

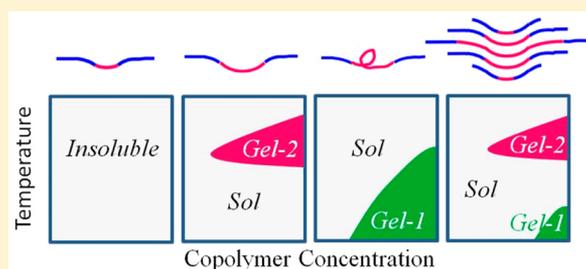
Effects of Molecular Weight and Its Distribution of PEG Block on Micellization and Thermogellability of PLGA–PEG–PLGA Copolymer Aqueous Solutions

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Supporting Information

ABSTRACT: While amphiphilic block copolymers have been extensively investigated, little is known about the effect of molecular weight distribution (MWD) of the hydrophilic blocks on corresponding physical gelation behaviors. Herein, we employed thermogelling poly(D,L-lactide-co-glycolide)-*b*-poly(ethylene glycol)-*b*-poly(D,L-lactide-co-glycolide) (PLGA–PEG–PLGA) as the model system. We synthesized the block copolymer and prepared a series of copolymers with similar PLGA blocks but varied lengths and distributions of the PEG block. The micellization in dilute solutions was detected by light scattering and fluorescence spectroscopy using the probe 1-anilino-8-naphthalenesulfonate (ANS) specifically for the micelle coronae. Vial-inverting observations and rheological measurements were carried out to judge sol or gel states for the concentrated aqueous systems. The gel-to-sol transition or sol-to-gel transition occurred only with appropriate molecular weight (MW) and molar mass dispersity (D_M). In this study, we denote the normal hydrogel with a sol-to-gel transition upon cooling as gel-1, and gel-2 refers to the thermogel with a reversed physical gelation in a sol-to-gel transition upon heating. We found that wider MWD of PEG block sometimes even led to coexistence of normal gel (gel-1) and reversed gel (gel-2). The corresponding concentrated aqueous system of copolymers underwent gel-1-to-sol-to-gel-2 transitions with an increase of temperature. The macroscopic physical gelation was further discussed based upon the mesoscopic micellization and the micellar aggregation.



INTRODUCTION

Most of amphiphilic block copolymers can form micelles in water,^{1–4} and some of them can form physical hydrogels over critical gel concentrations (CGCs).^{5–7} While various factors to influence micellization and physical gelation have been examined,^{8–13} a very common parameter of a synthetic macromolecule, molecular weight distribution (MWD), has not yet been paid sufficient attention. Only a few reports concern the influence of MWD on self-assembly.^{14–17} Eisenberg's group examined the effect of poly(acrylic acid) (PAA) block length distribution on polystyrene-*b*-poly(acrylic acid) vesicles in selective solvents.^{14,15} Ding's group made a corresponding dynamic Monte Carlo simulation of the vesicle formation.¹⁸ Micelles of mixed amphiphilic block copolymers have been experimentally investigated by Chu's group.¹⁹ Hillmyer's group prepared several sets of poly(ethylene-*alt*-propylene)-*b*-poly(D,L-lactide) and found the significant influence of dispersity on self-assembly of the diblock copolymers such as the dependence of domain spacing with MWD.¹⁶ The group led by An has dealt with statistical thermodynamics of the spinodal in polymer solution using the lattice fluid model.²⁰ The theoretical and experimental investigations of the dispersity effects on the melt-phase morphology of block copolymers have been well reviewed by Lynd et al.²¹ Leibler, Hillmyer, and some other researchers have explicitly suggested using dispersity as a

parameter to adjust the morphology and nanostructure of block copolymers.^{21–23}

The present study focused on the effect of MWD on the macroscopic self-assembly of a block copolymer poly(D,L-lactide-co-glycolide)-*b*-poly(ethylene glycol)-*b*-poly(D,L-lactide-co-glycolide) (PLGA–PEG–PLGA) in water. We are interested in PLGA–PEG–PLGA not only due to its rich physics but also due to its great potential as an injectable medical material. The aqueous solutions of block copolymers of polyether and polyester are, under appropriate composition, sols at room temperature and turn to physical hydrogels upon heating.^{24,25} It has been demonstrated via *in vitro* and *in vivo* studies that these thermogels are potentially applied in drug delivery,^{26–33} tissue engineering,^{34,35} postoperative antiadhesion,^{36,37} and so on.³⁸

Previous studies suggested that the internal structure of the thermogel of the block copolymer of polyether and polyester is a percolated micelle network.⁹ This unique reversed gelling with temperature is closely related to thermosensitivity of poly(ethylene glycol) (PEG) in water.^{39,40} We have recently illustrated the effect of the MWD of poly(D,L-lactide-co-glycolide) (PLGA) blocks on solubility of copolymer PLGA–PEG–PLGA

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and the sol-to-gel transition of the resultant aqueous system.⁴¹ The present paper examines the effects of the molecular weight (MW) and MWD of the PEG block on thermogellability of the block copolymers in water. Different from the hydrophobic blocks such as PLGA, hydrophilic blocks such as PEG seem to less influence micellization on the surface. Nevertheless, we herein found that it influenced thermogellability significantly. So, the present study has its own impact as a publication focused on the effect of MWD of hydrophilic blocks on macroscopic self-assembly of amphiphilic block copolymers in water. This polymer physics research might be helpful for guidance of the molecular design of corresponding biomaterials.

We synthesized PLGA-PEG-PLGA copolymers with different chain lengths of the PEG block under a similar length of the PLGA block. A series of copolymer samples with different mass distributions of PEG block were obtained by mixing. The sol-gel transition was examined by vial-inverting approach and rheological measurements. Following the IUPAC suggestion,⁴² we use the term molar-mass dispersity or molecular-weight dispersity (\mathcal{D}_M) instead of polydispersity index (PDI) to quantify the extent of MWD in this paper.

MATERIALS AND METHODS

Materials. A series of poly(ethylene glycol) with different molar masses (PEG400, PEG600, PEG800, PEG1K, PEG1500, PEG2K, PEG3K, PEG4K, and PEG6K) were purchased from Sigma-Aldrich Corporation. D,L-Lactide (LA) and glycolide (GA) were bought from Purac and stored at $-20\text{ }^\circ\text{C}$ before using. Stannous octoate (95%) from Sigma was dissolved in anhydrous toluene with concentration of 30 mg/mL. P123, a Pluronic product of block copolymer poly(ethylene glycol)-*b*-poly(propylene glycol)-*b*-poly(ethylene glycol) (PEG-PPG-PEG) with degree of polymerizations of blocks EG₂₀PG₇₀EG₂₀, was bought from Sigma-Aldrich Company. Methylene dichloride was of chemical reagent grade and purchased from Shanghai Chemical Reagents Co., Ltd., China.

Copolymer Synthesis. The triblock copolymer PLGA-PEG-PLGA was obtained by ring-opening polymerization of LA and GA in the presence of PEG. First the initiator PEG was dehydrated at $120\text{ }^\circ\text{C}$ under vacuum for 4 h; then the monomers LA and GA with molar ratio 8:1 were added at $80\text{ }^\circ\text{C}$ and dehydrated under vacuum for 3 h; third, the toluene solution of the catalyst stannous octoate was added, followed by removing toluene for 30 min. The reaction chemicals were stirred at $140\text{ }^\circ\text{C}$ under an argon atmosphere for 12 h; then the unreacted monomer and residual toluene were removed at $120\text{ }^\circ\text{C}$ under vacuum for other 3 h. Finally, the products were washed three times with water at $80\text{ }^\circ\text{C}$. After lyophilized, the products were stored at $-20\text{ }^\circ\text{C}$ before using.

Multiple-Component Mixing. The PEGs with wider MWD were obtained by mixing. The mixing ratio followed the logarithm normal distribution with the distribution function of molar mass $W(M)$ expressed as

$$W(M) = \frac{1}{\beta\sqrt{\pi}} \frac{1}{M} \exp\left(-\frac{1}{\beta^2} \ln^2 \frac{M}{M_p}\right) \quad (1)$$

Here $\beta = [2 \ln(M_w/M_n)]^{1/2}$ and $M_p = (M_w M_n)^{1/2}$. The PEG mixtures were dissolved in 0.1 M NaNO₃ aqueous solution, and the molecular-weight dispersity (\mathcal{D}_M) was characterized by GPC. The final mixing ratio was calculated based on the GPC results, until the MWD of the mixed PEGs obeyed the logarithm normal distribution with similar number-average molecular weight M_n or weight-average molecular weight M_w .

The copolymers with wider MWD of PEG blocks were obtained by mixing the copolymers with different chain lengths of PEG block. The mixing ratio was calculated from corresponding PEG mixing ratio and the PLGA/PEG ratio of the copolymers. The copolymer mixtures were dissolved in methylene dichloride. Then rotary evaporation and

lyophilizing were carried out to obtain copolymers with wider MWD of PEG block.

GPC Measurements. The MW and \mathcal{D}_M of PEGs were characterized by GPC with a differential refractometer (Agilent 1100). The mobile phase was 0.1 M NaNO₃ aqueous solution. MWs were calibrated with PEG standards. For PLGA-PEG-PLGA copolymers, the mobile phase was tetrahydrofuran and calibrated with polystyrene standards.

Nuclear Magnetic Resonance (NMR). The ¹H NMR spectra of copolymer were recorded in a NMR spectrometer (Bruker, AVANCE 400 MHz). The copolymers were dissolved in CDCl₃ (5 mg/mL) with tetramethylsilane as the internal reference.

Determination of State Diagram. The sol or gel states of the copolymer aqueous solutions at different concentrations or temperatures were determined by the vial-inverting method. We added 0.6 mL of solution into a 2 mL vial. The vials were immersed in a water bath. Temperature was adjusted between 1 and 50 °C. The sample was equilibrated at 1 °C for 3 h before measurement. In the process of heating, equilibration lasted for 15 min at each temperature. If no flow was noticed in 30 s after the vial was inverted, the copolymer aqueous system at this concentration and temperature was judged as in a gel state.⁴³

Dynamic Rheological Measurements. The viscoelasticity of the copolymer aqueous solution was tested in a stress-controlled rheometer (Malvern Kinexus). We added 1.5 mL of solution between a flat plate of diameter 60 mm above a larger basal plate; the gap between the two plates was set as 0.3 mm. Lowly viscous oil was added surrounding the margin of the liquid to minimize volatilization. The heating rate was 1 °C/min, and the angular frequency was 10 rad/s.

Critical Micelle Concentration (CMC). The CMC of the copolymer aqueous solution was measured by the UV absorbance method with a hydrophobic dye 1,6-diphenyl-1,3,5-hexatriene (DPH) as probe. We first added 0.6 μM DPH into copolymer solutions of different concentrations. The solutions were equilibrated in dark for 4 h. Then, the absorbance of the solution from 320 to 400 nm was detected in a UV-vis spectrophotometer (Lambda 35, PerkinElmer). The absorbance increases if the DPH is partitioned into the hydrophobic core upon micelle formation. The CMC value was calculated as the inflection point of the absorbance difference between 377 and 400 nm against logarithm concentration of copolymer solution.

Dynamic Light Scattering (DLS). The micelle sizes of the copolymer in a diluted aqueous solution was measured by laser light scattering (Zetasizer Nano ZS90 Malvern). Prior to measurement, the solutions were filtered through a 0.45 μm filter and equilibrated at each temperature for 15 min. The algorithm of non-negative least-squares (NNLS) was used to calculate distribution of the hydrodynamic sizes of micelles. Because all of the calculated dispersity indexes were less than 0.5, the presented micelle size distributions were simply intensity weighed instead of mass/volume weighting, which needs premeasurement of refractive index and absorption of the copolymer suspension according to the requirement of Mie theory.

Fluorescence Spectra. The water-soluble 8-anilino-1-naphthalenesulfonic acid (ANS) in the form of ammonium salt was used as a nonpolarity probe to investigate the change of micellar coronas as a function of temperature. The ANS aqueous solution was dropped into 10 mL of 0.5 wt % copolymer solution with the final ANS concentration 100 μM and equilibrated at 4 °C for 12 h. The fluorescence emission spectrum was recorded in a fluorescence spectrometer (PTI, QM40) upon excitation at 365 nm. Before measurement, the samples were equilibrated at each temperature for 15 min. Fluorescence spectra of P123 (0.5 wt %) and PEG (M_n 1370, 50 wt %) were also detected for comparison.

Transmission Electron Microscopy. The micelle images were observed in an electron microscope from FEI Company (Tecnai G2 20 TWIN). An ultrathin carbon network was dipped into a 1 wt % copolymer aqueous solution and then dried under an infrared lamp. The acceleration voltage was set as 200 kV.

RESULTS

Synthesis of Copolymer PLGA–PEG–PLGA with Different MWs of PEG Block. The copolymers were synthesized by ring-opening polymerization of LA and GA, and PEG served as the initiator. Copolymers with different PEG block lengths were obtained by using PEGs of different MWs (Figure S1) as initiators. As shown in Figure 1 and Table 1, the

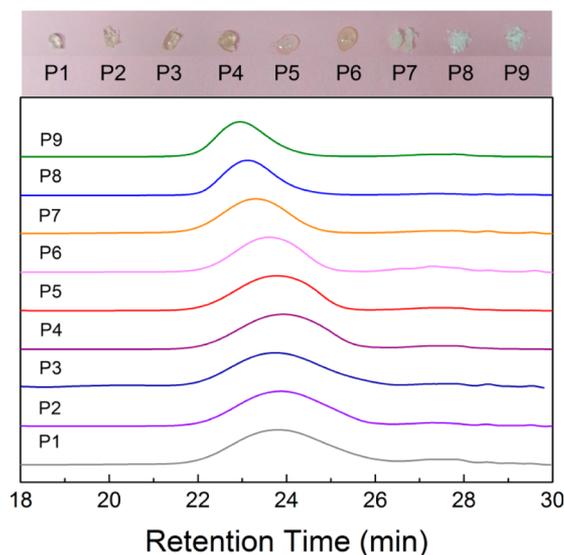


Figure 1. Molecular weight and its distribution of PLGA–PEG–PLGA copolymers. The upper row shows the global views of the copolymer samples at room temperature. The lower row presents their GPC profiles.

copolymers from P1 to P9 exhibited increased M_n 's of PEG block from 370 to 5380. The resultant molar masses of the PLGA block were changed between 1240 and 1670, and the LA/GA ratios were around 8.0, which were both less scattered compared with the hydrophilic block since we controlled the feeding amounts of the monomers LA and GA in polymerization. The M_n values of the PLGA block were calculated from ^1H NMR spectra (as typically presented in Figure S2), based upon the known M_n 's of PEG via GPC before polymerization (Table S1) and the ratios of the NMR peaks of the two blocks in the copolymer after polymerization (Figure S2).

Effects of MW of the PEG Block on Morphology, Solubility, and Thermogellability of the Copolymers. The M_n 's of the PEG blocks from P1 to P4 were less than 1000; the M_n 's of the PLGA blocks were around 1500. Because

of the high weight fraction of the hydrophobic PLGA block, the copolymers took on a blocky solid state and were insoluble in water. P5 and P6 with longer hydrophilic PEG blocks became sticky paste; P7, P8, and P9 with $M_{n,\text{PEG}}$ larger than 2600 exhibited powder morphology, as shown in the upper row of Figure 1 and described in the second-most right column of Table 1.

When the PLGA/PEG weight ratio was smaller than 2, the copolymers P5–P9 were soluble in water. The aqueous system of P5 underwent a sol-to-gel transition upon heating, as indicated in Figure 2a. The modulus of the 25 wt % P5 aqueous

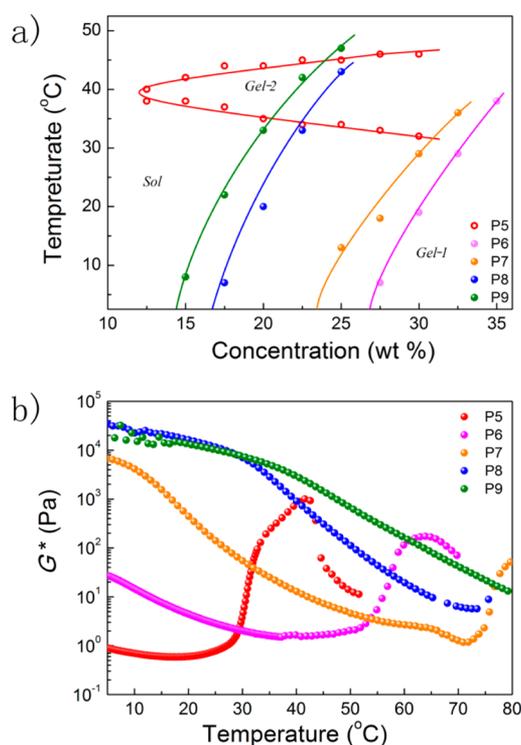


Figure 2. Phase transition of aqueous solutions of the synthesized copolymers with an increase of the PEG block length from P5 to P9. (a) Phase diagrams or state diagrams of the copolymer aqueous systems. Sol or gel states were estimated by the vial-inverting approach. (b) Complex shear modulus (G^*) of corresponding 25 wt % aqueous systems as a function of temperature at heating rate 1 °C/min.

solution was increased abruptly when the temperature was above 30 °C, as recorded in Figure 2b.

In contrast, the aqueous systems of P6 to P9 exhibited the gel-to-sol transition upon heating. With an increase of the PEG

Table 1. A Series of Copolymers with an Increase of PEG Block Length under Similar PLGA Block Length

| copolymer | M_n^a PLAG–PEG–PLGA | D_M^b | M_w^c | PLGA/PEG (M_n/M_n) ^a | LA/GA (M_n/M_n) ^a | morphology | in water |
|-----------|-----------------------|---------|---------|-------------------------------------|----------------------------------|--------------|--------------|
| P1 | 1410–370–1410 | 1.27 | 4050 | 7.60 | 7.5 | blocky solid | insoluble |
| P2 | 1430–590–1430 | 1.27 | 4390 | 4.86 | 7.8 | blocky solid | insoluble |
| P3 | 1540–770–1540 | 1.29 | 4960 | 3.97 | 7.6 | blocky solid | insoluble |
| P4 | 1500–970–1500 | 1.26 | 5020 | 3.09 | 8.6 | blocky solid | insoluble |
| P5 | 1370–1370–1370 | 1.18 | 4850 | 2.00 | 8.9 | sticky paste | sol-to-gel-2 |
| P6 | 1370–1800–1370 | 1.16 | 5250 | 1.52 | 7.8 | sticky paste | gel-1-to-sol |
| P7 | 1500–2630–1500 | 1.15 | 6460 | 1.14 | 7.7 | powder | gel-1-to-sol |
| P8 | 1240–3520–1240 | 1.12 | 6720 | 0.71 | 7.4 | powder | gel-1-to-sol |
| P9 | 1670–5380–1670 | 1.13 | 9850 | 0.62 | 7.5 | powder | gel-1-to-sol |

^a M_n 's, the PLGA/PEG ratios, and LA/GA ratios of the copolymers were calculated from ^1H NMR spectra. ^b D_M 's of copolymers were measured via GPC. ^c M_w 's of the copolymers were calculated from M_n 's and D_M 's.

block length, the gel-to-sol transition happened at lower concentrations and the gel state could maintain at higher temperatures. Along with temperature increase, the modulus decreased at most of detected temperatures for the samples of **P6–P9**. The rheological measurements (Figure 2b) are consistent with the vial-inverting tests (Figure 2a). While the PEG block length increased monotonically from **P5** to **P9**, a sudden change occurred between **P5** and **P6** in Figure 2. We suppose that a gel-1 region hidden in the lower part of the phase diagram for **P5** in Figure 2a and a gel-2 region hidden in the upper parts of the phase diagrams of **P6–P9**. These hidden regions are not available to detect.

Postmixing Preparation of Copolymer PLGA–PEG–PLGA with Dispersity of PEG Block. The copolymers with wider MWDs of the PEG block were obtained by multi-component blending. Resultant GPC profiles for the mixture copolymer **M1** to **M3** along with the unmixed copolymer **P5** are summarized in Figure 3. The data of weight percent for

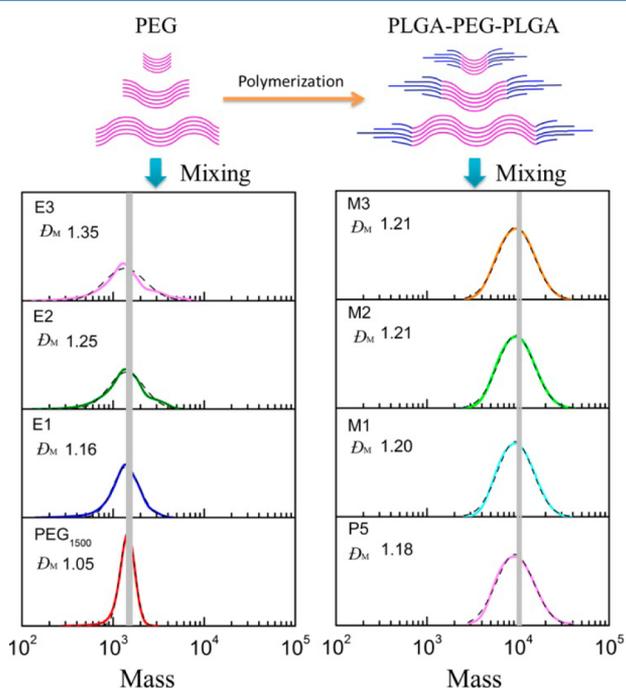


Figure 3. GPC profiles for the mixtures of PEG homopolymers (left) and corresponding mixtures of copolymers (right). The dashed vertical lines were fitting of logarithm normal distribution; the shadow vertical regions indicate the range of the M_w values of these polymers. The mixed PEG blocks have similar MWs with an increase of D_M . The corresponding mixed PLGA–PEG–PLGA copolymers have similar total MWs and D_M 's. The left column can indirectly reflect the MWDs of the PEG blocks in the copolymer mixtures.

mixing are listed in Table 2, which were calculated from the mixing ratios of PEG (Table S2). **P5**, **M1**, **M2**, and **M3** have similar MWs and D_M 's. The PLGA/PEG ratios were all around 2.1, and the D_M 's of PLGA block were around 1.8. The main difference among these copolymer samples was D_M 's of the PEG block, from 1.05 to 1.35.

In Table 2, D_M 's of PLGA block were calculated via

$$D_{M,B} = \frac{M_{n,BAB}^2 D_{M,BAB} - 4M_{n,A} M_{n,B} - 2M_{n,B}^2 - M_{n,A}^2 D_{M,A}}{2M_{n,B}^2} \quad (2)$$

Here we denote PLGA–PEG–PLGA as a BAB type block copolymer. Our derivation is based upon the theory introduced in a review about dispersity and block copolymer self-assembly.²¹ A detailed derivation is described in the Supporting Information.

In principle, the mixing could happen before polymerization (mixing the PEG initiators) and after polymerization (mixing copolymers). We ever tried to obtain the copolymer with different MWDs of the PEG block directly by ring-opening polymerization, using the mixed PEG as the initiator. But the different reactivities among PEGs of different MWs made the MWD of the eventual PEG blocks in the synthesized copolymer uncontrollable (Figure S3). That is why the samples reported in this paper were all from the postmixing. It seems worthy of noting that the GPC profiles of the PEG mixtures in the left column of Figure 3 did not mean that the copolymers shown in the right column of Figure 3 were synthesized using the left PEG mixtures as the initiators but just demonstrated the hidden distribution of PEG blocks in the corresponding block copolymers PLGA–PEG–PLGA after the postmixing.

Effect of MWD of the PEG Block on Thermogelling of Copolymer Aqueous Solutions. Copolymer **P5** with a relatively narrow MWD of the PEG block exhibited sol-to-gel transition upon heating in the detectable temperature and concentration ranges. Very interestingly, for copolymers with a wider MWD of the PEG block, the solutions of **M1**, **M2**, and **M3** underwent not only the sol-to-gel transition but also the gel-to-sol transition. Gel-1 (gel state at low temperatures) and gel-2 (gel state at high temperatures) coexist in Figure 4 for these mixture samples.

We further found that these two gel states exhibited different extents of MWD sensitivity. Gel-1 was more sensitive than gel-2. With the increase of D_M of the PEG block, the gel-to-sol transition happened at significantly lower concentration and higher temperature in the phase diagrams.

The rheological measurements strengthen this finding. Some data are shown in Figure 5. The viscosities and moduli of the copolymer aqueous solutions decreased with the increase of temperature at first, and then abruptly increased, indicating a phase transition; under the same concentration 25 wt %, the viscosities and moduli of the copolymer aqueous systems increased with D_M of the PEG block at low temperatures. G' of **M3** is 1 order of magnitude higher than that of **P5** at the starting temperature (1 °C) in Figure 5, which is consistent with the gel-1 state for **M3** versus the sol state for **P5** at low temperatures in the vial-inverting tests to determine the phase state diagram in Figure 4. All of samples exhibited a sol state at 30 °C. Further heating resulted in the gel-2 state with an abrupt increase of viscosity and modulus. In the gel-2 state, these two basic rheological quantities of the aqueous solution of **M3** with the widest MWD were significantly lower than those of **P5** with the narrowest MWD.

Effect of MWD of the PEG Block on Micellization of Copolymers in Dilute Aqueous Solutions. The critical micelle concentration (CMC) of the copolymer aqueous solution was detected by a hydrophobic probe DPH. The UV absorption of DPH in a copolymer aqueous solution was significantly increased upon micelle formation over CMC. The CMC values did not change significantly with D_M of PEG blocks, as shown in Figure 6a. In contrast, the hydrodynamic radius of the micelle under a given concentration over CMC increased significantly with increasing D_M of PEG block as well as with increasing the block length of PEG block, as illustrated

Table 2. Copolymers with Different MWD of PEG Block

| mixed copolymer | wt % ^a P1–P2–P3–P4–P5–P6–P7–P8–P9 | M_n^b | D_M^c | M_w^c | PLGA/PEG (M_w/M_n) ^b | D_M of PLGA ^d | D_M of PEG ^c |
|-----------------|--|----------------|---------|---------|-------------------------------------|----------------------------|---------------------------|
| P5 | 0–0–0–0–100–0–0–0–0 | 1370–1370–1370 | 1.18 | 4850 | 2.00 | 1.79 | 1.05 |
| M1 | 0–0–7–22–44–22–4–0–0 | 1340–1250–1340 | 1.20 | 4710 | 2.14 | 1.79 | 1.16 |
| M2 | 0–6–11–17–34–23–6–3–0 | 1300–1230–1300 | 1.21 | 4630 | 2.10 | 1.80 | 1.25 |
| M3 | 3–6–12–17–35–17–6–3–1 | 1270–1180–1270 | 1.21 | 4510 | 2.16 | 1.75 | 1.35 |

^aThe mixing weight percent of copolymer was calculated from the mixing weight percent of PEG. ^b M_n 's and the PLGA/PEG ratios were calculated from ¹H NMR spectroscopy. ^c D_M 's were measured via GPC, and M_w 's were calculated from corresponding M_n 's and D_M 's. ^d D_M 's of PLGA block were calculated from eq 2. ^e D_M 's of PEG block were measured via GPC, and the mixing weight percent values are shown in Table S2.

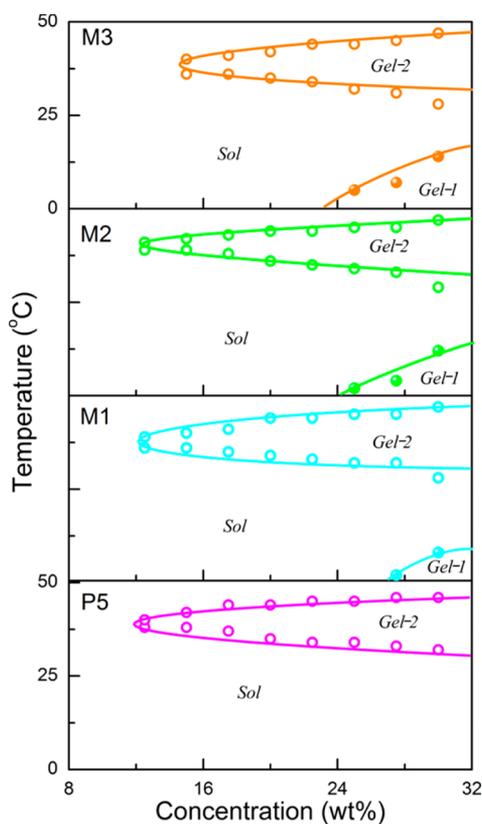


Figure 4. State diagrams of the copolymer aqueous solutions with an increase of D_M 's of the PEG block from P5 to M3. The solid spheres present the gel-1-to-sol transition upon increasing temperature or decreasing concentration. The hollow circles present the sol-to-gel-2 transition and corresponding gel-2-to-precipitate transition. The numerical scales for the temperature axes of the middle two figures are identical to those of the upper and lower two figures and omitted for clarity of the figures.

from the DLS test (Figure 6b). The TEM observations also presented much larger micelles of M3 ($D_{M,PEG}$ 1.35) than micelles of P5 ($D_{M,PEG}$ 1.05), as demonstrated in Figure 6c.

The temperature-induced aggregation of micelles was also monitored by DLS, as indicated in Figure 6b. For P5, the micelle size in 0.5 wt % aqueous solution increased obviously when heated to 35 °C. In contrast, for M3 with a wide MWD, the micelles in 0.5 wt % aqueous solution at 45 °C were even smaller than those at 15 °C and then increased when heated to 55 °C. The different temperature responses of the micelles in dilute aqueous solutions in Figure 6 were consistent with the different state changes of the concentrated solutions in Figures 4 and 5.

Fluorescence Detection Using ANS To Probe Micellar Coronas of Amphiphiles in Water. Fluorescence response is

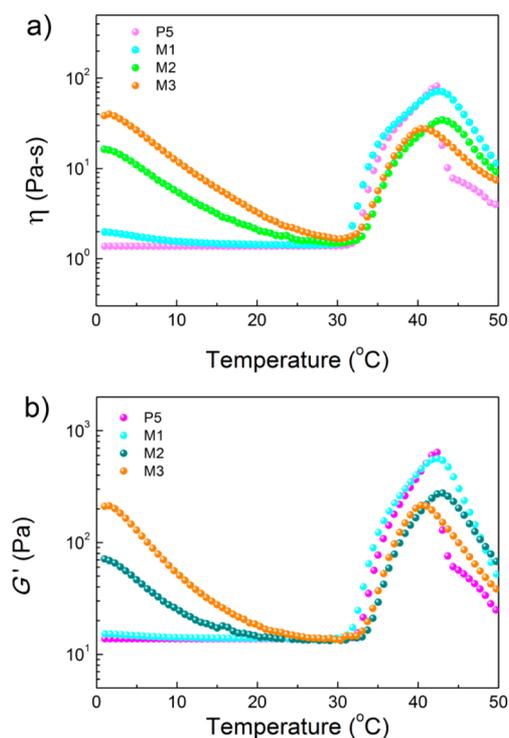


Figure 5. Viscosity (η) and storage modulus (G') of copolymer aqueous solutions as a function of temperature. Concentration: 25 wt %; heating rate: 1 °C/min.

another effective way to investigate the micellar behaviors.^{44,45} The ANS fluorescence is sensitive to nonpolarity of the local medium: decrease of polarity leads to increased quantum yields and blue-shift of the emission maximum. So ANS would be regarded as a nonpolarity probe. This dye has been used to study biomembranes.⁴⁶ It has also been applied to observe coronae of other micelles by other groups such as Wu et al. in monitoring ultrafast heating-induced micellization kinetics of PEG–PPG–PEG in water.⁴⁷ They reported a significant increase of fluorescence signal with heating.

As demonstrated in Figure 7a, ANS emitted only very weak fluorescence in water but quite strong fluorescence upon micelle formation. We confirmed the observation by Wu's group that the ANS fluorescence became stronger at temperatures crossing over critical micelle temperature (CMT).⁴⁷ And we also observed the decrease curve upon further heating for P123 in water, as shown in Figure 7b. Such a difference is reasonable, since Wu et al. observed the kinetics around CMT, and we examined equilibrium behaviors in a broader temperature range. The micelles of the copolymer PLGA–PEG–PLGA exhibited a decreased fluorescence of ANS with temperature, as shown in Figure 7b. The increase curve was hidden

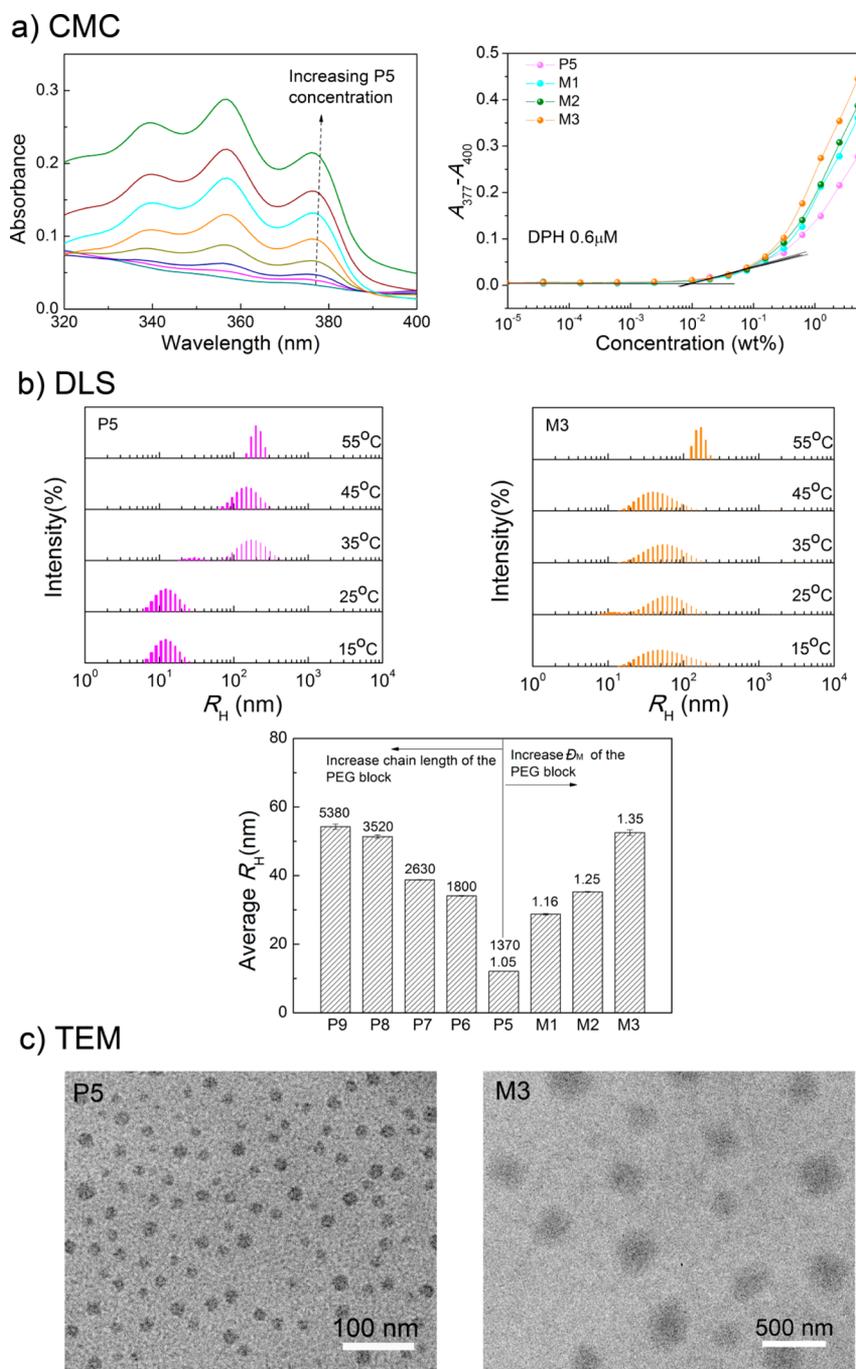


Figure 6. Micellization of copolymers with a series of MWD of PEG block. (a) Typical absorption spectra of DPH in the aqueous solution of the copolymer P5 (left) and UV absorption difference as a function of copolymer concentration for the indicated four copolymer samples (right). The temperature was 30 °C. (b) Hydrodynamic radius distribution of copolymer micelles at indicated temperatures for the copolymers P5 (left) and M3 (right), calculated by the NNLS function. The middle is shown for comparison of the micelle sizes (cummulants mean value) among copolymers with different MWs or MWDs of PEG block illustrated from DLS detection. In the histogram, chain lengths are marked above the columns for P5 to P9, and for P5 to M3, the D_M of PEG block is marked above the corresponding columns. The copolymer concentrations were 0.5 wt %. (c) TEM images. Samples were not prestained; the copolymer concentrations were 1.0 wt %.

just due to a very low CMT for our triblock copolymers in water.

Here ANS was used to detect the local nonpolarity of our micelle corona, where PEG blocks were located. The water-soluble dye ANS is located at the micelle coronae more than micelle cores. Although most of the dye molecules are dissolved in water medium outside micelles, the less polar coronae contributed to the fluorescence very significantly and thus resulted in

strong signals as shown in Figure 7b. We found that even a 0.5 wt % aqueous solution of the copolymer PLGA–PEG–PLGA led to stronger fluorescence of ANS than 25 wt % PEG aqueous solution as shown in Figure 7a, so we deduced that some minor hydrophobic blocks joining in the coronae might also have a contribution.

In order to interpret the decrease of the fluorescence intensity of ANS with temperature above CMT, we tried to observe the

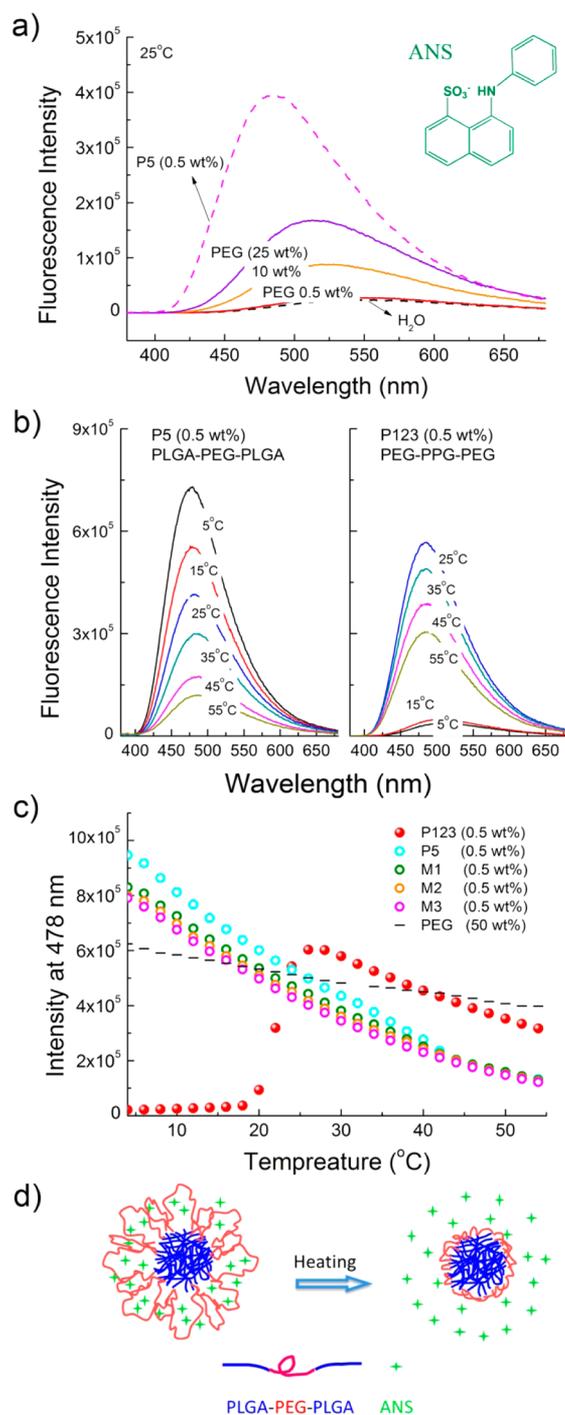


Figure 7. ANS experiments. (a) The fluorescence intensity of ANS in copolymer aqueous solutions as a function of temperature. The molecular structure of ANS is marked on the right side. Its emission intensity increased with PEG1500 concentration at 25 °C. The dashed curve is the fluorescence spectrum in 0.5 wt % P5 aqueous solution. (b) Temperature dependence of the fluorescence spectra of ANS in P5 aqueous solutions (0.5 wt %) or P123 aqueous solutions (0.5 wt %) upon heating. (c) Fluorescence intensity of ANS at 478 nm as a function of temperature. In all of tests, the concentrations of ANS were 100 μM . (d) Schematic presentation of ANS localization in the coronae of PLGA-PEG-PLGA copolymer micelles and the micelle shrinking with increasing temperature.

temperature dependence of this dye in a highly concentrated PEG aqueous solution (50 wt %), with the result shown in

Figure S4c in Supporting Information. A similar decrease trend was observed. This might arise from the decrease of medium viscosity, as shown in Figure S4d. However, the decrease extent in a 0.5 wt % PLGA-PEG-PLGA solution was much more significant than that for a 50 wt % PEG solution (Figure 7c), and the local viscosity in the micellar coronae might be increased along with the shrinkage of PEG chains upon heating. So, the temperature dependence of ANS in the PEG homopolymer solution cannot account for that in the copolymer micelle dispersion. Another candidate reason seems to be related to the dehydration of PEG with increasing temperature. However, the dehydration might, in our opinion, decrease local polarity, for PEG chains eventually precipitate from water at a sufficiently high temperature. So, dehydration can neither account for the temperature dependence of ANS fluorescence in the copolymer micelle dispersion. The main reason might be very simple: the shrinkage of micelle coronae squeezed out a large amount of water-soluble dye ANS, as schematically illustrated in Figure 7d. So, the decreases of the “efficient” amount of dyes due to micelle shrinkage led to the decrease of fluorescence intensity, if the global probe concentration was below the fluorescence quenching concentration.

It seems be worthy of indicating that the above interpretation does not conflict with the DLS detection about the enlargement of apparent “micelles” with increase of temperature in Figure 6b, although one large micelle might encapsulate more ANS than a smaller micelle. Here, the so-called large micelles refer to micelle clusters in our opinion. Considering the total number of the micelles or micelle clusters, the total volume fraction of the micelle coronae might be decreased upon heating, which in turn led to the decrease of ANS fluorescence. Hence, the ANS experiments illustrated the decrease of the total corona fraction upon heating, which is very important in our later discussion about internal structural change underlying the state transition.

And for copolymers with different MWDs of the PEG block, the fluorescence intensity decreased with the increase of \bar{D}_M of the PEG block at low temperatures, as shown in Figure 7c. This reflects the looser micelle coronae of copolymers with wider length distribution of the hydrophilic blocks.

DISCUSSION

MWD is one of the basic molecular characteristics of a synthesized polymer. This study examined the effects of the MW and MWD of the PEG block on the micellization and phase transition of the PLGA-PEG-PLGA aqueous system for the first time. The effects and underlying internal structures of the physical hydrogels will be discussed as follows.

Wide MWD of PEG Block Led to Larger PLGA-PEG-PLGA Micelles and Looser Coronae in Water. PEG is a unique polymer due to its properties, especially its water solubility and increase of hydrophobicity with temperature.^{48–50} The chemical structure of PEG is in favor of formation of hydrogen bonds with water. Considering the structures of poly(butylene oxide) and poly(methylene oxide), the distances between neighboring oxygen atoms are too long or too short to fit with H_2O molecules. So even though poly(methylene oxide) has more oxygens than PEG, it is still insoluble in water. As poly(propylene glycol) (PPG) is concerned, the methyl side group forms a steric hindrance against the hydrogen bonding, so PPG is also a hydrophobic polymer. The natural structure of PEG makes it combine with water at the greatest degree via hydrogen bonding. The highly local ordering of water molecules

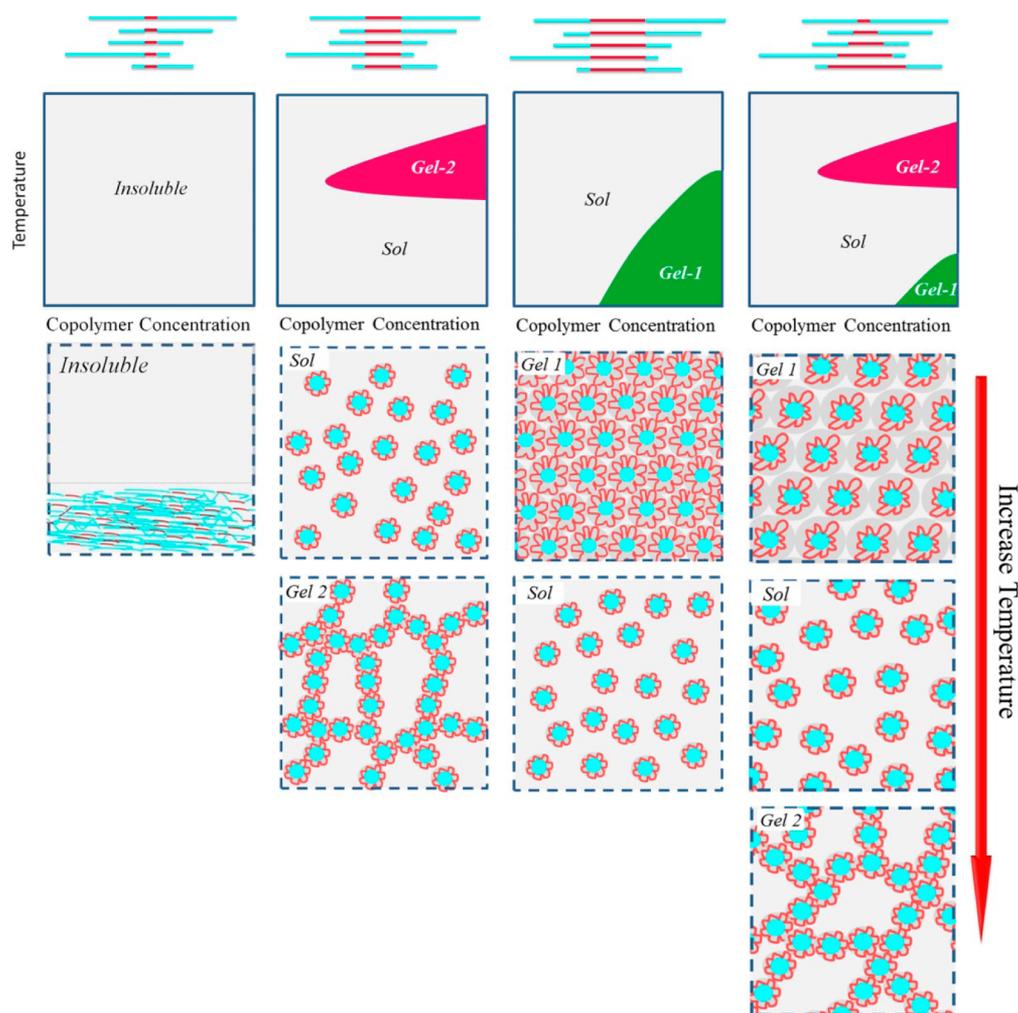


Figure 8. Schematic presentation of the phase transition of aqueous systems of copolymer PLGA–PEG–PLGA with different MWs or different MWDs of the PEG block at different temperatures. The first row shows that copolymers have similar average length of PLGA block but different chain lengths or MWD of the PEG block. The second row demonstrates the state diagrams of these copolymers in water. All of pictures below show the internal mesoscopic structures with respect to those macroscopic states. The copolymer with a small PEG block cannot be dissolved in water at any temperature. The copolymer is soluble in water with an increase of the PEG block length. The solution of copolymer with a narrow MWD of the PEG block is a sol at low temperatures and can spontaneously gel at body temperature. In the case of an even longer PEG block, the aqueous system presents a gel state at low temperature and becomes a sol upon heating. For copolymers with a wider MWD of the PEG block, its aqueous system exhibits, upon heating, a normal gel due to micelle jamming, to a sol (micelle dispersion), and then to a thermogel with a percolated micelle network.

surrounding a PEG chain leads to a significant entropy loss of water. Such an entropy effect contributes to the free energy more at higher temperatures, which is, in our opinion, the origin of the increased hydrophobicity of PEG (shrinkage or aggregation in water) upon heating.

Benjamin Chu's group had investigated the micellization of series of oxyethylene/oxybutylene ($B_nE_mB_n$) triblock copolymers in water.^{2,51} Their micelles were enlarged significantly with the elongation of the hydrophilic PEG block. For our copolymer PLGA–PEG–PLGA, the hydrophilic PEG blocks are also located in the micellar coronae, and the micelle sizes might highly depend on the longer PEG chains in a dispersed sample. So, a wider dispersity of PEG block resulted in larger micelles, as indicated in Figure 6b,c, and looser micelle coronae, as implied by the ANS fluorescence tests in Figure 7c.

Wide MWD of PEG Block Induced Coexistence of Gel-1 and Gel-2 States in Concentrated Block Copolymer Aqueous Systems upon Heating. Aqueous solutions of block copolymers composed of PEG and polyester can, if the concentrations are over CGCs, form either low-temperature gel

(gel-1) or high-temperature gel (gel-2), by adjusting the molecular parameters. According to the study by Lee's group,⁵² methoxy poly(ethylene glycol)-*block*-poly(caprolactone) (MPEG-PCL) exhibited a normal gel in water with the PCL block at molar mass 1750 and the MPEG block between 750 and 5000, and the elongation of the MPEG block led to the gel-1 formation at larger concentration and temperature ranges. Jeong's group found the reversed hydrogel in the aqueous system of PCL–PEG–PCL, and when the PCL/PEG ratio was around 2, an increase of the block length of PEG from 1000 to 1500 shifted the gel-2 window to higher temperatures.⁵³ Jeong's group also examined the thermogelation (similar to formation of gel-2) of poly(ethylene glycol)–poly(L-alanine) block copolymers in water, and a nonmonotonic effect of the PEG block length was found due to the change of secondary structure of the polypeptide.⁵⁴ The combination of this two gelation behaviors in one chemical system was observed by our group in the study of MPEG-PLGA (M_n 2000–2150) by adding a large amount of appropriate salts.⁴⁰ The present study achieved the coexistence of these two gel states in one

copolymer system without dependence of additives, only if a wider MWD of the PEG block was introduced under appropriate PEG and PLGA length ranges. The second gelling in the progress of heating was “reentrant”, so we called the resultant thermogel as a reentrant gel. Such a reentrant physical gelation with the gel-1-to-sol-to-gel-2 transition is very interesting and also affords a model system for the later research in polymer physics and biomaterial sciences.

Gel-1 and Gel-2 Have Different Structures and Interaction Mechanisms. In the PLGA–PEG–PLGA aqueous systems, both normal gel and reversed gel take micelles as the basic building blocks. Gel-1 (normal physical hydrogel) formed at low temperatures along with large micelles. We suggested that gel-1 is formed due to a close packing of plenty of micelles. This is reminiscent of jamming of repulsive colloids in the field of soft condensed matter physics.^{55,56} The decisive parameter is the volume fraction of micelles, and thus the copolymer with bigger micelles can form gel-1 in lower concentrations. A high temperature always enhances the contribution of the hydrophobicity of the PEG block (entropy loss of surrounding water molecules) to the free energy. So, the volume fraction of micelles decreased with an increase of temperature, leading to dissolving of gel-1 upon heating.

Gel-2 formed at high temperatures. For such a physical gelation reversed with temperature, the micelles started to aggregate upon heating. The driving force comes from the hydrophobic interaction. The ratio of PLGA/PEG is the key molecular parameter when holding similar chain length. The temperature sensitivity of PEG might also play a role in the thermogelation. For copolymers from P6 to P9, their PEG blocks are too large to micelle aggregation. So upon increasing temperature, no gel-2 formation was observed until precipitating at high temperature (Figure 2). The series from P5 to M3 were collected to examine the effect of MWD of the PEG block, and the fluorescence intensity of ANS decreased to a low value while temperature was raised over 40 °C, indicating significant shrinking of the micelle coronae of PEG (Figure 7). Along with the micelle shrinkage, the micelle aggregation happened with an increase of temperature, which is reflected by our laser scattering tests (Figure 6b). So, gel-2 occurred if a percolated micelle network was formed in heating.

The micelles in the gel-2 state might not be closely packed. Nevertheless, the micelle core is dense, and the sticking between micelles due to the hydrophobic interaction at high temperature might be much stronger than that of gel-1 phase formed just due to jamming. For gel-1, the micelles are closely packed, but each micelle has a loose corona, and the “building block” is very soft at low temperatures. So the G' value of gel-2 phase is probably higher than that of gel-1 phase, which interprets the rheological behaviors as shown in Figure 5.

Mesoscopic Interpretation of the Phase Transition and the Corresponding MW/MWD Effects. The effects of MW and D_M of PEG blocks on the solubility and the physical gelation of the corresponding aqueous systems are schematically summarized in Figure 8. The chain length of PEG block has, under a similar average length of PLGA block, a great effect on the properties of copolymer aqueous system. When the PLGA block length was around 1500, the copolymer was insoluble if the block length of PEG was under 1000. In the case of the block length of PEG over 2000, the aqueous solution of the copolymer underwent a gel-to-sol transition. The copolymer aqueous system exhibited sol-to-gel transition upon heating, only if the block length of PEG was between

1000 and 2000 for the series of copolymers examined in this paper.

It is then easy to understand why gel-1 is very sensitive not only to MW of PEG block but also to its MWD. With an increase of D_M of PEG block, the copolymer micelles were enlarged, and thus the volume fraction of micelles became higher. So, the gel-1 window was extended to wider temperature and concentration ranges.

Under appropriate composition, a broad MWD might induce a reentrant physical gelling with a gel-1-to-sol-to-gel-2 transition upon heating. Plenty of loose micelles jam to form the normal gel at low temperatures. With the decrease of the total volume fraction of micelle coronae upon heating, the jammed state cannot be maintained, and micelle dispersion occurs. Further heating leads to micelle aggregation, and gel-2 appears if a percolated micelle network is formed. The extremely high temperature always over amplifies the hydrophobic interaction and results in precipitation, and such an ordinary state is not depicted in Figure 8.

CONCLUSIONS

This is the first report of the effect of MWD of PEG block on the phase transition of an amphiphilic copolymer aqueous system. We reveal that even the hydrophilic blocks can influence the macroscopic self-assembly of amphiphilic block copolymers to a large extent. The MWD of the PEG block has great effects on the micelle sizes and global condensed states of copolymers in selective solvents. A wider MWD of PEG block enlarged copolymer micelles, and the corresponding concentrated aqueous solution became more viscous and even physically gelled at low temperatures. The block copolymer with a relatively low D_M exhibited a gel-1-to-sol transition (normal gelling) or sol-to-gel-2 transition upon heating (reverse gelling), depending on molecular composition including the length of PEG block. A large D_M even induced a reentrant physical gelation with gel-1-to-sol-to-gel-2 transitions. Hence, MWD is not just a flaw of synthetic polymers but offers a regulator of mesoscopic and macroscopic states of macromolecules.

ASSOCIATED CONTENT

Supporting Information

Molecular information on PEG and the mixed PEG used in this study; ¹H NMR spectrum of a PLGA–PEG–PLGA copolymer; the GPC profiles of PEG homopolymer mixtures and the corresponding supernatant products during the purification progress of synthesizing copolymer PLGA–PEG–PLGA using this PEG mixture as initiator; fluorescence intensity of ANS in water and in 50 wt % PEG solution; viscosity of the 50 wt % PEG solution as a function of temperature; derivation of formulas to calculate molar mass dispersity of each block in block copolymers. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.macromol.5b00168.

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Notes

The authors declare no competing financial interest.

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