Communications to the Editor

Synthesis and Characterization of Star-Shaped PLLA−PEO Block Copolymers with Temperature-Sensitive Sol−Gel Transition Behavior

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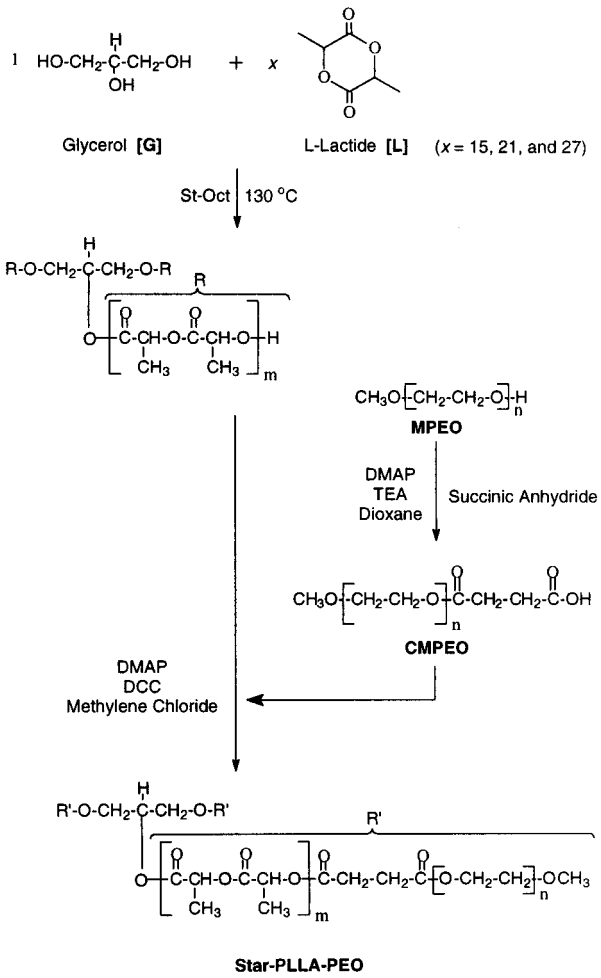
Introduction. One of the most widely investigated temperature-sensitive polymers is Pluronic, which is poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) (PEO−PPO−PEO) triblock copolymer.[1] The aqueous solution of Pluronic showed sol-to-gel (lower temperature region) and gel-to-sol (higher temperature region) transition behaviors with increasing temperature,[2] and it is proposed that Pluronic had possibility to be used as injectable drug delivery system.[3] The transition behaviors were studied by many researchers with various instruments such as light scattering,[4] SANS,[5] UV,[6] DSC,[7] cryo-TEM,[8] dielectric measurement,[9] and rheology.[10] It has been proved that the transition behavior is due to the formation of micelle and micelle packing over specific temperature and concentration.[1−10] However, Pluronic was limited in use as injectable drug delivery system because of its nondegradability, toxicity, and dissolution of the gel after administration.[11]

Some investigators developed biodegradable block copolymers to produce nontoxic injectable drug carriers for long-term delivery of polypeptides and protein drugs.[12,13] Biodegradable poly(ethylene oxide-*b*−L-lactic acid) (PEO−PLLA) diblock and poly(ethylene oxide-*b*−L-lactic acid-*b*-ethylene oxide) (PEO−PLA−PEO) triblock copolymers with PEO fixed chain length (MW = 5000) were prepared by Kim et al.[12] These block copolymers showed gel-to-sol transition behavior over a specific concentration in aqueous solution with increasing temperature. Poly(ethylene glycol-*b*-(DL-lactic acid-*co*-glycolic acid)-*b*-ethylene glycol) (PEG−PLGA−PEG) triblock copolymers with short-chain PEG (MW = 550 and 750) were also produced.[13] These materials displayed sol-to-gel and gel-to-sol transition behaviors in aqueous solution, and the gel state was maintained for more than 1 month in the body of a rat.[14] In addition, star-shaped PEO−PLA and PEO−PCL block copolymers were prepared with using multiarm PEO, and their equilibrium swellings, thermal properties, drug release rates, and degradation behaviors were evaluated.[15]

Scheme 1. Synthetic Scheme of Star PLLA−PEO



Star-PLLA-PEO

However, star-shaped PLLA−PEO block copolymers have not yet been studied. Star PLLA−PEO block copolymers are expected to form micelles in aqueous solution and to show temperature-sensitive gel-to-sol transition behavior that may be different from linear di- and triblock copolymers of PLLA and PEO because of their star architecture. It is worth while to investigate the solution properties of new block copolymers. The purpose of this study is to synthesize biodegradable, nontoxic, and temperature-sensitive star-shaped PLLA−PEO amphiphilic block copolymers and to investigate their sol−gel transition behavior in aqueous solution.

Experimental Section. Scheme 1 shows the synthetic reactions of star PLLA−PEO block copolymers. Star block copolymers were formed from two reactive precursors, a hydroxy-terminated 3-armed poly(L-lactide) and α-monocarboxy-ω-monomethoxy poly(ethylene oxide) (CMPEO). First, star poly(L-lactide)s (star PLLAs) were synthesized by bulk ring-opening polymerization of L-lactide (Aldrich) using glycerol (Aldrich, spectrophotometric grade) as a trifunctional initiator and using

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Table 1. Molecular Weights and Thermal Properties of Star PLLAs, CMPEO, and Star PLLA−PEOs

			GPC				DSC		
sample	theor M_n	NMR M_n	M_n	M_w	PDI	PEO content (%)	T_g	T_{mL}[a]	T_{mE}[b]
star PLLA5	2250	2280	3400	4700	1.38		35.2	116.3	
star PLLA7	3120	3140	4940	6710	1.36		38.6	127.6	
star PLLA9	3980	4000	6540	8200	1.25		41.0	129.2	
CMPEO5K	5100	5060	7510	8340	1.11				62.7
star PLLA5−PEO5K	17550	17310	16690	22190	1.32	85			60.3
star PLLA7−PEO5K	18420	18110	17090	23870	1.39	83			59.9
star PLLA9−PEO5K	19280	18880	18600	24550	1.32	79			59.5

[a] Melting temperature of PLLA or PLLA block in star-block copolymer. [b] Melting temperature of PEO or PEO block in star-block copolymer.

stannous 2-ethylhexanoate (stannous octoate, St-oct, Sigma) as a catalyst.[16] Second, dried monomethoxy poly(ethylene oxide) (MPEO, Aldrich) was carboxylated with succinic anhydride (Aldrich) to produce CMPEO.[17] Finally, star PLLA and CMPEO were coupled using 1,3-dicyclohexylcarbodiimide (DCC, Aldrich) and 4-(dimethylamino)pyridine (DMAP, Aldrich) to produce a star PLLA−PEO block copolymer. The typical procedure is as follows: 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 μm 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 mole of glycerol ([L]/[G]) was varied with 15, 21, and 27 to produce star PLLA5, 7, and 9, respectively. In all cases, the molar ratio of L-lactide over St-oct ([L]/[St-oct]) was fixed with 200.

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.[17] The solvent was evaporated with a rotary evaporator, the residue taken up in carbon tetrachloride, filtered, and precipitated in ethyl ether twice. After drying in vacuo overnight, white powder CMPEO was obtained.

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-block copolymers were purified by solvent extraction using ethyl ether and benzene cosolvent and characterized by ^{1}H NMR.

Gel permeation chromatography (GPC, Waters 410) was used to obtain number-average molecular weight (M_n), weight-average molecular weight (M_w), and polydispersity index (PDI).[18] The thermal behaviors of star PLLAs and star PLLA−PEOs were studied by using differential scanning calorimeter (TA DSC 2010). The heating rate was 10 °C/min, and the scanning range was from −30 to 190 °C.

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

The effective diameters of micelle in aqueous solution of star PLLA7−PEO5K were measured by dynamic light scattering (DLS, Brookhaven Instruments BI-200SM) with 532 nm laser source at 90° angle with increasing temperature. The samples were prepared with 0.005 wt % aqueous solution and filtered by 0.45 μm syringe filter.

Results and Discussion. L-Lactide (*x* mol per glycerol mol; $x = 15$, 21, and 27) was ring-opening polymerized using nontoxic glycerol as a trifunctional initiator to yield biodegradable nontoxic star PLLA5, 7, and 9.[19,20] It was found by ^{1}H NMR that each of the hydroxy group of glycerol initiated polymerization of L-lactide, specifically by the disappearance of **G**-CH_2OH (3.73 ppm) and by the ratio of **L**-C*H* (5.16 ppm) to **G**-CH_2 (4.35−4.20 ppm).[21] The molecular weights of star PLLAs were calculated from the peak integral ratio between 4.35 and 4.20 ppm (**G**-CH_2 and **L**-C*H*-OH) and 1.55 ppm (**L**-CH_3).[22] The calculated molecular weights of star PLLAs are listed in Table 1 and are compared with theoretical value and those obtained by GPC. The GPC data showed a unimodal-shaped curve.

The successful derivatization of MPEO to CMPEO was found, specifically by the appearance of the peak of methylene proton of PEO which is next to the ester linkage at 4.23 ppm (−OCH$_2$CH_2COO−).[23] The ratio between the peak of 3.35 ppm (−OCH_3) and 4.23 ppm (−OCH$_2$CH_2COO−) was equal to 3 to 2. This observation demonstrates that all the hydroxy group of the MPEO was substituted by succinic anhydride. The molecular weight of CMPEO was calculated from the ratio of integration of peak at 3.35 ppm (−OCH$_3$) and 3.61 ppm (−OCH$_2$CH$_2$O−).[24]

The coupling of star PLLA and CMPEO was found to be successful,[25] specifically by disappearance of **L**-CH−O*H* (2.66 ppm) and increase of molecular weight by NMR and GPC. The molecular weights that are theoretically calculated and calculated from the NMR peak area and obtained by GPC are listed in Table 1. The molecular weights of star-block copolymers were calculated by the following equation:

$$A_{3.35}:A_{3.61} = 3 \times 3:3 \times (4 \times n)$$

$$M_{n,PEO} = 3 \times \{(44 \times n) + 115\}$$

$$A_{3.35}:A_{1.55} = 3 \times 3:3 \times (6 \times m)$$

$$M_{n,PLLA} = 3 \times (144 \times m) + 89$$

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

$A_{3.35}$, $A_{3.61}$, and $A_{1.55}$ are integration of peak at 3.35, 3.61,

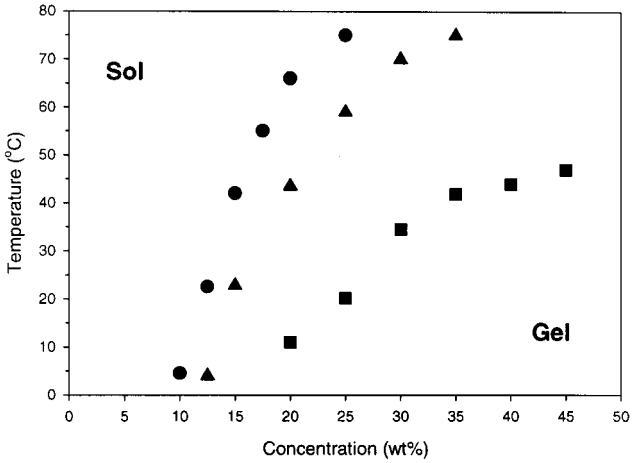

Figure 1. Temperature-sensitive sol−gel transition behavior of aqueous solution of star PLLA−PEOs: ■, star PLLA5−PEO5K; ▲, star PLLA7−PEO5K; ●, star PLLA9−PEO5K.

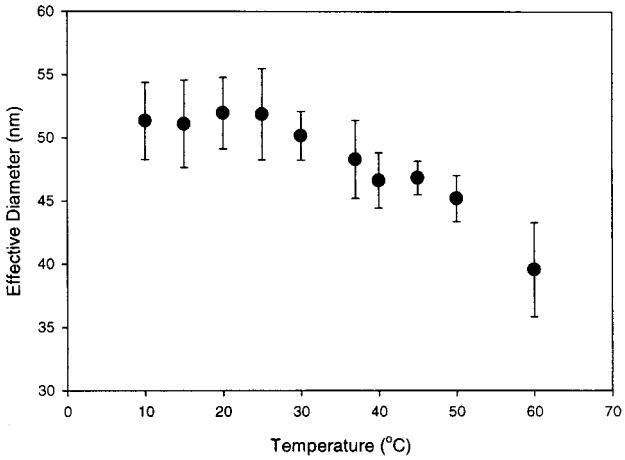

Figure 2. Effective diameter of micelle measured by DLS in 0.005 wt % aqueous solution of star PLLA7−PEO5K ($n = 3$).

and 1.55 ppm, 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.

The glass transition temperature (T_g) and melting temperature (T_m) of the polymers were measured (Table 1). With increasing the molecular weight of star PLLA the T_g and T_m increased. In star-block copolymers, the T_g and T_m of the PLLA block were not found possibly because PLLA content is relatively low. The T_m of CMPEO was 62.7 °C, whereas, the T_m 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. The T_m of PEO block decreased with increasing the molecular weight of attached PLLA block. It is thought that the partial phase mixing reduced the T_m of PEO block. This result is consistent with other results.[12b]

The temperature-dependent sol−gel transition behaviors of star PLLA−PEOs are shown in Figure 1. The lower right region of the curve corresponds to gel phase and the opposite region sol phase. In the case of 15 wt % aqueous solution of star PLLA9−PEO5K, there was no flow at low temperature, the gel state was maintained until 42 °C, and, moreover, further increase of temperature makes the gel start to flow in the vial tilting test. 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−PEO5K, star PLLA7−PEO5K, and star PLLA9−PEO5K were 17.5, 12.5, and 10 wt %, respectively. With increasing the molecular weight of PLLA block, the CGC decreased, the boundary curve shifted to the left, and the gel regions were expanded. As in the case of di- and triblock copolymers of PLLA and PEO5K, the transition of lower boundary demonstrated by Pluronic was not found.

Figure 2 shows the effective diameter of micelle in 0.005 wt % aqueous solution of star PLLA7−PEO5K. The effective diameter was about 52 nm at 10−25 °C, but it decreased to 39 nm at 60 °C. These findings provide evidence that star PLLA−PEO block copolymer forms micelle in aqueous solution. It is thought that while hydrophobic PLLA block aggregates and forms core, hydrophilic PEO chains exist outside and forms the shell of the micelle. Over the CGC, micelles are ordered and packed to show gel phase; however, these structures were destroyed by the decrease of micelle size at higher temperature.

In summary, we synthesized star PLLA−PEO copolymers successfully, and they showed sol−gel transition behavior over the CGC with varying temperature. The location and slope of boundary curve were highly affected by the molecular weight of PLLA while conventional triblock PEO−PLLA−PEO was not.[12b] These materials have possibility to be used as injectable drug delivery system for long-term delivery of bioactive agents.

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(18) For the calibration, monodiperse polystyrene standards of which MW were 52000, 22000, 9200, 5460, 1060, and 580 were used and M_n and M_w of star polymers were overestimated.

(19) PLLA5 has average 5 lactidyl units or 10 lactyl repeats per arm, similar to PLLA 7 and 9.

(20) Star PLLA: δ 1.55 (**L**-CH_3), δ 2.66 (**L**-OH), δ 4.35−4.20 (**L**-CH−OH, **G**-CH_2), δ 5.16 (**L**-CH, **G**-CH).

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(22) $A_{1.55}$:$A_{4.35}$ = 3 × (6 × m):7 and M_n = 3 × (144 × m) + 92, where $A_{1.55}$ is the area of peak at 1.55 ppm and $A_{4.35}$ is the area of peak at 4.35−4.20 ppm.

(23) CMPEO: δ 2.65 (−COCH_2CH_2CO−), δ 3.35 (−OCH_3), δ 3.61 (−OCH_2CH_2O−), δ 4.23 (−OCH$_2$CH_2COO−).

(24) $A_{3.35}$:$A_{3.61}$ = 3:(4 × n) and M_n = (44 × n) + 132, where $A_{3.35}$ is area of peak at 3.35 ppm and $A_{3.61}$ is area of peak at 3.61 ppm.

(25) Star PLLA−PEO: δ 1.55 (**L**-CH_3), δ 2.65 (−COCH_2CH_2CO−), δ 3.35 (−OCH_3), δ 3.61 (−OCH_2CH_2O−), δ 4.23 (−OCH$_2$CH_2-COO−, **G**-CH_2), δ 5.16 (**L**-CH, **G**-CH).

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