Thermoresponsive Phase Transitions of PLA-*block*-PEO-*block*-PLA Triblock Stereo-Copolymers in Aqueous Solution

Hyung Tak Lee and Doo Sung Lee*

*Center for Advanced Functional Polymers, Department of Polymer Science and Engineering,
Sungkyunkwan University, Suwon, Kyungki 440-746, Korea*

Abstract: A series of PLA-PEO-PLA triblock stereo-copolymers with varying PLA/PEO and L-/DL-LA ratios were synthesized via ring opening polymerizations. Aqueous solutions of these copolymers undergo thermo-responsive phase transitions as the temperature monotonically increases. Further study shows that there is a critical gel concentration (CGC), and also lower and upper critical gel temperatures (CGTs), at which the thermo-responsive phase transition occurs. The CGC and CGTs are affected by various factors such as block length, as well as the compositions of the PLA blocks and of the additives. In particular, the changes in the phase diagram produced by varying the L-/DL-LA ratio in the PLA blocks were determined to be mainly due to consequent stereo-regularity changes in the PLA blocks.

Keywords: biodegradable, thermo-responsive, phase transition, triblock stereo-copolymer.

Introduction

The thermo-responsive gelation of synthetic polymers has been extensively investigated for possible medical applications in recent decades.[1-3] Commercially available block copolymers, such as poly(ethylene oxide)-*block*-poly(propylene oxide)-*block*-poly(ethylene oxide) (PEO-PPO-PEO), poly(butylene oxide)-*block*-poly(ethylene oxide) (PBO-PEO, Dow Chem),[4,5] Pluronics (BASF) and Poloxamers (ICI)[6] undergo gel-sol transitions in aqueous solution when the polymer concentration is above a critical value and as the temperature increases, because of micelle aggregation or packing. However, these synthetic polymers are all non-biodegradable and potentially toxic. For example, Poloxamer 407 (P-407) has been found to cause toxic hypertriglyceridemia and hypercholesterolemia in rodents.[7,8] It is obviously very important for medical applications to synthesize polymers that are biocompatible, biodegradable and safe. Because of these features, block copolymers composed of PEO and biodegradable polyesters, such as PLLA, PDLLA, PLGA, PCL, and PDLLA-*co*-PCL, have been investigated for use in biomedical applications as controlled-release drug carriers in hydrogel and nanosphere forms.[9-18] Kim and coworkers have reported thermo-responsive phase transitions occurring for diblock and triblock copolymers composed of

PEO and various aliphatic polyesters.[9-13] In particular, aqueous solutions of PEO-PLGA-PEO triblock copolymers always undergo a sol-to-gel (the lower transition) and gel-to-sol (the upper transition) transitions as the temperature monotonically increases. The lower transition is important for drug delivery applications because materials that undergo this transition can be found to flow freely at room temperature but become a gel at body temperature. The lower transition may arise because of micellar growth and intra- and inter-micelle phase mixing and packing, while the upper transition involves breakage of the micellar structure.[12] On the other hand, we have previously concluded that the gel-sol transition mechanism of the aqueous solution of PLGA-PEO-PLGA triblock copolymer is different to that of the PEO-PLGA-PEO system.[19,20] PLGA-PEO-PLGA triblock copolymers can form micelles, with bridging between grouped micelles in aqueous solution produced by the hydrophobic PLGA end blocks, which can be located in different micelles. The variation in the critical gel concentration (CGC) between the PLGA-PEO-PLGA triblock copolymers system and the PLGA-PEO-PLGA triblock copolymers system (the former, less than 10%, while the latter exhibits values higher than 20%) is due to the different sol-gel mechanisms of the two systems. Vert *et al.* described the protein release properties of a hydrogel composed of PLA-PEO-PLA triblock copolymers synthesized via the ring opening polymerization of D,L-lactide.[21] However, the thermo-responsive gelation of those copolymers was not mentioned in their study.

In this study, poly(D,L-co-L)LA-poly(ethylene glycol)-poly (D,L-co-L)LA (PLA-PEO-PLA) triblock stereo-copolymers with varying poly(D,L-co-L)LA block composition and block length were synthesized. The thermo-responsive phase transition behaviors of these triblock copolymers in water were investigated in detail. These novel thermo-responsive hydrogels will be useful in long-term drug delivery systems as the carriers of proteins or other pharmaceuticals.

Experimental

Materials. Poly(ethylene oxide)s (PEOs) (M_n, 1,000 and 1,500, M_w/M_n: 1.10) were acquired from Aldrich and purified before use, first by dissolution in dried chloroform and then by precipitation in n-hexane. L-lactide(L-LA) and D,L-lactide (DL-LA) supplied by Boehringer Ingelheim and Polyscience were purified by recrystallization in dry ethyl acetate. D,L-lactide is racemic mixture composed of D-lactide (50%) and L-lactide(50%). Stannous octoate (Sn(Oct)$_2$, Sigma) was dried for 24 h under vacuum at ambient temperature prior to use. Toluene was thoroughly purified by distillation over calcium hydride (CaH$_2$). All other reagents were of analytical grade and used without further purification.

Synthesis. The syntheses of various PLA-PEO-PLA triblock copolymers with different PLA/PEO and L-lactide/ D,L-lactide (L-/DL-LA) ratios were carried out through ring-opening copolymerizations using PEO as the initiator. The synthetic procedure was as follows: PEO (M_n = 1,500, 2 g) was added to 60 mL dried toluene and the residual water in the PEO/toluene solution was removed by azeotropic distillation to a final volume of 30 mL. L-lactide, D,L-lactide and stannous octoate (0.03 g) were added to this PEO/toluene solution and refluxed under a dry nitrogen atmosphere for 60 h. The reaction was ended by precipitation in diethyl ether, and then the final copolymer was dried under vacuum at 20 °C for over 48 h. The conversions of these copolymers were found after drying to all be greater than 80%.

Characterizations. An FT-IR spectrometer (UNICAM Mattson 5000) and an ^{1}H-NMR spectrometer (Varian Unity Inova 500 instrument, 500 MHz) were used to determine the structures and compositions of the triblock copolymers.[11,13] The molecular weight distributions (MWDs) of these triblock copolymers were determined by gel permeation chromatography (GPC) on a Waters Model 410 apparatus equipped with four μ-styragel columns from 500 to 10 Å in series at a flow rate of 1.2 mL/min (eluent: THF, 36 °C, polystyrene as standard).

Phase Diagram. Block copolymer aqueous solutions (0.5 g) of various concentrations were prepared by dissolving the copolymers at 2 °C in 4 mL vials with an inner diameter of 11 mm. The phase transition temperatures of the sol (flow) to gel (no flow) transitions of these solutions were measured using the inverting test method with heating in increments of 2 °C. The detailed steps were as described previously.[19]

Results and Discussion

Molecular Structure Characterization. The GPC traces of these PLA-PEO-PLA triblock stereo-copolymers show unimodal peaks with molecular weight distributions (M_w/M_n) varying between 1.06 and 1.12. The M_n of the PLA blocks in the triblock stereo-copolymers can be calculated from their ^{1}H-NMR spectra, as reported in previous papers.[13] Detailed characterizations of the triblock stereo-copolymers are listed in Table I.

Solubility of PLA-PEO-PLA in Water. PLA-PEO$_{1500}$-PLA triblock stereo-copolymers with different PLA block lengths and L-/DL-LA ratios were synthesized. The solubilities of these copolymers are listed in Table II. In the table, "I" indicates that the copolymer is insoluble in water, and "S" indicates that the copolymer is soluble without a sol-gel transition. Copolymers showing a sol-gel transition in distilled water are marked with sample codes such as "A-1".

Table I. Molecular Characteristics of the Synthesized PLA-PEO-PLA Triblock Copolymers

PLA-PEO-PLA $M_n{}^a$	PLA/PEO (wt/wt)^a	L-LA/D,L-LA (wt/wt)^b	$M_w/M_n{}^c$	Yield (%)
1692-1500-1692 (A-1)	2.25/1	0.6/1	1.06	91
1732-1500-1732 (B-1)	2.30/1	0.4/1	1.12	85
1691-1500-1691 (C-1)	2.25/1	0.2/1	1.11	87
1683-1500-1683 (D-1)	2.24/1	0/1	1.07	90
1550-1500-1550 (A-2)	2.06/1	0.6/1	1.08	88
1556-1500-1556 (B-2)	2.07/1	0.4/1	1.08	93
1576-1500-1576 (C-2)	2.10/1	0.2/1	1.10	81
1552-1500-1552 (D-2)	2.07/1	0/1	1.09	86
1049-1000-1049 (E-1)	2.09/1	0.4/1	1.08	93

^{a}Number-average molecular weight and block ratio calculated from ^{1}H-NMR peak integration. ^{b}Ratio in feed. ^{c}Measured by GPC.

Table II. The Sol-Gel Transition Regions of PLA-PEO-PLA Triblock Copolymers in Water

| PLA/PEO (wt/wt) \ L-LA/D,L-LA (wt/wt)	0.8/1	0.6/1	0.4/1	0.2/1	0/1 (only DL-LA)
2.5/1 (1875-1500-1875)	I	I	I	I	I
2.3/1 (1725-1500-1725)	I	A-1	B-1	C-1	D-1
2.1/1 (1575-1500-1575)	~	A-2	B-2	C-2	D-2
1.8/1 (1350-1500-1350)	~	S	S	S	S

Note : 1. PLA/PEO ratios and M_n of polymer are target values. Samples prepared have some deviation from these values.
2. "I" = insoluble; "S" = soluble; bold line-framed zone, sol-gel region.

Table II shows that all the triblock stereo-copolymers composed of a central PEO (M_n, 1,500) bearing two PLA blocks of molecular weight above about 1,875 were insoluble in water, whereas all the triblock stereo-copolymers with PLA blocks of molecular weight under about 1,350 were soluble without a sol-gel transition in water. These phenomena can be understood in terms of the difference in hydrophobicity of the triblock copolymers produced by variation of the PLA block length. Only those triblock copolymers with PLA blocks of molecular weight in the approximate range of 1,575 to 1,727 and with an L-/DL-LA ratio under 0.8 showed a thermo-responsive sol-gel transition, as listed in the bold line framed box in Table II.

Phase Diagrams. The sol-gel transitions of the PLA-PEO-PLA triblock stereo-copolymers produced by concentration and temperature changes are represented by the phase diagrams in Figures 1 to 4. The phase diagrams show the critical gel concentrations (CGCs) and two critical gelation temperature (CGT) curves. The lower CGT curves indicate transitions from sol to gel, while the upper CGT curves indicate transitions from gel to sedimentation. Taking the phase diagram for PLA-PEO-PLA (1692-1500-1692, A-1) in Figure 1(a) as an example, the lower CGT is 25 °C at 10 wt%, 24 °C at 15 wt%, and 23 °C at 20 wt%. The lower CGT changes only a few degrees with large variation of concentration. The CGC is below 10 wt%, similar to that of the PLGA-PEO-PLGA triblock copolymer.[19,20] As expected from the copolymer structure, two hydrophobic PLA blocks can be located in different adjacent micelles; these bridges facilitate the aggregation of micelles. Dehydration of the hydrogel inducing the sedimentation of polymer phase appears at upper CGTs for the PLA-PEO-PLA block copolymer. It is little different mechanism from gel to sol transition at upper CGTs in PLGA-PEO-PLGA system. The observed difference may arise because PLA blocks are more hydrophobic than PLGA blocks.

Figure 1(a) displays the phase diagrams of the PLA-PEO-PLA triblock stereo-copolymers with varying L-/DL-LA ratios and similar PLA/PEO molecular weight ratios (2.3/1). It shows that the lower CGTs of a 15% copolymer concentration water solution decrease from 30 to 23 °C with variation of the L-/DL-LA ratios from 0/1 (D-1) to 0.6/1 (A-1). How-
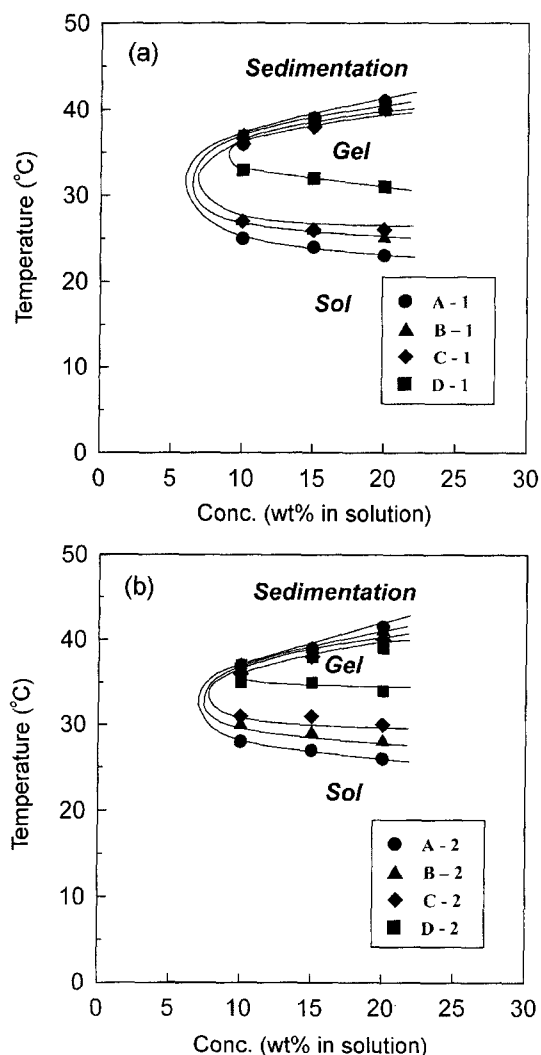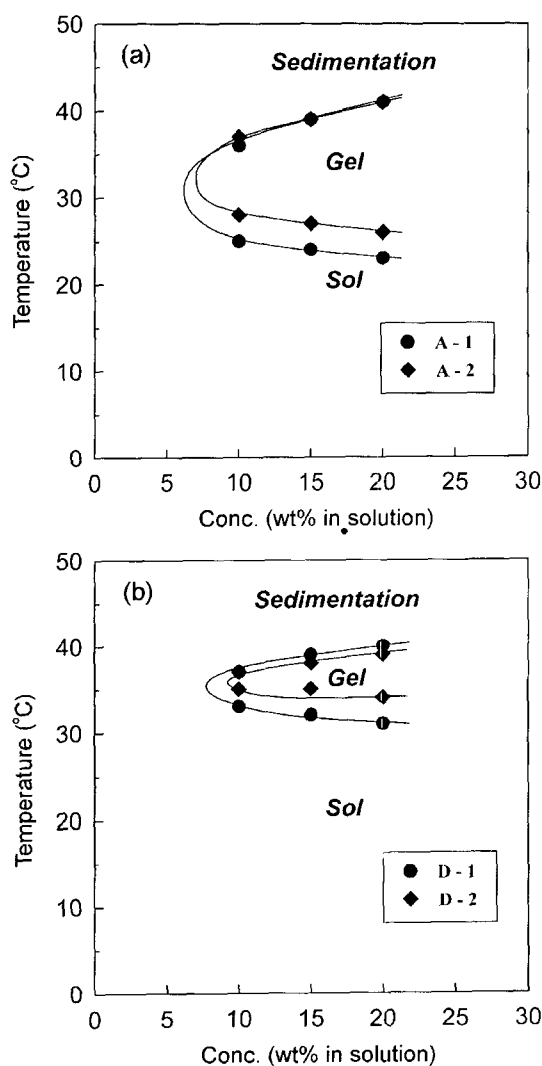

Figure 1. Phase diagrams of PLA-PEG-PLA triblock stereo-copolymers with various L-/DL-LA ratios in water: (a) PLA/PEO (2.3/1) wt. ratio; (b) PLA/PEO (2.1/1) wt. ratio.

ever, the upper CGTs are hardly affected by variation of the L-/DL-LA ratios. If the L-/DL-LA ratio of the PLA block is increased, its stereo-regularity increases because of the presence of the LLA repeat unit. PLLA and PDLA are semi-

crystalline and amorphous in the bulk state respectively, due to their different stereo-regularity. The degree of stereo-regularity of the PLA block determines the ease of micellization and aggregation because it alters the hydrophobicity of the PLA block. As PLA blocks become more stereo-regular, micelle association is made easier by the increased establishment of bridging connections between micelles. Therefore, as the L-/DL-LA ratio in the PLA blocks increases, the lower CGTs and the CGC gradually decrease. In other words, the gel range in the phase diagram becomes larger with the increase of the L-/DL-LA ratio in the PLA blocks. The same tendency was observed for the series of copolymers (A-2) to (D-2) (in Table II) with the same PLA/PEO ratio (2.1/1), as shown in Figure 1(b).
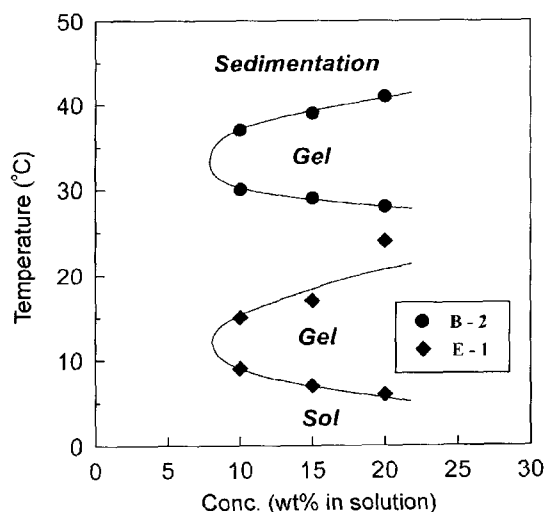
Figure 2 displays the phase diagrams of PLA-PEO-PLA triblock stereo-copolymers with different PLA/PEO ratios


and comparable L-/DL-LA ratios. In Figure 2(a), the CGC and lower CGTs of A-1 (PLA/PEO = 2.25/1, L-/DL-LA = 0.6/1) are lower than those of A-2 (PLA/PEO = 2.06/1, L-/DL-LA = 0.6/1). This indicates that the gel range becomes larger as the length of the PLA blocks increases. A similar tendency was observed for D-1 (PLA/PEO = 2.24/1) and D-2 (PLA/PEO = 2.07), which have lower L-/DL-LA ratios (0/1), as shown in Figure 2(b). Longer PLA blocks in the triblock copolymers produce stronger hydrophobic interactions, leading to an increase in the micelles tendency to associate. In Figures 1 and 2, note that the gel region is mainly dependent on the L-/DL-LA ratio but not on the PLA block length. This result is different from that for the PLGA-PEO-PLGA system, in which the PLGA block length has a more significant influence on the gel region than the DLLA/GA ratio in PLGA blocks.[20] This is because the variations of the hydrophobicity and stereo-regularity of PLA blocks with changing L-/DL-LA ratio is greater than those of PLGA blocks with changing DLLA/GA ratio.

The phase diagrams of PLA-PEO$_{1000}$-PLA (1049-1000-1049, PLA/PEO = 2.09/1, L-/DL-LA = 0.4/1, E-1) and PLA-PEO$_{1500}$-PLA (1556-1500-1556, PLA/PEO = 2.07/1, L-/DL-LA = 0.4/1, B-2) are compared in Figure 3. E-1 has the same PLA/PEO and L-/DL-LA ratios as B-2, but has a different total molecular weight. The phase diagram of E-1 occurs at a lower temperature than that of B-2, but the gel range is the same for both copolymers. This indicates that the shape of the gel-sol diagram does not change much as long as the composition of the copolymer is unchanged, but that the transition temperatures strongly depend on the total molecular weight of the block copolymer, as was shown in a previous paper.[20] This effect seems to be related to the fact that

Figure 2. Phase diagrams of PLA-PEG-PLA triblock stereo-copolymers with various PLA/PEO ratios in water: (a) L-/DL-LA (0.6/1) ratio; (b) L-/DL-LA (0/1) ratio (only DL-lactide).

Figure 3. Phase diagrams of PLA-PEO-PLA triblock stereo-copolymers with different PEO molecular weights and PLA/PEO, L-/DL-LA ratio (B-2: 1556-1500-1556; E-1: 1049-1000-1049) in water.
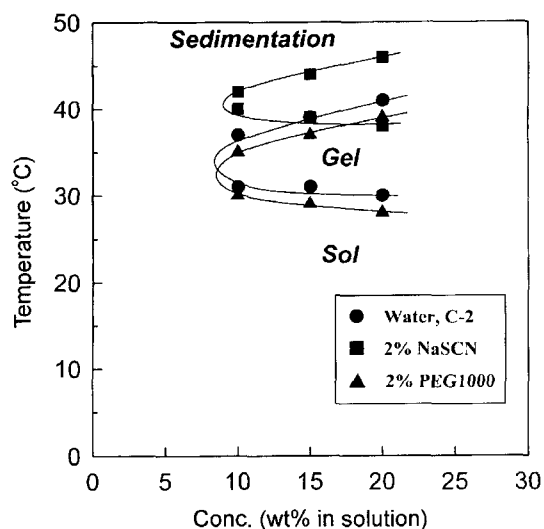
Figure 4. Phase diagrams of PLA-PEO-PLA triblock stereo-copolymers (C-2: 1576-1500-1576) in water with different additives.

amphiphilic copolymers with low molecular weight micellize more easily than those with high molecular weight.[12]

The phase diagram of C-2 (in Table I) vertically shifts

without influencing the gel range when different additives such as PEO1000 (M_n = 1,000) and NaSCN (2% in solution) are added to the copolymer solution (Figure 4). NaSCN is known as a water structure breaking salt. It decreases the strength of the hydrogen bond interactions between water molecules, resulting in an increase in copolymer solubility in water.[22,23] Thus it shifts gelation to higher temperatures, raising the phase diagram. In contrast, adding a small amount of PEO1000 shifts the phase diagram to lower temperatures. Free PEO1000 in aqueous solution produces bridging connections between micelles because of the interaction between PEO1000 molecules and the PEO blocks of copolymer molecules. Therefore, PEO1000 molecules in solution can increase the strength of interaction between micelles and make micelles pack better, which induces gelation at lower temperatures, as observed for the PLGA-PEO-PLGA triblock aqueous solution.[20]

Gelation and Phase Separation Mechanisms. The gelation mechanism of PLGA-PEO-PLGA, which has the same B-A-B structure as PLA-PEO-PLA, has been schematically illustrated in previous papers.[19,20] At lower CGTs, the gelation mechanism of PLA-PEO-PLA in water may be similar to that of PLGA-PEO-PLGA because of the similar locations of the hydrophobic (side block) and hydrophilic (central block) parts of the triblock copolymers. However, the dehy-
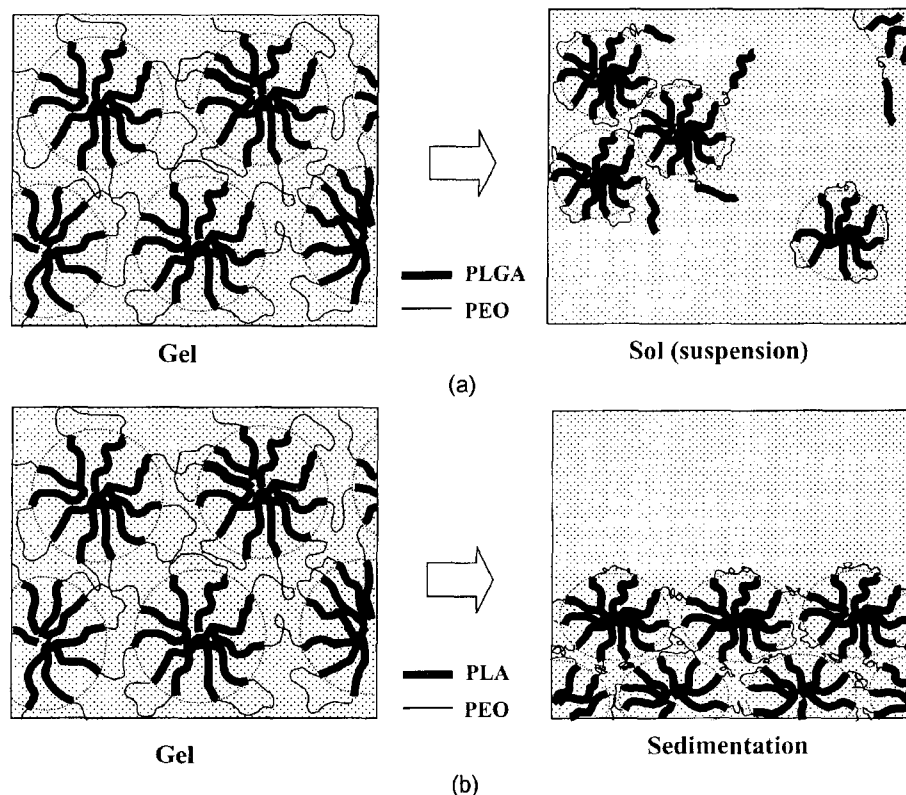
Figure 5. A schematic diagram of the micellar gel to sol mechanism in aqueous solution at upper CGTs (a) for PLGA-PEO-PLGA triblock copolymer and (b) for PLA-PEO-PLA triblock stereo-copolymer.

dration at upper CGTs of the PLA-PEO-PLA hydrogel occurs in a different manner to that of PLGA-PEO-PLGA. This difference in phase behavior at upper CGTs may be because PLA blocks are more hydrophobic than PLGA blocks. The gel to sol transitions of PLGA-PEO-PLGA and PLA-PEO-PLA at upper CGTs are schematically illustrated in Figure 5. In the case of PLGA-PEO-PLGA, when the temperature is raised above the upper CGTs the PLGA blocks in the micelle core shrink. At the same time, the hydrophilic PEO chains dehydrate and shrink. This shrinking of PEO chains during the gel to sol transition produces breaking of bridge connections and disruption of the loose packing between micelles (see Figure 5(a)). In the case of PLA-PEO-PLA, the hydrophobicity of the PLA blocks in micelle cores is so strong that bridging connections between micelles are maintained, even though the PEO chains dehydrate and shrink at upper CGTs. For this reason, the sedimentation of the PLA-PEO-PLA hydrogel occurs without flow above the upper CGTs (Figure 5(b)).

Conclusions

The thermo-responsive phase transitions of PLA-PEO-PLA triblock stereo-copolymers in aqueous solution were investigated. The effects on the phase diagrams of varying the PEO/PLA ratio, the block lengths, and of adding additives (NaSCN, PEO) were studied in detail. The lower CGTs and CGC were found to decrease for increasing L-/DL-LA ratios in PLA blocks and for increasing PLA/PEO ratios. Phase diagrams were shifted to higher temperatures without change in gel range by increasing the total molecular weight without changing the composition of the copolymers. The locations of the phase diagrams could be adjusted by adding small amounts of additives such as NaSCN and PEO. These results indicate that the controlling factors of the gel-sol transition of the PLA-PEO-PLA block copolymer are the same as those for the PLGA-PEO-PLGA block copolymer. These controlling factors can be used for design of similar block copolymer hydrogel systems.

References

(1) G. Chen and A. S. Hoffman, *Nature*, **373**, 49 (1995).
(2) R. Yoshida, K. Uchida, and Y. Kaneko, *Nature*, **374**, 240 (1995).
(3) J. M. Guenet, *Thermoreversible Gelation of Polymers and Biopolymers*, Academic Press, New York, 1992.
(4) Y. Zhuo, P. Simon, N. J. Deng, J. B. Ramound, D. Attwood, and C. Booth, *Macromolecules*, **27**, 2371 (1994).
(5) Y. W. Yang, Z. Yang, Z. K. Zhou, D. Attwood, and C. Booth, *Macromolecules*, **29**, 670 (1996).
(6) M. Malmsten and B. Lindman, *Macromolecules*, **25**, 5446 (1992).
(7) Z. Wout, E. A. Pec, and J. A. Maggiore, *J. Parenteral Sci. & Technol.*, **46(6)**, 192 (1992).
(8) W. K. Palmer, E. E. Emeson, and T. P. Johston, *Atherosclerosis*, **136**, 115 (1998).
(9) H. Suh, B. M. Jeong, R. Rathi, and S. W. Kim, *J. Biomed. Mater. Res.*, **42(2)**, 331 (1998).
(10) B. M. Jeong, Y. H. Bae, D. S. Lee, and S. W. Kim, *Nature*, **388**, 860 (1997).
(11) B. M. Jeong, D. S. Lee, J. Shon, Y. H. Bae, and S. W. Kim, *J. Polym. Sci., Polym. Chem. Ed.*, **37(6)**, 751 (1999).
(12) B. M. Jeong, Y. H. Bae, and S. W. Kim, *Macromolecules*, **32**, 7064 (1999).
(13) S. W. Choi, S. Y. Choi, B. M. Jeong, S. W. Kim, and D. S. Lee, *J. Polym. Sci., Polym. Chem. Ed.*, **37(13)**, 2207 (1999).
(14) Y. H. Bae, K. M. Huh, Y. G. Kim, and K. H. Park, *J. Control. Release*, **64**, 3 (2000).
(15) K. M. Huh and Y. H. Bae, *Polymer*, **40**, 6147 (1999).
(16) Y. X. Li, V. Christian, and T Kissel, *J. Control. Release*, **32**, 121 (1994).
(17) Y. K. Sung and S. W. Kim, *Korea Polym. J.*, **8(5)**, 199 (2000).
(18) J. W. Lee, F. Hua, and D. S. Lee, *J. Control. Release*, **73**, 315 (2001).
(19) M. S. Shim, S. W. Kim, H. J. Lee, T. Chang, and D. S. Lee, *Macromol. Rapid Commun.*, **22**, 587 (2001).
(20) M. S. Shim, H. T. Lee, W. S. Shim, I. S. Park, H. J. Lee, T. H. Chang, S. W. Kim, and D. S. Lee, *J. Biomed. Mater. Res.*, **61(2)**, 188 (2002).
(21) I. Molina, S. Li, M. B. Martinez, and M. Vert, *Biomaterials*, **22**, 363 (2001).
(22) B. Y. Zaslavsky, *Aqueous Two Phase Partitioning*, Marcel Dekker, New York, 1995.
(23) C. A. Finch, *Chemistry and Technology of Water-Soluble Polymers*, Plenum Press, New York, 1983.