

# Micellization and Gelation of Aqueous Solutions of Star-Shaped PLLA–PEO Block Copolymers

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**ABSTRACT:** Nontoxic and biodegradable star-shaped poly(L-lactide-*b*-ethylene oxide) (star PLLA–PEO) block copolymers were synthesized by the coupling reaction of two reactive precursors, a hydroxy-terminated 3-armed poly(L-lactide) (star PLLA) and  $\alpha$ -monocarboxy- $\omega$ -monomethoxypoly(ethylene oxide) (CMPEO). The chemical structure and physical properties of the resulting star-block copolymers were characterized. In the dilute aqueous solutions of star-block copolymers, micellization behavior was investigated, and over specific concentration and specific temperature, the unimer to micelle transition occurred. 1,6-Diphenyl-1,3,5-hexatriene (DPH) was solubilized in the micelle core, and the absorbance of DPH at 356 nm increased when micelle was formed. In addition, the effective diameter was highly affected by temperature. With increasing temperature, micelle size decreased dramatically. In high-concentration solution, star-block copolymer showed temperature-sensitive sol–gel transition behavior. Over specific concentration, the whole system cannot flow by the packing of micelles. However, with increasing temperature, packing structure was destroyed by the decrease of the micelle volume due to the dehydration and contraction of the PEO chain, and the system flew. In the sol–gel transition phase diagram obtained by the vial tilting method, the critical gel concentration decreased, the boundary curve shifted to the left, and the gel regions were expanded with increasing the molecular weight of PLLA block and PEO block.

## Introduction

Temperature-sensitive micellization and gelation behaviors of block copolymers in aqueous solution have attracted significant attention.<sup>1–9</sup> Especially, the sol–gel transition of Pluronic, which is poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) (PEO–PPO–PEO), in aqueous solution has been studied intensively by many researchers varying the molecular weight and block ratio with using various instruments including UV spectrophotometer,<sup>2</sup> light scattering,<sup>3</sup> small-angle neutron scattering,<sup>4</sup> differential scanning calorimeter,<sup>5</sup> cryo-TEM,<sup>6</sup> dielectric measurement,<sup>7</sup> rheometer,<sup>8</sup> and atomic force microscope.<sup>9</sup>

In the dilute aqueous solution of Pluronic, a unimer to micelle transition occurs over the critical micelle concentration (cmc) and/or over the critical micelle temperature (cmt). This behavior is mainly due to the dehydration and aggregation of the PPO block while the PEO block is still hydrated and remained in a soluble state.<sup>2</sup> It is generally accepted that the aggregated hydrophobic PPO formed the core of the micelle while the hydrophilic PEO remained outside and formed the shell; however, Malmsten et al. concluded that there was a modest miscibility of the PPO and PEO segment, and there was no complete segregation.<sup>10,11</sup>

Over the critical gel concentration (cgc) and over the critical gel temperature (cgt), the fluidity of the system dramatically decreased, and a sol to gel transition (lower boundary curve) occurred.<sup>12,13</sup>

It has been proved by SANS (small-angle neutron scattering) and SAXS (small-angle X-ray scattering)

study that this transition is due to the arrangement of micelles with special long-range order.<sup>14–16</sup>

The aqueous solution of Pluronic showed not only sol to gel transition (lower boundary curve) but also gel to sol transition behavior (upper boundary curve) over the critical gel concentration and the other specific temperature due to the dehydration of PEO block.<sup>12</sup> Finally, the polymer was precipitated in water with further increase of temperature and reaching the forth critical temperature, called the lower critical solution temperature (LCST) of Pluronic.<sup>13</sup>

It has been proposed that aqueous solution of Pluronic can be used as an injectable drug carrier for both hydrophilic and hydrophobic drugs.<sup>17</sup> However, Pluronic has disadvantages due to its nondegradability, toxic side effect, and dissolution of the gel after administration.<sup>18,19</sup> Kim et al. studied biodegradable poly(ethylene oxide-*b*-L-lactic acid) (PEO–PLLA) diblock and poly(ethylene oxide-*b*-L-lactic acid-*b*-ethylene oxide) (PEO–PLLA–PEO) triblock copolymers with fixed PEO chain length ( $M_n = 5000$ ).<sup>20,21</sup> These block copolymers showed temperature-sensitive micellization and gelation behavior in aqueous solution, though the lower boundary curve of the sol to gel transition of Pluronic was not shown. The cmc of these block copolymers was investigated with UV/vis spectrophotometer by the dye solubilization method, and gel to sol transition behavior was investigated by the tube inversion method.

In our previous report,<sup>22</sup> we briefly introduced the synthesis and characterization of biodegradable, nontoxic, and temperature-sensitive star-shaped PLLA–PEO (poly(L-lactide-*b*-ethylene oxide)) with 3-armed star architecture with PEO fixed chain length ( $M_n = 5000$ ). The gel to sol transition behavior was investigated and briefly compared with that of the conventional linear block copolymer.

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In this work, we synthesized and characterized star-shaped PLLA-PEO with PEO molecular weights of 2000 and 5000, and their temperature-sensitive micellization and gelation behavior was investigated with a UV/vis spectrophotometer, dynamic light scattering, and the vial tilting method.

## Experimental Section

**Materials.** L-Lactide (Aldrich) was recrystallized from ethyl acetate. Monomethoxypoly(ethylene oxide) (MPEO, Aldrich) with molecular weights of 5000 and 2000 was dried by azeotropic distillation with benzene. Glycerol (Aldrich; spectroscopic grade, 99.5+%), stannous 2-ethylhexanoate (Stannous octoate, St-oct, Sigma), triethylamine (TEA, Acros, Belgium; 99.7%, H<sub>2</sub>O ≤ 0.06%), 4-(dimethylamino)pyridine (DMAP, Aldrich; 99%), succinic anhydride (Aldrich; 99+%), and 1,3-dicyclohexylcarbodiimide (DCC, Aldrich) were used as received. For the reaction media, anhydrous grade of 1,4-dioxane (Aldrich; 99.8%, H<sub>2</sub>O < 0.003%) and methylene chloride (MC, Aldrich; 99.8%, H<sub>2</sub>O < 0.001%) were used without purification. All other reagents were of reagent grade and used as received.

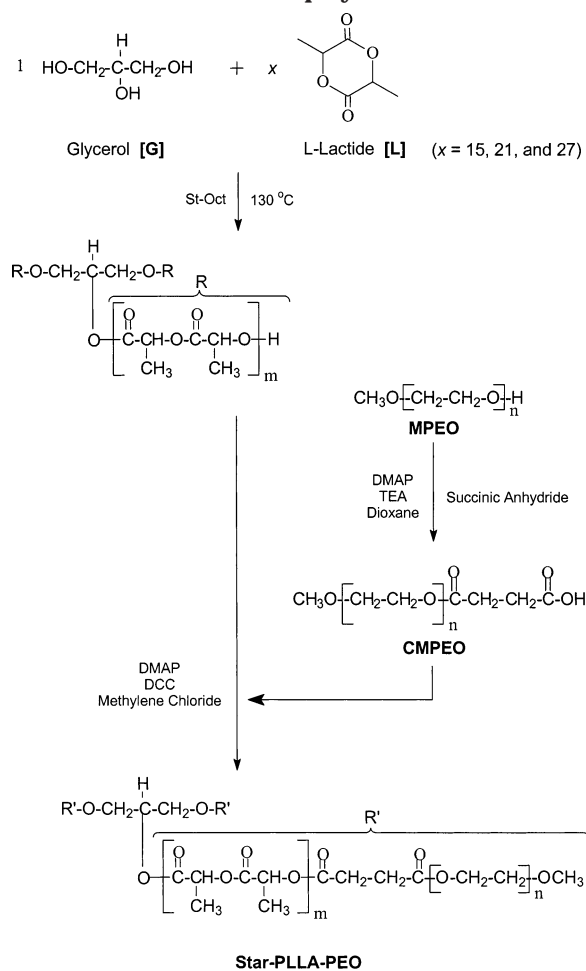
**Synthesis.** A star-shaped block copolymer was formed from two reactive precursors, hydroxy-terminated 3-armed poly(L-lactide) and  $\alpha$ -monocarboxy- $\omega$ -monomethoxypoly(ethylene oxide) (CMPEO). First, star poly(L-lactide) (star PLLA) was synthesized by bulk ring-opening polymerization of L-lactide using glycerol as a trifunctional initiator and stannous 2-ethylhexanoate (stannous octoate, St-oct) as a catalyst.<sup>23–25</sup> Second, dried monomethoxypoly(ethylene oxide) (MPEO) was carboxylated with succinic anhydride to produce CMPEO.<sup>26,27</sup> Finally, 1 mol of hydroxy-terminated star PLLA was coupled with 3 mol of carboxy-terminated CMPEO using 1,3-dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP) to produce 1 mol of star PLLA-PEO block copolymer. The reaction scheme of star PLLA-PEO is shown in Scheme 1.

**Synthesis of Star-Shaped PLLA.**<sup>23–25</sup> L-Lactide (30 g, 208 mmol), glycerol (0.913 g, 9.9 mmol), and St-oct (0.422 g, 1.04 mmol) were added into a round-bottomed flask and reacted in melt at 130 °C for 6 h under argon. The product was dissolved in chloroform, microfiltered through a 0.45  $\mu$ m pore membrane filter, and precipitated in *n*-hexane twice. The residual solvent was eliminated by vacuum at 55 °C overnight, and white powder star PLLA7 was obtained. The number of moles of L-lactide added per 1 mol of glycerol ([L]/[G]) was varied with 15, 21, and 27 to produce star PLLA5, 7, and 9, respectively. Theoretically, star PLLA5 has on average five lactidyl units (monomeric unit of lactide) in the one arm of star PLLA, and star PLLA7 and star PLLA9 have on average seven and nine lactidyl units in the one arm of star PLLA. In all cases, the molar ratio of L-lactide to St-oct ([L]/[St-oct]) was fixed at 200.

**Synthesis of CMPEO.**<sup>26,27</sup> MPEO (MW = 5000, 40 g, 8 mmol), succinic anhydride (0.961 g, 9.6 mmol), DMAP (0.977 g, 8 mmol), and triethylamine (TEA, 0.81 g, 8 mmol) were dissolved in 240 mL of anhydrous dioxane and stirred for 24 h at room temperature. The solvent was evaporated with a rotary evaporator; the residue was taken up in carbon tetrachloride, filtered, and precipitated in ethyl ether twice. After drying in vacuo overnight, white powder CMPEO5K was obtained. MPEO2K was carboxylated by the same procedure to produce CMPEO2K.

**Synthesis of Star-Shaped PLLA-PEO.** Star PLLA7 (6.24 g, 2 mmol), CMPEO5K (30.8 g, 6.04 mmol), DCC (1.246 g, 6.04 mmol), and DMAP (0.148 g, 1.208 mmol) were dissolved in 200 mL of anhydrous methylene chloride and reacted at room temperature for 24 h under dry nitrogen. The reaction byproduct dicyclohexylcarbodiurea (DCU) was removed by filtration and then precipitated in ethyl ether twice. The obtained star PLLA7-PEO5K was purified by solvent extraction using ethyl ether and benzene cosolvent. Star PLLA7-PEO2K was synthesized by the same procedure with using

**Scheme 1. Synthetic Scheme of Star PLLA-PEO Block Copolymer**



CMPEO2K. The resulting star-shaped block copolymers were denoted as follows: star PLLA $m$ -PEO $n$ K, where  $m$  (5, 7, or 9) is the number of repeat units of lactide and  $n$  (2 or 5) is the molecular weight ( $K = 1000$ ) of CMPEO both in one arm of star-block copolymer. The synthesized reactive precursors and star-block copolymers in this study are shown in Table 1.

**Characterization.** The structure of synthesized star PLLA, CMPEO, and star PLLA-PEO block copolymers were confirmed by <sup>1</sup>H and <sup>13</sup>C NMR that were recorded by a Bruker AMX 500 spectrometer with CDCl<sub>3</sub>-*d* (Aldrich) solvent.

Gel permeation chromatography (GPC; Waters 150C) was used to obtain number-average molecular weight ( $M_n$ ), weight-average molecular weight ( $M_w$ ), and polydispersity index (PDI). The eluent was tetrahydrofuran with 1 mL/min flow rate. For calibration, monodisperse polystyrene standards of which MW were 52 000, 22 000, 9200, 5460, 1060, and 580 were used.

The thermal properties of synthesized polymers were studied by using a differential scanning calorimeter (TA DSC 2010) connected with a liquid nitrogen cooling accessory (LNCA). The heating and cooling rate was 10 °C/min, and the scan range was from -40 to 200 °C. The middle of the step change in heat capacity was determined as glass transition temperature ( $T_g$ ), and the endotherm peak was determined as melting temperature ( $T_m$ ), in the second heating run.

The aqueous solution properties of star PLLA-PEO block copolymers were studied with a UV/vis spectrophotometer and dynamic light scattering in dilute solution (less than 1 wt % solution) and the vial tilting method in concentrated solution (more than 5 wt % solution).

The critical micelle temperature (cmt) values of star PLLA-PEO block copolymers in aqueous solution were determined employing the hydrophobic dye solubilization method using 1,6-diphenyl-1,3,5-hexatriene (DPH, Aldrich). DPH was dis-

**Table 1.** Molecular Weights of Star PLLAs, CMPEOs, and Star PLLA-PEOs

sample	theor $M_n$ (g/mol)	NMR $M_n$ (g/mol)	GPC			PEO content (wt %)
			$M_n$ (g/mol)	$M_w$ (g/mol)	PDI <sup>a</sup>	
star PLLA5	2252	2280	3400	4700	1.38	
star PLLA7	3116	3140	4940	6710	1.36	
star PLLA9	3980	4000	6540	8200	1.25	
CMPEO2K	2100	2010	3120	3370	1.08	
CMPEO5K	5100	5060	7510	8340	1.11	
star PLLA5-PEO2K	8552	8570	10320	12120	1.18	70.2
star PLLA7-PEO2K	9416	9500	11480	14190	1.23	63.7
star PLLA9-PEO2K	10280	12960	11760	15230	1.29	58.4
star PLLA5-PEO5K	17552	17310	16690	22190	1.32	85.5
star PLLA7-PEO5K	18416	18110	17090	23870	1.39	81.4
star PLLA9-PEO5K	19280	18880	18600	24550	1.32	77.8

<sup>a</sup> Polydispersity index ( $M_w/M_n$ ).

solved in methanol to produce 0.5 mM DPH methanol solution. Copolymer aqueous solutions were prepared by dissolving 0.2 g of polymer in 19.8 g of Milli-Q water (18 Mohm cm) to produce 1 wt % solution that was placed at 80 °C for 20 min and diluted to the desired concentration (ranging from 1 to 0.0005 wt %). In a quartz cell, 2.4 mL of copolymer solution, 24  $\mu$ L of DPH solution (0.5 mM), and a small spin bar were added and capped with Teflon. To prevent the evaporation of water during the measurement, the sample cell was sealed with Teflon film and Parafilm. The final copolymer solution contained 1% v/v methanol and 0.005 mM DPH. The samples were stored in a dark place to equilibrate for 1 h 30 min at 10 °C with stirring before measurement. UV/vis absorption spectra of the samples were recorded in the 300–600 nm range using a Jasco V-530 UV/vis spectrophotometer. The temperature range employed was 10–60 °C. Temperature control was achieved using an ETC-505T thermostat system (Jasco), and the temperature was raised in 5 °C per each step with 15 min equilibrium time before the measurement.

The effective diameters of micelle of star-block copolymers in aqueous solution were measured by dynamic light scattering (DLS, Brookhaven Instruments BI-200SM) with a 532 nm laser source with varying temperature from 10 to 80 °C. The temperature was increased by 5 °C per each step and was controlled within  $\pm 0.1$  °C in a water bath, and the samples were immersed for 20 min before each measurement to reach thermal equilibrium. To minimize the effect of the stray light, a 90° angle was chosen for measurement. Copolymer aqueous solutions were prepared by the same method as the UV/vis spectrophotometer measurement described above. The aqueous solutions of star-block copolymers were filtered by a 0.45 or 0.2  $\mu$ m syringe filter and put in a glass cell with a polypropylene cap and sealed with Teflon film to prevent evaporation of water. The sample cell was positioned at the center of brass thermostat block filled with refractive index matching fluid, decaline. The digital autocorrelator BI9000AT was used for data analysis. The effective diameter of formed micelle of star PLLA-PEO block copolymer in aqueous solution was calculated by the following Stokes–Einstein relationship.

$$d = \frac{k_B T}{3\pi\eta_0 D}$$

where  $D$  is the diffusion constant,  $k_B$  is Boltzmann's constant,  $T$  is absolute temperature in K,  $\eta_0$  is the solvent viscosity, and  $d$  is the diameter of the particle that a solid sphere may possess to have the same diffusion constant as the particle producing the scattering.

The sol–gel transition behaviors of aqueous polymer solution were investigated by the vial tilting method. Star PLLA-PEO block copolymers were dissolved in ultrapure water to make 0.5 g of aqueous copolymer solution in tightly capped 5 mL vials at 80 °C for 20 min. The block copolymer solutions were kept at 4 °C for 12 h prior to measurement. The temperature increased by 2 °C per each step, and samples were immersed for 20 min at measuring temperature. The sol–gel

transition temperature was determined by tilting the vials with 90° for 1 min. If there is no flow, it was regarded as a gel state.

## Results and Discussion

L-Lactide ( $x$  mol per glycerol mol;  $x = 15, 21$ , and  $27$ ) was polymerized by the ring-opening mechanism using nontoxic glycerol as a trifunctional initiator to yield biodegradable nontoxic star PLLA5, -7, and -9, which have average 5, 7, and 9 monomeric units in one arm of star PLLA. The  $^1\text{H}$  NMR spectra of star PLLA7, CMPEO5K, and star PLLA7-PEO5K with peak assignment and  $^{13}\text{C}$  NMR spectra are presented in Figure 1 and Figure 2, respectively. It was found by  $^1\text{H}$  NMR of star PLLA7 (Figure 1a) that each hydroxy group of glycerol initiated polymerization of L-lactide, specifically by the disappearance of  $\text{G-CH}_2\text{OH}$  (3.54, and 3.65 ppm) and  $\text{G-CHOH}$  (3.71 ppm) that were shifted to 4.35–4.20 and 5.16 ppm, respectively.<sup>24</sup> The molecular weights of star PLLAs were calculated from the peak integral ratio between 4.35 and 4.20 ppm ( $d$  and  $b'$  peak;  $\text{G-CH}_2$  and  $\text{L-CH-OH}$ ) and 1.55 ppm ( $a$  peak;  $\text{L-CH}_3$ ) by the following equation.

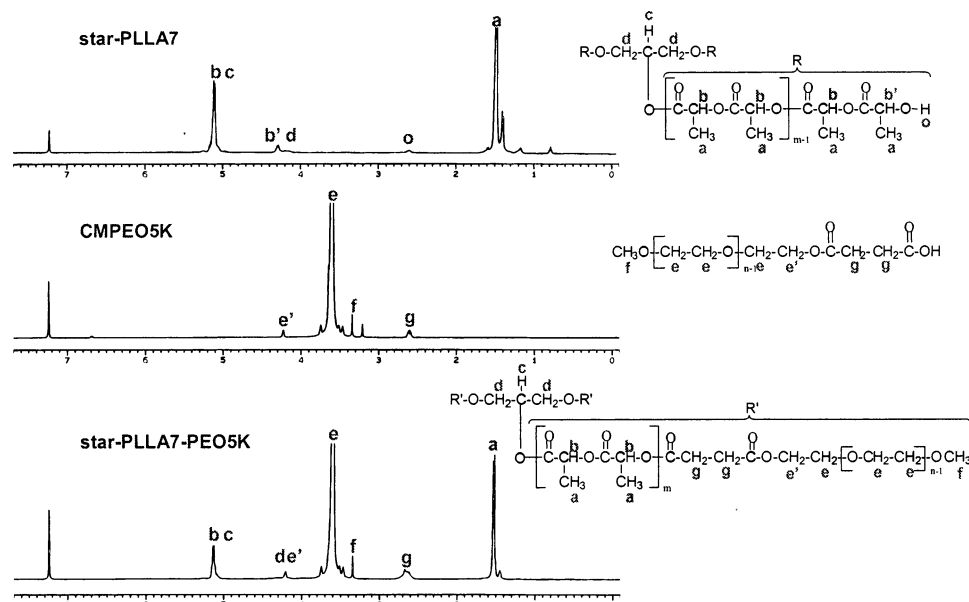
$$A_a: A_{b',d} = 3 \times 6 \times m : 7$$

$$M_n = 3 \times (144 \times m) + 92$$

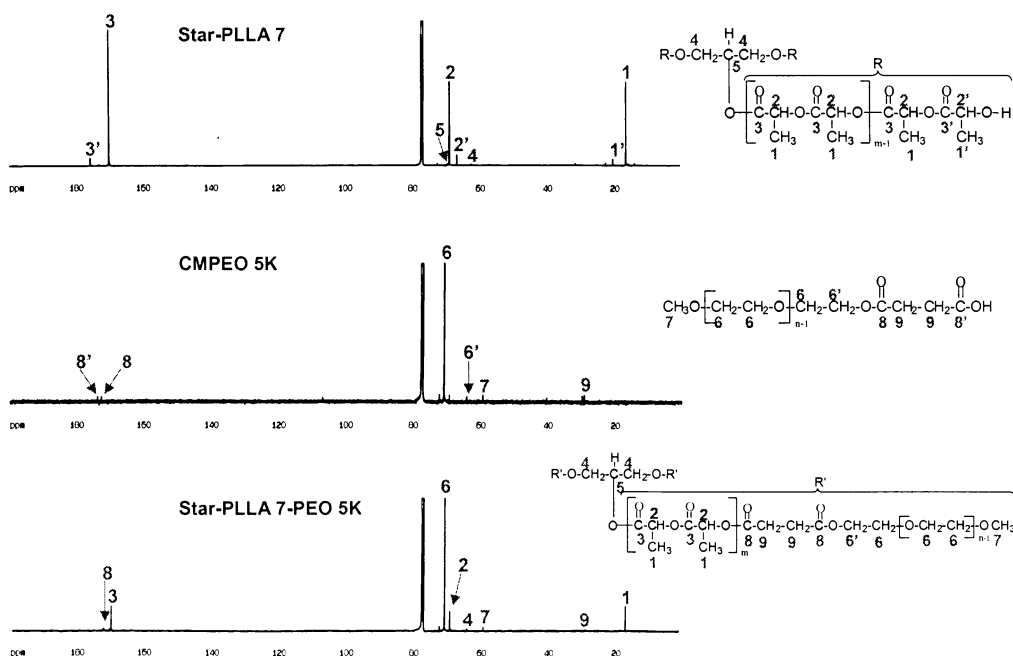
where  $A_a$  and  $A_{b',d}$  are the area of peaks at 1.55 and 4.35–4.20 ppm, respectively, and  $m$  is the calculated average number of monomeric units in one arm. The calculated molecular weights of star PLLAs are listed in Table 1 and are compared with the theoretical value and those obtained by GPC. In  $^{13}\text{C}$  NMR spectra of star PLLA (Figure 2a), it is confirmed that all the hydroxy group of glycerol initiated polymerization of L-lactide by the disappearance of the peak of  $\text{G-CH}_2\text{OH}$  (66.47 ppm) and  $\text{G-CHOH}$  (76.4 ppm) which were shifted to 62.5 and 69.8 ppm, respectively. The peak of L-lactide are assigned as peaks 1, 2, and 3 for  $\text{L-CH}_3$  (16.6 ppm),  $\text{L-CH}$  (69.0 ppm), and  $\text{L-Cd=O}$  (169.5 ppm). The carbon peaks next to the end hydroxyl group were shifted to 20.4 (peak 1'), 66.6 (2'), and 175 ppm (3'), respectively.

The successful coupling of MPEO to CMPEO<sup>26,27</sup> was found specifically by the appearance of the peak of methylene proton of PEO that was next to the ester linkage at 4.23 ppm ( $e'$ ;  $-\text{OCH}_2\text{CH}_2\text{OCO}-$ ) (Figure 1b). The ratio between the peak of 3.35 ppm ( $f$ ;  $-\text{OCH}_3$ ) and 4.23 ppm ( $e'$ ;  $-\text{OCH}_2\text{CH}_2\text{OCO}-$ ) was equal to 3:2. This observation demonstrates that all of the hydroxy groups of the MPEO were substituted by succinic anhydride.





**Figure 1.**  $^1\text{H}$  NMR spectra of (a) star PLLA7, (b) CMPEO5K, and (c) star PLLA7-PEO5K



**Figure 2.**  $^{13}\text{C}$  NMR spectra of (a) star PLLA7, (b) CMPEO5K, and (c) star PLLA7-PEO5K

The molecular weight of CMPEO was calculated from the ratio of integration of peak at 3.35 ppm ( $f$ ,  $-\text{OCH}_3$ ) and 3.61 ppm ( $e$ ,  $-\text{OCH}_2\text{CH}_2\text{O}-$ ) by the following equation, and the results are shown in Table 1.

$$A_f : A_e = 3 : (4 \times n) - 2$$

$$M_n = (44 \times n) + 132$$

where  $A_f$  and  $A_e$  are the area of peak at 3.35 and 3.61 ppm, respectively, and  $n$  is the average number of monomeric units in CMPEO.

The coupling of star PLLA and CMPEO was found to be successful, specifically by the disappearance of  $\text{L-CH-OH}$  ( $o$  peak; 2.66 ppm) in  $^1\text{H}$  NMR (Figure 1c) and the disappearance of the  $1'$ ,  $2'$ , and  $3'$  peaks in  $^{13}\text{C}$  NMR (Figure 2c). It was concluded that all of the hydroxy group in PLLA were reacted with the carboxy group of CMPEO. The molecular weights of star-block

copolymers were calculated by the following equation.

$$A_f : A_e = 3 \times 3 : 3 \times ((4 \times n) - 2)$$

$$M_{n,\text{PEO}} = (44 \times n) + 115$$

$$A_f : A_a = 3 \times 3 : 3 \times (6 \times m)$$

$$M_{n,\text{PLLA}} = 3 \times (144 \times m)$$

$$M_{n,\text{star PLLA-PEO}} = 3 \times M_{n,\text{PEO}} + M_{n,\text{PLLA}} + 89$$

where  $A_f$ ,  $A_e$ , and  $A_a$  are the integration of peaks at 3.35, 3.61, and 1.55 ppm in  $^1\text{H}$  NMR spectra of star PLLA-PEO, respectively.  $M_{n,\text{star PLLA-PEO}}$ ,  $M_{n,\text{PEO}}$ , and  $M_{n,\text{PLLA}}$  are  $M_n$  of star-block copolymer,  $M_n$  of PEO block in star-block copolymer, and  $M_n$  of PLLA block in star-block copolymer, respectively.

**Table 2.**  $T_g$  and  $T_m$  of Star PLLAs, CMPEOs, and Star PLLA-PEOs

sample	DSC		
	$T_g$ (°C)	$T_{mL}^a$ (°C)	$T_{mE}^b$ (°C)
star PLLA5	35.2	116.3	
star PLLA7	38.6	127.6	
star PLLA9	41.0	129.2	
CMPEO2K			55.6
CMPEO5K			62.7
star PLLA5-PEO2K			52.8
star PLLA7-PEO2K			52.4
star PLLA9-PEO2K			50.5
star PLLA5-PEO5K			60.3
star PLLA7-PEO5K			59.9
star PLLA9-PEO5K			59.5

<sup>a</sup>  $T_m$  of PLLA or PLLA block in star-block copolymer. <sup>b</sup>  $T_m$  of PEO or PEO block in star-block copolymer.

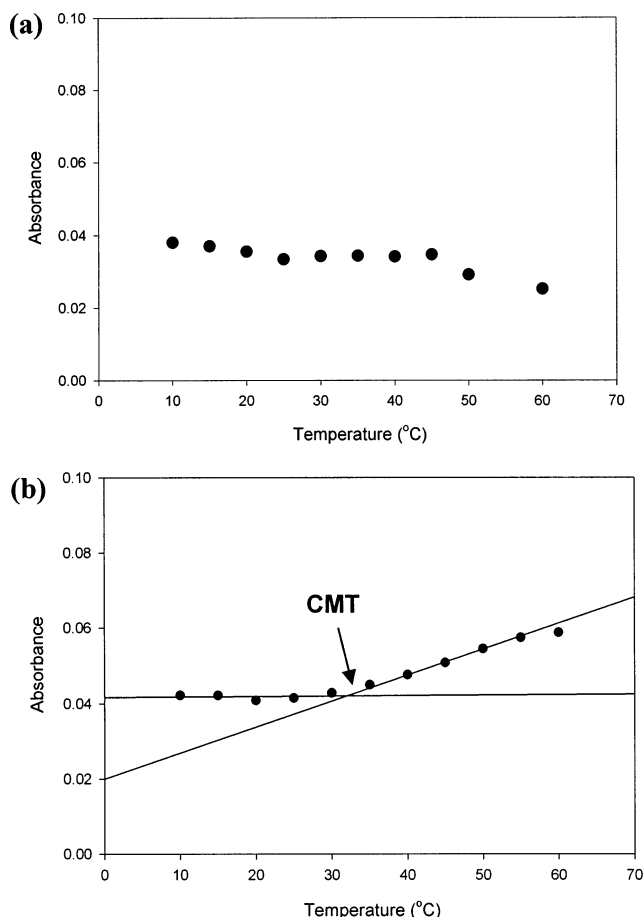
The molecular weights that were calculated from the feed molar ratio, calculated from the ratio of  $^1\text{H}$  NMR peak area, and obtained by GPC are listed in Table 1. The molecular weight calculated from  $^1\text{H}$  NMR matches well with theoretical value for all polymer samples. However, the molecular weights of star PLLAs, CMPEOs, and star PLLA-PEOs that are obtained by GPC were a little bit different from those calculated theoretically, possibly because molecular weights are overestimated by using monodisperse polystyrene standards. The PDI of star PLLA, CMPEO, and star-block copolymers was around 1.3, 1.1, and 1.18–1.39, respectively.

In star-shaped PLLA, the glass transition temperature ( $T_g$ ) and melting temperature ( $T_m$ ) that are measured by DSC decreased with decreasing the molecular weight (see Table 2). The  $T_g$  of star PLLA9 ( $M_n = 3980$ ) was 41 °C, and the  $T_m$  was 129.2 °C. The  $T_g$  and  $T_m$  of star PLLA7 was 38.6 and 127.6, and those of PLLA5 were 35.2 and 116.3.

In star-block copolymers, the  $T_g$  and  $T_m$  of the PLLA block were not found possibly because the PLLA content was relatively low (15–42%). The  $T_m$  of CMPEO2K was 55.6 °C, whereas those of PEO block in star PLLA5-PEO2K, star PLLA7-PEO2K, and star PLLA9-PEO2K decreased to 52.8, 52.4, and 50.5 °C, respectively. The same behavior was observed in star PLLA-PEO5K series. The  $T_m$  of CMPEO5K was 62.7 °C, whereas those of PEO block in star PLLA5-PEO5K, star PLLA7-PEO5K, and star PLLA9-PEO5K decreased to 60.3, 59.9, and 59.5 °C, respectively. It is thought that the partial phase mixing reduced the  $T_m$  of PEO block. This result is consistent with other results.<sup>21</sup>

Alexandridis et al.<sup>2</sup> determined the critical micellization temperature (cmt) and critical micellization concentration (cmc) values of 12 Pluronic block copolymers employing the dye solubilization method. They added 1,6-diphenyl-1,3,5-hexatriene (DPH) as a probe molecule to the Pluronic aqueous solution, and the absorbance was measured by a UV/vis spectrophotometer. Below the cmc, the absorbance of DPH in copolymer solution was the same as that in water in the absence of surfactant. Above the cmc, however, the absorbance increased sharply as the total micelle concentration increased and the amount of solubilized DPH in the hydrophobic micelle interior increased.

In our experiment, we determined the cmt of star PLLA-PEO block copolymer in aqueous solution by measuring the absorbance of DPH with a UV/vis spectrophotometer. As a control, 24 mL of pure water and 24  $\mu\text{L}$  of DPH were added into a quartz cell, and the



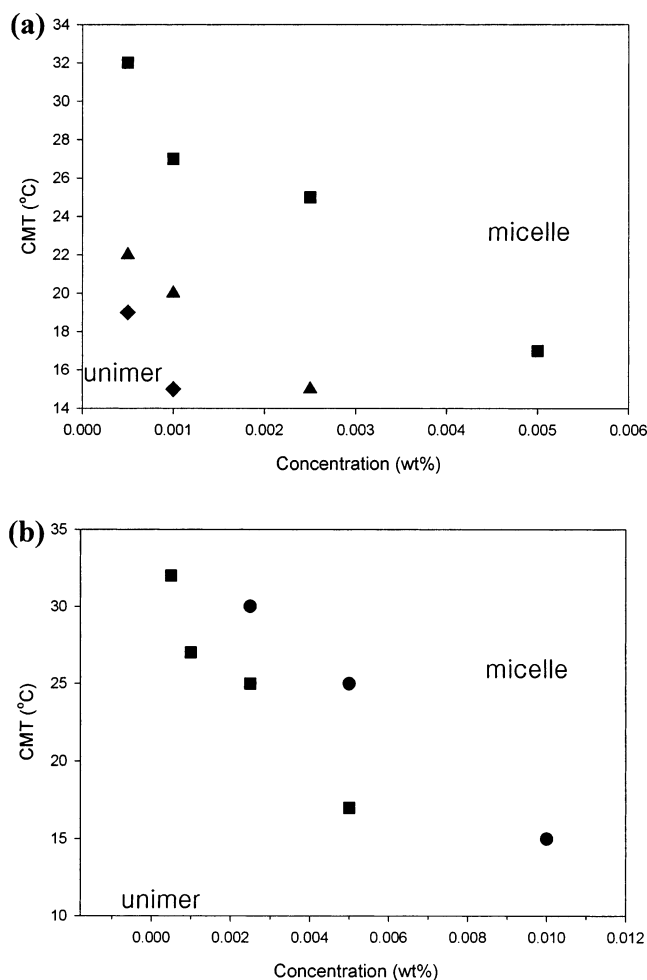
**Figure 3.** (a) Absorption intensity selected at 356 nm for DPH in pure water. (b) Absorption intensity at 356 nm for DPH in a 0.005 wt % solution of star PLLA5-PEO2K. The estimation of the critical micellization temperature from the data is indicated.

absorbance was measured with varying temperature with the experimental procedure mentioned previously. The absorption intensities at 356 nm are selected and are shown as a function of temperature in Figure 3a because those are the spectral maximum due to DPH in the measured absorbance spectra (300–600 nm, which is not shown). Absorbance of control at 356 nm was 0.04 and remained constant as temperature was increased.

However, the absorption intensity of star-block copolymers was highly affected by both temperature and concentration. In Figure 3b, the absorbance selected at 356 nm of DPH in aqueous solution of star PLLA5-PEO2K at the concentration of 0.0005 wt % are presented as a function of temperature. The absorbance was almost constant at low temperature; however, they start to increase over 30 °C. It is thought that while the hydrophobic PLLA block aggregated and formed core, the hydrophilic PEO chains existed outside and formed the shell of the micelle. The cmt was determined at the onset point of the increase of absorption intensity at 356 nm. For 0.0005 wt % solution, the absorbance at 356 nm was 0.042 until 30 °C; however, it started to increase with further increase of temperature. Similar behavior was observed at 0.001, 0.0025, and 0.005 wt % solutions (which are not shown). Above 0.01 wt % (which are not shown), the absorbance, even at 10 °C, showed a large value enough to observe micelle formation. The cmt values obtained for star PLLA5-PEO2K,

Table 3. Cmt and Standard Free Energy of Micellization of Star PLLA-PEO

sample	concn (wt %)	cmt (°C)	concn (mol/L)	concn (mol fr)	$\Delta G^\circ_{\text{micelle}}$ (kJ/mol)
star PLLA5-PEO2K	0.0005	32	$5.5 \times 10^{-7}$	$9.0 \times 10^{-9}$	-47.00
	0.0010	27	$1.2 \times 10^{-6}$	$2.1 \times 10^{-8}$	-44.12
	0.0025	25	$2.9 \times 10^{-6}$	$5.2 \times 10^{-8}$	-41.58
	0.0050	17	$5.8 \times 10^{-6}$	$1.1 \times 10^{-7}$	-38.65
star PLLA7-PEO2K	0.0005	22	$5.3 \times 10^{-7}$	$9.0 \times 10^{-9}$	-45.46
	0.0010	20	$1.1 \times 10^{-6}$	$1.9 \times 10^{-8}$	-43.33
	0.0025	15	$2.7 \times 10^{-6}$	$4.7 \times 10^{-8}$	-40.43
star PLLA9-PEO2K	0.0005	19	$4.9 \times 10^{-7}$	$8.0 \times 10^{-9}$	-45.29
	0.0010	15	$9.7 \times 10^{-7}$	$1.7 \times 10^{-8}$	-42.86
star PLLA5-PEO5K	0.0025	30	$1.4 \times 10^{-6}$	$2.5 \times 10^{-8}$	-44.12
	0.0050	25	$2.8 \times 10^{-6}$	$5.1 \times 10^{-8}$	-41.63
	0.0100	15	$5.7 \times 10^{-6}$	$1.0 \times 10^{-7}$	-38.62



**Figure 4.** (a) The cmt of aqueous solution of star PLLA-PEO2K series: star PLLA5-PEO2K (■), star PLLA7-PEO2K (▲), and star PLLA9-PEO2K (◆). (b) The cmt of star PLLA5-PEO2K (■) and star PLLA5-PEO5K (●).

star PLLA7-PEO2K, star PLLA9-PEO2K, and star PLLA5-PEO5K are listed in Table 3.

The cmt values for star PLLA5-PEO2K, star PLLA7-PEO2K, and star PLLA9-PEO2K, which have the same hydrophilic (PEO) segment and varying hydrophobic (PLLA) segment, are plotted in Figure 4a as a function of concentration. The upper right regions are the micelle phase, and the lower left regions are the unimer phase. The boundary curve shifted to the left and downward with increasing PLLA molecular weight in the star PLLA-PEO2K series, indicating that star-block copolymers with a larger hydrophobic block form micelles at lower concentrations.

The cmt values for star PLLA5-PEO2K and star PLLA5-PEO5K, which have the same hydrophobic (PLLA) segment and varying hydrophilic (PEO) segment, are plotted in Figure 4b. The boundary curve was shifted to the right and upward with increasing the shell block molecular weight. The tendency of micelle formation decreased as the shell block molecular weight increased. This indicates that the micelle formation is more difficult as the block copolymer hydrophilicity increases. These results are consistent with that of the Pluronic block copolymers.<sup>2</sup> However, the star PLLA-PEO block copolymer formed micelles at much lower concentrations (0.005 wt % at 25 °C for star PLLA5-PEO5K) than Pluronics (0.695 wt % at 25 °C for Pluronic F127, which is PEO4400-PPO3800-PEO4400),<sup>2</sup> possibly because of the higher hydrophobicity of PLLA block than PPO block.

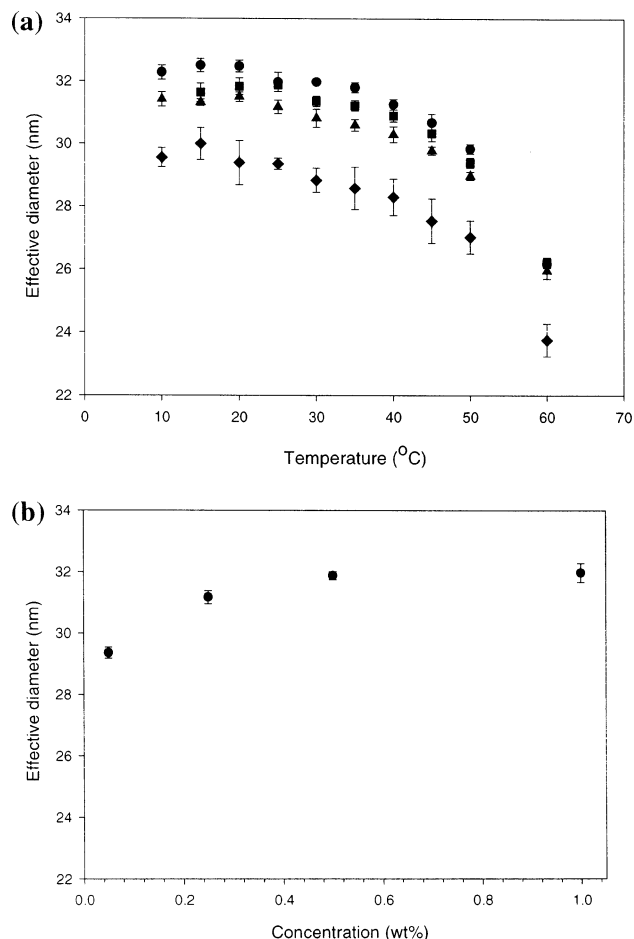
The free energies of micellization of star-block copolymers are also shown in Table 3. In the case of nonionic surfactant, there is no electrostatic interactions, and the standard free energy change for the transfer of 1 mol of amphiphilic block copolymer from solution to the micellar phase is given by the following equation:<sup>2</sup>

$$\Delta G^\circ_{\text{micelle}} = RT \ln(X_{\text{cmc}})$$

where  $R$  is the gas constant,  $T$  is the absolute temperature, and  $X_{\text{cmc}}$  is the critical micelle concentration in mole fraction.

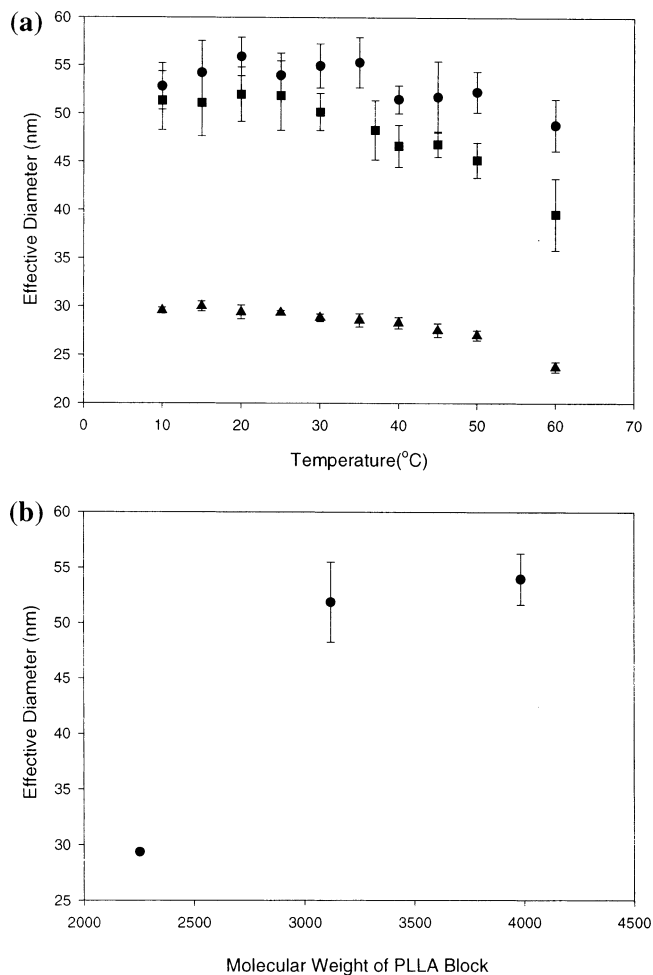
Star-block copolymers have standard free energies with negative values, which imply that the micellization of them in aqueous solution is spontaneous.

Alexandridis et al.<sup>2</sup> calculated standard free energies, enthalpies, and entropies of micellization for various Pluronics. The standard enthalpy of micellization was positive value for all Pluronics (e.g., 253 kJ/mol for Pluronic F127 at 1 wt % solution), indicating that the transition of unimers to the micelle is an enthalpically unfavorable endothermic process. The free energy was negative (e.g., -27.5 kJ/mol for Pluronic F127 at 1 wt % solution), indicating spontaneous micelle formation. Therefore, it was concluded that a negative entropy contribution must be the driving force for micellization of Pluronics (0.944 kJ/(mol K) for Pluronic F127 at 1 wt % solution). The higher values of standard free energies of micellization were observed for relatively hydrophobic Pluronics while the lower values for the relatively hydrophilic ones. In our study, the standard free energy of star PLLA9-PEO2K (-45.29 kJ/mol at 0.0005 wt %) was higher value than that of star PLLA5-PEO2K (-47.00 kJ/mol at 0.0005 wt %) because the former is more hydrophobic.



**Figure 5.** (a) Effective diameter of micelle of star PLLA5-PEO5K at 1.0 (●), 0.5 (■), 0.25 (▲), and 0.05 wt % (◆) aqueous solution measured by DLS as a function of temperature. Each data point represents an average  $\pm$  standard deviation ( $n = 3$ ). (b) Effective diameter of micelle of star PLLA-PEO5K at various concentrations selected at 25 °C.

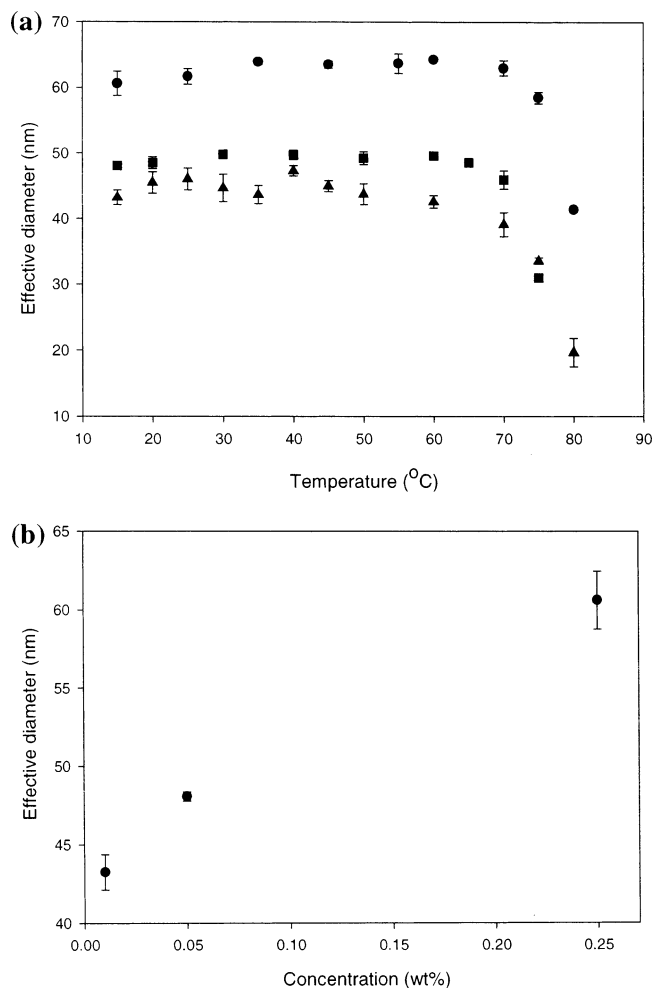
The effective diameters of micelles of star PLLA5-PEO5K in 0.05, 0.25, 0.5, and 1.0 wt % aqueous solution were measured with dynamic light scattering, and the results are presented as a function of temperature in Figure 5a. In 0.05 wt % aqueous copolymer solution, the measured micelle size was about 29.5 nm at 10 °C, and the curve makes a plateau at the low-temperature region; however, it starts to decrease with further increasing temperature and reached 24 nm at 60 °C. Similar behavior was observed for higher concentrations as 0.25, 0.5, and 1 wt % aqueous copolymer solutions. The size of the micelles was around 32 nm at low temperature; however, they started to decrease above 25 °C and reached about 26 nm at 60 °C. The sizes of micelles of star PLLA5-PEO5K block copolymer showed dramatic temperature dependency possibly due to the partial dehydration of PEO block with the elevation of temperature though complete dehydration did not occur at that temperature. Hydrophilic interaction of PEO and water is stronger at lower temperatures; however, hydrophobic interaction between PEO intermolecular chains is stronger at higher temperatures. The precipitation temperature due to the complete dehydration was not measured in this study; however, that of linear triblock copolymer of PLLA and PEO, for example PEO5K-PLLA5K-PEO5K, was 103 °C in 5–35 wt % aqueous solution.<sup>21</sup>



**Figure 6.** (a) Effective diameter of micelle of star PLLA9-PEO5K at 0.005 wt % (●), star PLLA7-PEO5K at 0.005 wt % (■), and star PLLA5-PEO5K at 0.05 wt % (▲) aqueous solution measured by DLS as a function of temperature ( $n = 3$ ). (b) Effective diameter of micelle of star PLLA-PEO5K with various PLLA block molecular weight selected at 25 °C.

The effective diameter of micelle of star PLLA5-PEO5K selected at 25 °C is shown as a function of concentration in Figure 5b. The effective diameter of star-block copolymer micelle was almost not affected by the concentration of the copolymers within the investigated concentration range 0.05–1 wt %. It is likely that the number of micelle increased with increasing concentration whereas the size of the micelle remained constant.

In Figure 6a, the effective diameters of micelle of star PLLA7-PEO5K and star PLLA9-PEO5K at 0.005 wt % are presented and compared with that of star PLLA5-PEO5K at 0.05 wt % to see the effect of core PLLA block molecular weight. The effect of the concentration can be neglected in star PLLA-PEO5K series (see Figure 5). The micelle sizes of star PLLA7-PEO5K and star PLLA9-PEO5K were around 52 and 56 nm at low temperature and decreased with increasing temperature and reached 40 and 49 nm at 60 °C, respectively. Figure 6b shows the effect of core molecular weight on micelle size at 25 °C. It seems that the core block with higher molecular weight constitutes a larger core of micelle so the size of micelle was larger. It is concluded that micelle size increases as hydrophobic core molecular weight increased when they have the same shell block molecular weight though their temperature-dependent behavior is similar.

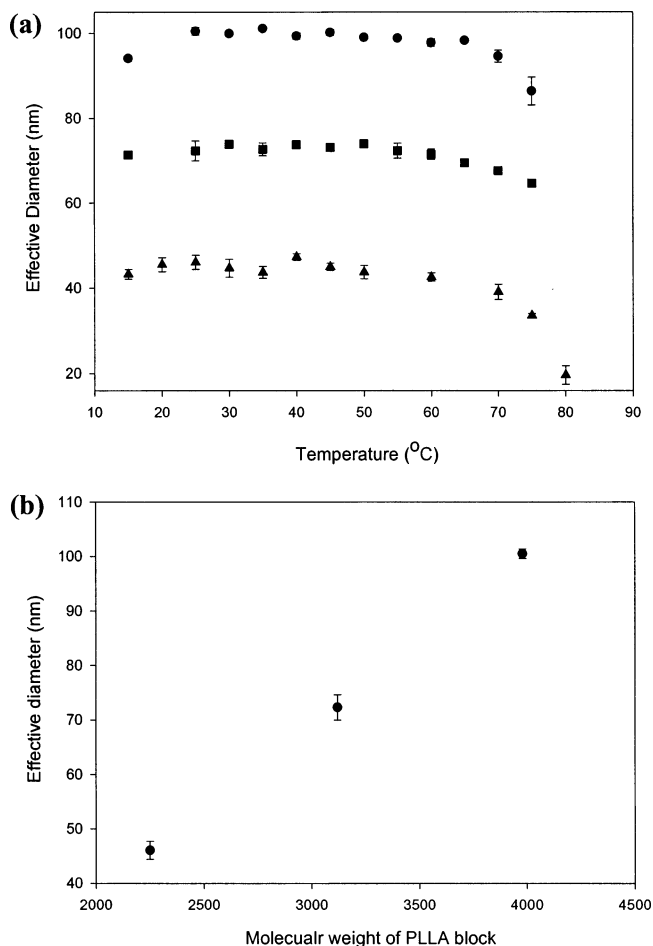


**Figure 7.** (a) Effective diameter of micelle of star PLLA5-PEO2K at 0.25 (●), 0.05 (■), and 0.01 wt % (▲) aqueous solution measured by DLS as a function of temperature ( $n = 3$ ). (b) Effective diameter of micelle of star PLLA5-PEO2K at various concentrations selected at 15 °C.

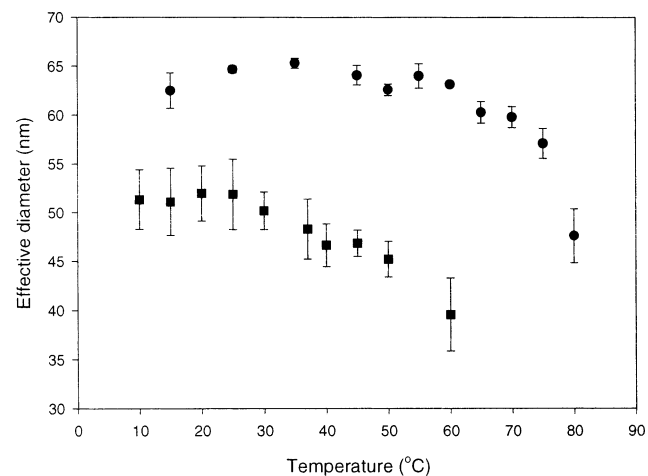
Figure 7 shows the effective diameter of micelle of star PLLA5-PEO2K in 0.01, 0.05, and 0.25 wt % aqueous solution as a function of temperature. The micelle size of star PLLA5-PEO2K also showed a dramatic temperature sensitivity. In 0.01 wt % solution, the micelle size was 45 nm at low temperature; however, above 50 °C, micelle size decreased and reached 20 nm at 80 °C. The temperature dependency behavior was similar for other concentrations though the measured micelle diameter was highly affected by concentration.

The micelle sizes of star PLLA5-PEO2K series increased with increasing concentration (Figure 7b), while that of star PLLA5-PEO5K series was not affected by concentration (Figure 5b). It is likely that micelles of star PLLA5-PEO2K series form micelle clusters, and the size of them increased as the concentration increased.

In Figure 8a, the effective diameters of micelle of star PLLA7-PEO2K and star PLLA9-PEO2K are presented and compared with that of star PLLA5-PEO2K to see the effect of core PLLA block molecular weight. The micelle sizes of star PLLA7-PEO5K and star PLLA9-PEO5K were around 73 and 100 nm at low temperature and decreased with increasing temperature and reached 66 and 87 nm at 80 °C, respectively. Figure 8b shows the effect of core molecular weight on micelle size at 25 °C. It seems that core block with higher



**Figure 8.** (a) Effective diameter of micelle of star PLLA9-PEO2K at 0.01 wt % (●), star PLLA7-PEO2K at 0.01 wt % (■), and star PLLA5-PEO2K at 0.01 wt % (▲) aqueous solution measured by DLS as a function of temperature ( $n = 3$ ). (b) Effective diameter of micelle of star PLLA-PEO2K with various PLLA block molecular weight selected at 25 °C.

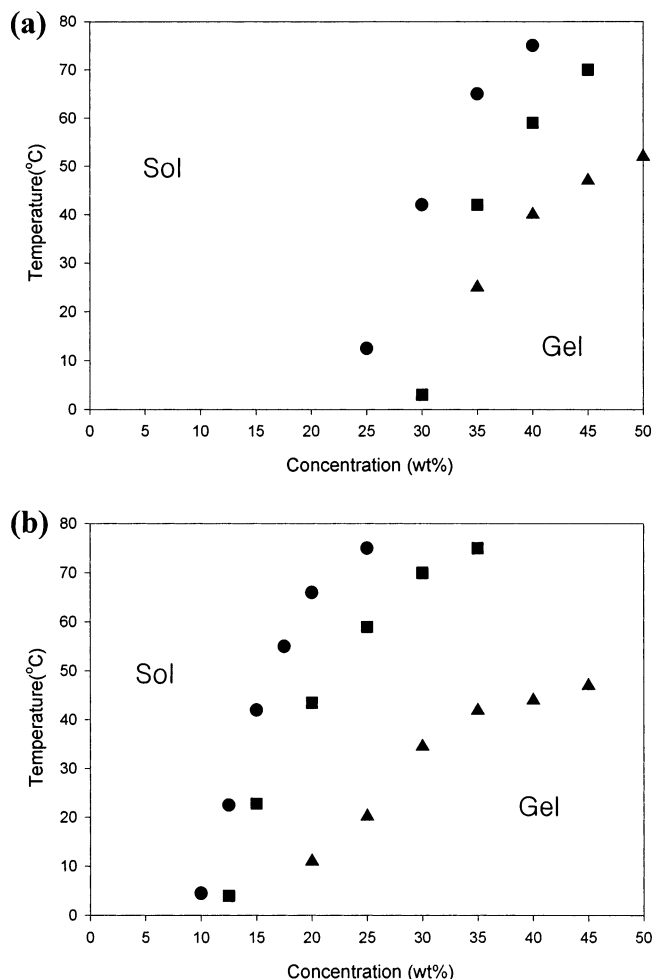


**Figure 9.** Effective diameter of micelle of star PLLA7-PEO2K at 0.005 wt % (●) and star PLLA7-PEO2K at 0.005 wt % (■) aqueous solution measured by DLS as a function of temperature ( $n = 3$ ).

molecular weight constitute larger core of micelle so the size of micelle cluster was larger.

The effect of shell block molecular weight on micelle size is presented in Figure 9. The micelle size of star PLLA7-PEO2K was larger than that of star PLLA7-PEO5K both in 0.005 wt % aqueous solution though the

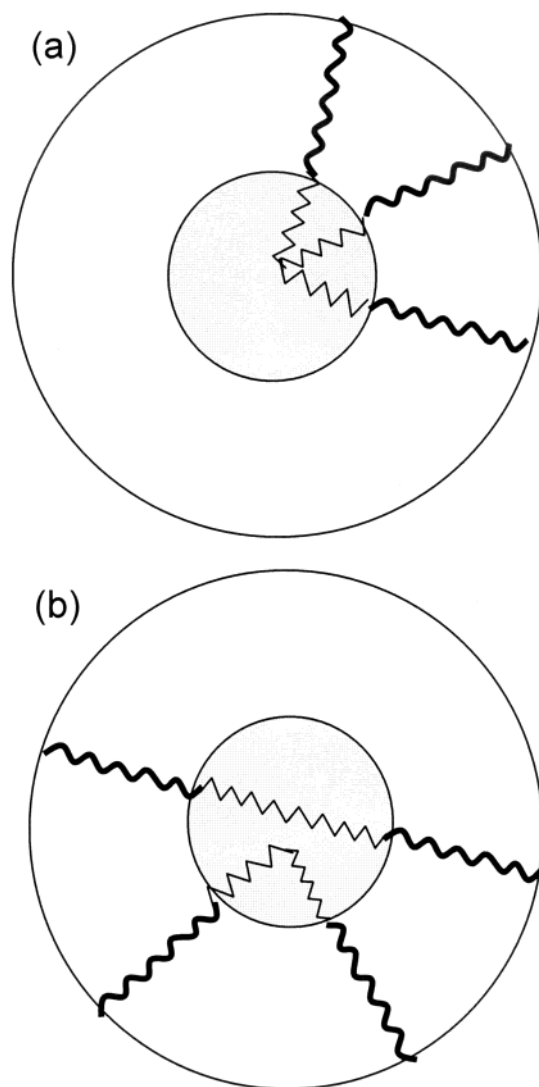




**Figure 10.** Sol-gel transition phase diagrams of star-block copolymers: (a) star PLLA9-PEO2K (●), star PLLA7-PEO2K (■), and star PLLA5-PEO2K (▲); (b) star PLLA9-PEO5K (●), star PLLA7-PEO5K (■), and star PLLA5-PEO5K (▲).

former has smaller shell block molecular weight. This result suggests that the formation of micelle cluster only in the case of star PLLA-PEO2K series. The micelle size of star PLLA7-PEO5K decreased above 30 °C whereas that of star PLLA7-PEO2K decreased above 65 °C. These results can be explained by considering the LCST of PEO in aqueous solution. It was reported that the LCST of PEO5K and PEO3K were about 130 and 170 °C at 0.2 wt %, respectively.<sup>28</sup> Partial dehydration of star PLLA7-PEO5K took place at lower temperature, because the LCST is lower as the molecular weight is higher.

The sol-gel transition behavior of star-block copolymer was investigated by the vial tilting method in aqueous solution at high concentration (more than 5 wt %). The temperature-dependent sol-gel transition phase diagram of star PLLA-PEOs is shown in Figure 10. The lower right region of the curve corresponds to gel phase and the opposite region sol phase. In the case of 30 wt % aqueous solution of star PLLA9-PEO2K, there was no flow at low temperature, and the gel state was maintained until 43 °C; however, further increase of temperature made the gel flow in the vial tilting test. The gel to sol transition behavior with increasing temperature was found over the critical gel forming concentration (cgc) for every sample. The cgc of star PLLA5-PEO2K, star PLLA7-PEO2K, and star PLLA9-PEO2K were 32.5, 29, and 23 wt %, respectively. With



**Figure 11.** Schematic illustration of micelle of (a) 3-armed star shaped block copolymer and (b) 2-armed linear triblock copolymer.

increasing the molecular weight of PLLA block, the cgc decreased, the boundary curve shifted to the left, and the gel regions were expanded. Similar behavior was observed in star PLLA-PEO5K series (Figure 10b).

The cgc's of star PLLA-PEOs affected not only by core block molecular weight but also by shell block molecular weight. With increasing shell block molecular weight, the cgc decreased and gel regions were expanded. It is likely that the longer PEO chain is advantageous for entanglement in the micelle packing structure. Higher concentration is required for the gel formation in the case of star PLLA-PEO2K series.

The gel to sol transition with increasing temperature over cgc is explained by the decrease of effective diameter of micelle followed by the breaking of the micelle packing structure at higher temperatures.

In the star PLLA-PEO block copolymer system, the upper boundary of gel to sol transition was observed; however, the lower boundary of sol to gel demonstrated by Pluronic was not found, possibly because the core PLLA block is much more hydrophobic than PPO and it is insoluble in water. So immediately they aggregated and formed core in aqueous solution.

The cgc of star PLLA-PEO with lower PEO content is smaller value than that with higher PEO content

when they have same PEO block length. However, the cgc of star PLLA9-PEO5K (PEO content is 77.8%) was smaller value (by 5 wt %) than that of linear PEO-PLLA-PEO (5000-3000-5000; PEO content is 76.9%),<sup>11</sup> which was reported by Kim et al. though they have the same PEO block length but different architecture. Star-block copolymer was advantageous for gel formation. The schematic illustration of the formed micelle in aqueous solution of the star and triblock copolymer is shown in Figure 11.

## Conclusion

Biodegradable, nontoxic star PLLA-PEO block copolymers were prepared by the coupling reaction of hydroxy-terminated 3-armed poly(L-lactide) and  $\alpha$ -monocarboxy- $\omega$ -monomethoxypoly(ethylene oxide) (CMPEO). The DPH solubilization method was used to determine the cmt of star-block copolymers. Star-block copolymers with larger hydrophobic block form micelles at lower temperatures. The tendency of micelle formation decreased as increasing the shell block molecular weight. Star-block copolymers have standard free energies with negative values, which imply that the micellization in aqueous solution is spontaneous.

The effective diameters of star PLLA-PEO5K and star PLLA-PEO2K series in aqueous solution were measured with increasing temperature. The micelle sizes decreased with increasing temperature after showing a plateau at low-temperature regions. These results can be explained by the dehydration of PEO with increasing temperature. In star PLLA-PEO5K series, the effective diameter was not highly affected by the concentration of solution. It is thought that the number of micelle increased with increasing concentration whereas the size of micelle remained constant. However, the effect of concentration was significant for the star PLLA-PEO2K series because of formation of micelle cluster. The micelle size was highly affected by the molecular weight of core block molecular weight. With increasing core block length, the micelle size increased. The micelle size of star PLLA-PEO2K was larger than that of star PLLA-PEO5K though the former had a smaller shell block molecular weight. These results suggest that the number of molecules that formed micelle is more in star PLLA-PEO2K, and the micelle size of star PLLA7-PEO5K decreased from 30 °C whereas that of star PLLA7-PEO2K decreased from 65 °C. The dehydration of star PLLA-PEO5K took place at lower temperature because the LCST is lower as the molecular weight is larger.

The temperature-sensitive sol-gel transition behaviors of star-block copolymers were investigated by the tube tilting method with increasing temperature. The star-block copolymer with larger core block and/or larger shell block had a lower critical gel concentration (cgc).

The investigated star-block copolymers, in this study, formed micelle in aqueous solution at very low concentration. In high-concentration solution (above 10 wt %), they showed temperature-sensitive sol-gel transition

behavior at relatively low concentration. They are composed of nontoxic glycerol, PLLA, and PEO. Star-block copolymers showed high possibility to be used as injectable drug delivery system for long-term delivery of drugs such as protein drugs and insulin.

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