_w^{copolymer}} \tag{3}$$

On the other hand, Zimm plot can be applied to micellar systems for the determination of molecular weight from SLS measurements. The molecular weight of micelles is obtained instead of unimers. The N_{agg} is then calculated according to Eq. (3) as in the case of aqueous GPC, where the average molecular weight of the copolymer unimers was determined by ^{1}H NMR (Tanodekaew et al., 1997; Jeong et al., 1999). All the results derived from SLS and aqueous GPC are summarized in Table 3.

It is noteworthy that aqueous GPC-derived M_w data were based on PEG standards and thus slightly different from the results derived from SLS. Comparing the aggregation number of different micelles in Table 3, it appears that mixed micelles formed by L-PLA/PEG and D-PLA/PEG copolymers have lower N_{agg} than single L-PLA/PEG copolymer micelles. In fact, the mixed micelle presents a more compact structure due to strong interactions between L-PLA and D-PLA blocks. Consequently, fewer molecular chains are needed to aggregate and to form a micelle. This finding is in contrast to the work reported by Kang et al. The authors found that mixed micelles were characterized by a higher N_{agg} than those obtained from single ones (Kang et al., 2005). This difference could be ascribed to the different micelle structures. In Kang's work, micelles were prepared by solvent (acetone) evaporation method, and PLA chains crystallized in the inner core. Thus PLA crystallites in stereocomplexes are stabilized by strong van der Waals interactions and adopt a more compact conformation than enantiomeric crystals, allowing more molecular chains and denser polymeric packing. Whereas in our work, polymeric micelles were prepared by direct dissolution method, and the structure is not as compact as that by solvent evaporation method. The much shorter PLA blocks cannot crystallize and thus excess copolymer chains are squeezed out of the micelles due to the interactions between L-PLA and D-PLA blocks.

On the other hand, with similar EO/LA ratio, the PLA/PEG5K-3 copolymer micelles with higher PEG block length present much lower N_{agg} than PLA/PEG2K-3 micelles. This finding can be assigned to the longer hydrophobic blocks of PLA/PEG5K-3. It is well known that amphiphilic copolymer micelles are formed due to thermodynamic interactions between hydrophobic blocks. PLA/PEG5K-3 copolymers present stronger aggregation capability due to longer hydrophobic blocks, thus leading to lower N_{agg}. The same phe-

Table 3
Average molecular weight and aggregation number of PLA/PEG diblock copolymer micelles obtained by aqueous GPC and SLS at 25 °C.

Sample	Aqueous GPC		SLS	
	M_w	N_{agg}	M_w	N_{agg}
L-PLA/PEG2K-3	409,000	105	285,000	100
L/D-PLA/PEG2K-3	381,000	97	239,000	84
L-PLA/PEG5K-3	292,000	38	174,000	26
L/D-PLA/PEG5K-3	215,000	33	121,000	18
L-PLA/PEG5K-4	231,000	63	251,000	40
L/D-PLA/PEG5K-4	184,000	46	157,000	25

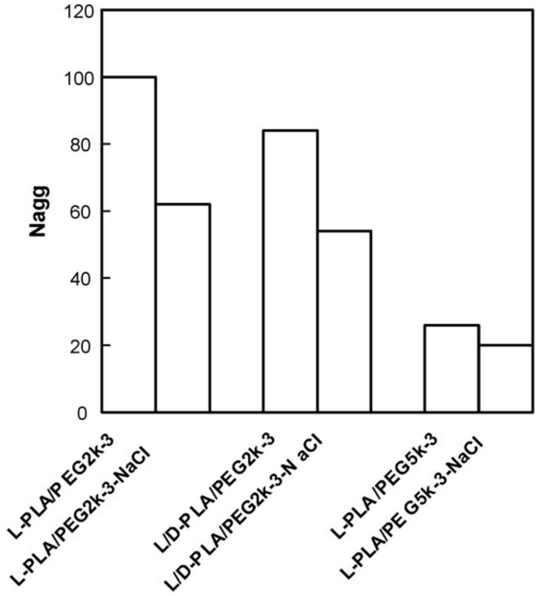


Fig. 3. Electrolyte addition effect on the aggregation number of PLA/PEG micelles as evaluated from SLS measurements of micelle aqueous solutions containing 0.15 M NaCl.

nomenon is observed when comparison is made between the two kinds of PLA/PEG5K micelles: higher N_{agg} is obtained for PLA/PEG5K-4 copolymers with shorter hydrophobic blocks. It is noteworthy that the decrease in N_{agg} for amphiphilic block copolymer with increasing hydrophobic block length has been previously reported (Li et al., 2006; Hussain et al., 2008a).

The effect of electrolyte addition on the N_{agg} of the micelles was also evaluated by SLS measurements. As shown in Fig. 3, the N_{agg} of micelles decreased after addition of 0.15 M NaCl in the solution. This is because salt addition results in a more compact micelle structure containing fewer molecular chains, as mentioned above. Figs. 4 and 5 present the effect of temperature on the N_{agg} of polymeric micelles determined by SLS measurements from 15 °C to 35 °C. The N_{agg} increases with elevating temperature since higher temperature favors chain mobility and micelle formation, which is consistent with literature data (Zhou and Chu, 1988; Wanka et al., 1990; Brown et al., 1991; Alexandridis and Hatton, 1995; Liu et al., 1998; Altinok et al., 1999; Dimitrov et al., 2006; Pioge et al., 2009). In fact, the equilibrium between unimers and micelles can be described by a closed association model with a dynamic association–dissociation process. With increasing temperature, molecular chain mobility is improved and thus more copolymer molecules can be incorporated into the micelles, leading to higher N_{agg} (Cau and Lacelle, 1996).

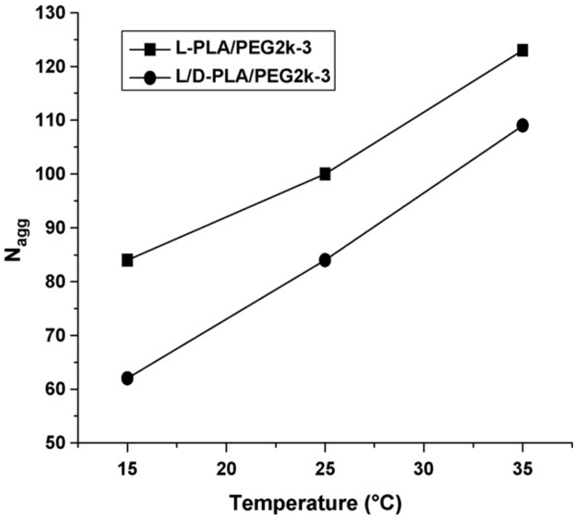


Fig. 4. N_{agg} variation of PLA/PEG2K-3 single or mixed micelles as a function of temperature.

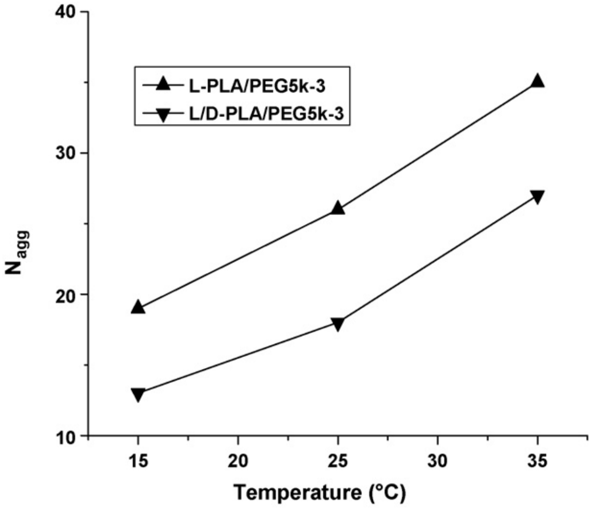


Fig. 5. N_{agg} variation of PLA/PEG5K-3 single or mixed micelles as a function of temperature.

3.4. Structure of PLA/PEG micelles determined by SLS

It is well established that the ratio of R_g/R_h is a characteristic parameter related to the conformation of polymer chains. The values of R_g/R_h for hard-sphere micelles, random coils, and rod-like structures have been reported to be 0.78, 1.78, and ≥2, respectively (Li et al., 2006; Hussain et al., 2008a). However, it is also found that the R_g/R_h value for spherical micelles with swollen shells equals to 1.3 (Dimitrov et al., 2007). The calculated R_g and R_g/R_h ratio at various temperatures are regrouped in Table 4. Both R_g and R_g/R_h increase with increasing temperature. At 15 °C, the R_g/R_h ratios of the various micelles are close to 0.78, which means that the micelles at this temperature exhibit a compact hard-sphere structure. When the temperature is elevated to 35 °C, the R_g/R_h values increase to about 1.30, revealing that the micelles present a swollen structure with water penetrating into the inner core. The micelles present R_g/R_h values in the range of 0.78–1.30 at 25 °C, in agreement with the intermediate structure from hard-sphere to looser structure due to water swelling effect (Yao et al., 2008).

The second virial coefficient (A_2) evaluated by SLS measurements is often used to identify the interactions between polymers and solvents. In the case of copolymer micelles, A_2 can be used as an indication of micellar interactions in solution. A negative value of A_2 means strong interactions among solute particles, indicating that the solute is in a poor solvent. The magnitude of A_2 values denotes the strength of such interactions. The variation of A_2 with temperature depends on the hydrophilic/hydrophobic ratio of the block copolymers (Liu et al., 1998; Jeong et al., 1999; Castro et al., 2008). Table 4 shows that, as the temperature increases from 15 °C to 35 °C, the A_2 value of PLA/PEG micelles turns from slightly negative to slightly positive. This means that at lower temperatures, water is a poor solvent for PLA/PEG copolymers. The strong interactions between polymer chains could lead to a more compact micellar structure with hard-sphere configuration as discussed above. While at higher temperatures, water becomes a good solvent for copolymers. The micelles will be in a more swollen state,

Table 4
Radius of gyration (R_g), R_g/R_h ratio, second Virial coefficient (A_2) and average density (ρ) of PLA/PEG copolymer micelles estimated by SLS measurement at various temperatures.

Sample	Temperature (°C)	R_g (nm)	R_g/R_h	A_2 (mol·cm^3/g^2)	ρ (g/cm^3)
L-PLA/PEG2K-3	15	46.4 ± 2.5[a]	0.80	-2.00×10^{-3}	4.81×10^{-4}
	25	70.6 ± 4.4	1.16	3.04×10^{-6}	4.54×10^{-4}
	35	93.3 ± 3.4	1.29	2.75×10^{-3}	3.72×10^{-4}
L/D-PLA/PEG2K-3	15	40.6 ± 4.8	0.82	-2.60×10^{-3}	5.91×10^{-4}
	25	68.5 ± 3.3	1.17	2.90×10^{-6}	4.74×10^{-4}
	35	83.1 ± 5.9	1.31	2.42×10^{-3}	4.38×10^{-4}
L-PLA/PEG5K-3	15	51.2 ± 2.8	0.78	-1.88×10^{-3}	1.83×10^{-4}
	25	84.0 ± 4.8	1.15	4.08×10^{-6}	1.78×10^{-4}
	35	115.6 ± 3.3	1.31	3.62×10^{-3}	1.36×10^{-4}
L/D-PLA/PEG5K-3	15	42.2 ± 2.5	0.74	-1.96×10^{-3}	1.91×10^{-4}
	25	72.7 ± 1.8	1.11	2.97×10^{-6}	1.80×10^{-4}
	35	108.1 ± 2.3	1.37	2.71×10^{-3}	1.49×10^{-4}

and more polymer chains will join the micelles so as to keep the micellar stability by increased hydrophobic interactions between PLA blocks, thus leading to higher R_h and N_{agg}. It is of interest to compare these findings with the behavior of PEG homopolymers. In fact, water is good solvent of PEG at lower temperatures, and becomes poor solvent at higher temperatures because of loss of polarity (Mansur et al., 1997; Letchford et al., 2004). The attachment of hydrophobic PLA blocks apparently changes the behavior of the copolymers in aqueous media. Similar findings have been observed for diblock and triblock copolymers (Liu et al., 1997; Castro et al., 2008).

The average density (ρ) of PLA/PEG diblock copolymer micelle was calculated from $M_w^{micelle}$ and R_h according to the following equation (Mya et al., 2005; Li et al., 2006; Yao et al., 2008):

$$\rho = \frac{3M_w^{micelle}}{4\pi N_A R_h^3} \tag{4}$$

where N_A is Avogadro constant. The ρ value decreases with increasing temperature, as shown in Table 4. This finding can be assigned to the swelling effect, i.e. more water molecules could penetrate into the micelles at higher temperatures as discussed above, leading to a looser structure and lower density. On the other hand, the mixed copolymer micelles present higher ρ value than single L-PLA/PEG micelles, which suggests that mixed micelles exhibit a more compact structure due to stereocomplexation effect between L-PLA and D-PLA blocks. In addition, comparison of the two series of micelles with different PEG blocks shows that PLA/PEG2K micelles present higher ρ values than PLA/PEG5K ones, which can be assigned to the much higher N_{agg} and lower R_h of the former.

4. Conclusion

Bioresorbable polymeric micelles can be obtained from water soluble PLA/PEG diblock copolymers by direct dissolution method. The hydrodynamic radius (R_h) and aggregation number (N_{agg}) of L-PLA/PEG and D-PLA/PEG mixed micelles were found to be lower than those of L-PLA/PEG single micelles due to the stereocomplexation effect between L-PLA and D-PLA blocks. The R_h value increases with increasing hydrophobic block length or temperature and decreases upon salt addition. In contrast, the N_{agg} decreases with hydrophobic block length increase or electrolyte addition, and increases with elevating temperature. The gyration radius (R_g) and R_g/R_h ratio data indicate that micelles change from hard-sphere to swollen structure as the temperature increases from 15 °C to 35 °C. Meanwhile, the second virial coefficient (A_2) of micelles turns from slightly negative to slightly positive, showing that water changes from poor solvent to good solvent for PLA/PEG as temperature increases. The average density (ρ) of micelles decreases with increasing temperature. The mixed micelles present higher ρ values, in agreement with a more compact structure. Finally, PLA/PEG2K micelles with lower copolymer molecular weight present higher ρ values due to much higher N_{agg} and lower R_h in comparison with PLA/PEG5K micelles. In conclusion, amphiphilic PLA/PEG diblock copolymers present interesting aggregation behaviors. The elucidation of the effect of various influential factors on the aggregation property and stability of the micelles should be very helpful potential drug delivery applications.

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