Roles of Hydrophilic Homopolymers on the Hydrophobic-Association-Induced Physical Gelling of Amphiphilic Block Copolymers in Water

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ABSTRACT: Recently, the addition of poly(ethylene glycol) (PEG) homopolymers was found to significantly lower the sol−gel transition temperature T_{gel} of the aqueous system of a block copolymer composed of poly(lactic acid-*co*-glycolic acid) (PLGA) and PEG, different from the "normal" phenomenon of a T_{gel} increase for Pluronic hydrogels of block copolymers of PEG and poly(propylene glycol) (PPG). While such an effect is useful for applications of PLGA−PEG−PLGA physical hydrogels as an injectable biomaterial, it is puzzling why the addition of a hydrophilic homopolymer probably enhances the hydrophobic-association-induced physical gelation of an amphiphilic block copolymer. A series of PEG homopolymers with different molecular weights and concentrations were examined in the aqueous systems of PEG/PLGA block copolymers with three compositions in the present paper. An immiscibility mechanism was put forward by us to account for the earlier spontaneous thermogelling under the assumption of the structure of a randomly percolated micelle network. Meanwhile, the late physical gelling of Pluronics in the presence of PEG homopolymers was interpreted by an order-interfering mechanism. We further found that the immiscibility mechanism might also work for Pluronics upon addition of ethylene glycol and oligo(ethylene glycol).

Introduction

Amphiphilic block copolymers in water can not only form micelles[1,2] but also spontaneously develop into a physical gel in some cases, for instance, upon a thermoreversible sol−gel transition.[3−6] The thermogelling polymers have been of great interest for applications in drug delivery[7−10] and tissue engineering[11] as a novel unique injectable biomaterial and in other fields such as biomolecular separation.[12] The pertinent fundamental researches of the gelling behaviors and structure−property relationship have gotten to be an interesting topic in polymer physics.[12−22]

Some physical gels might result from the formation of percolated micelle networks. Two exampled thermogelling systems pregnant of micelles are concentrated aqueous suspensions of block copolymers composed of poly(ethylene glycol) (PEG) and either poly(propylene glycol) (PPG)[6,12,16−18] or an aliphatic polyester.[3,8−10,13−15,19] It has been found that their phase diagrams could be adjusted by many factors such as chain compositions,[6,19,23] end groups,[24] and salt additives.[19,25] Interestingly, the addition of hydrophilic PEG homopolymer was also found to lead to earlier[19] or later[16] thermogelling. In 1993, Malmsten and Lindman found that PEG homopolymers could increase the gelling temperature of a PEG−PPG−PEG triblock copolymer, Pluronic F127.[16] In contrast, an early gelling phenomenon induced by the addition of PEG homopolymers was observed by Jeong et al. when they studied the thermoreversible gelation of an aqueous solution of a triblock copolymer composed of PEG and biodegradable poly(lactic acid-*co*-glycolic acid) (PLGA) in 1999.[19] Such a kind of polyester−polyether block copolymers is biodegradable and biocompatible and exhibits great promise as an injectable biomaterial.[9,14,23,24,26−33]

A question thus arises: why does the addition of a hydrophilic homopolymer enhance the hydrophobic-association-induced physical gelling of an amphiphilic block copolymer in water? Jeong et al. anticipated that PEG homopolymers might act as bridges between PEG−PLGA−PEG micelles and thus lead to gelation at a lower temperature.[19] While their pioneering work of this polymeric system is important and stimulating, such a bridging mechanism is, in our opinion, questionable because we cannot find any driving force to make a free PEG chain attach to either a PLGA core or a PEG corona. The elucidation of the underlying mechanism is required.

This paper is just focused on answering the above question. Two PLGA−PEG−PLGA triblock copolymers and a PEG−PLGA diblock copolymer (Scheme 1) were employed as the model block copolymers. In synthesis of the diblock copolymer, monomethoxypoly(ethylene glycol) (MPEG) was used. Both concentration and molecular weight (MW) effects of the addition of PEG homopolymers into the copolymer aqueous systems were examined. We also observed the Pluronic F127/PEG/water system for comparison. Our analysis illustrates that the bridging mechanism should be taken into minor consideration, and our interpretation of the underlying physical gelation will be given.

An appropriate gelling temperature is crucial for an injectable medical hydrogel. A simple mixing with PEG homopolymers, which have been approved to be used in an implantable material by Food and Drug Administration (FDA), affords a convenient approach to tune the sol−gel transition temperature of the corresponding block copolymer aqueous system. The ”mixing” approach is much more practical than chemical modification in medical applications. Considering also that the mechanism of physical gelation is a challenging fundamental problem, the studies of the present paper are thus meaningful in both biomaterials and polymer physics.

Experimental Section

Materials. Ethylene glycol (EG), PEG with varied MWs (PEG 200, PEG 400, PEG 800, PEG 1K, PEG 2K, PEG 4K, PEG 6K, PEG 8K, and PEG 10K), and a poly(ethylene glycol) methyl ether (MPEG 550) were products of Sigma. DL-Lactide (LA) and glycolide (GA) were purchased from Purac and used without further treatments. Stannous 2-ethylhexanoate (stannous octoate) was obtained from Sigma and used as received. All other chemicals

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Scheme 1. Chemical Structures of PLGA−PEG−PLGA Triblock Copolymer and MPEG−PLGA Diblock Copolymer

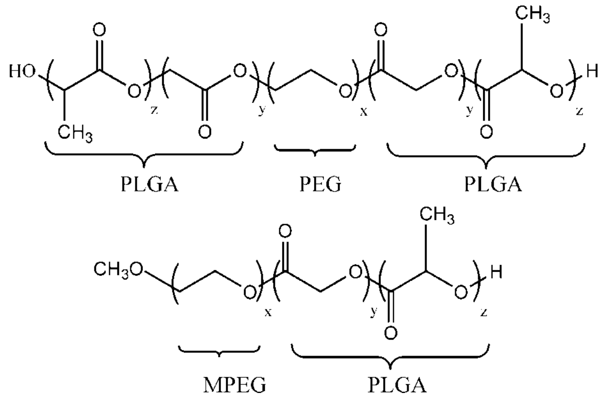

Table 1. Characteristics of the Synthesized PLGA−PEG−PLGA and MPEG−PLGA Block Copolymers

sample	M_n[a]	PEG M_n[a]	LA/GA[a]	M_w[b]	M_w/M_n[b]	CMC[c] (wt %)
Copolymer-1	1280−1000−1280	1000	1.9/1	4100	1.21	0.02
Copolymer-2	1709−1500−1709	1500	15/1	6110	1.21	0.05
Copolymer-3	550−1540	550	3/1	2630	1.25	0.03

[a] The number-average MW, M_n, of PEG was provided by Aldrich. The molar ratio of lactide/glycolide (LA/GA) and M_n of each PLGA block were calculated by ^{1}H NMR. [b] M_n of block copolymers and their polydisperse indexes denoted by weight-averaged MW M_w over M_n were measured via GPC. [c] Measured by the hydrophobic dye method at $T = 25$ °C.

were reagent grade and used as purchased without further purification.

Synthesis. The details of synthesis and characterization of the triblock copolymers and diblock copolymer could be found elsewhere.[23] Briefly, ring-opening polymerization of LA and GA in the presence of PEG or MPEG was performed to synthesize PLGA−PEG−PLGA triblock copolymers or MPEG−PLGA diblock copolymer using stannous octoate as a catalyst. ^{1}H NMR spectrometry (Bruker, DMX500) and gel permeation chromatography (GPC, Agilent1100) were used to determine the structure, composition, and MW of the copolymer. $CDCl_3$ was as solvent in ^{1}H NMR measurements; tetrahydrofuran was as eluent and polystyrene as standard in GPC measurements. The critical micelle concentration (CMC) in an aqueous solution was also measured via the hydrophobic dye method, using 1,6-diphenyl-1,3,5-hexatriene (DPH) as the probe.[28] The basic characteristic results of the triblock copolymer are listed in Table 1.

Phase Diagram Determination. The phase diagrams of the PLGA/PEG block copolymer aqueous solutions with and without addition of PEG homopolymers were measured by the tube inverting approach. 0.5 mL of the polymer solution was added into each 2 mL vial. All vials were sealed and kept individually in a water bath and then heated by a step of 1 °C from 5 to 60 °C. Each temperature was kept for more than 15 min to ensure samples to reach equilibrium. A gel was thought to be formed, if no visual flow was observed 30 s after a vial was inversed, while a precipitation state occurred when excess water was excluded out of the gel. The sol−gel transition temperature (T_{gel}) and gel-precipitation transition temperature ($T_{precipitation}$) were both measured in a heating process. The results were reproducible within ±1 °C.

Rheological Measurements. Viscoelastic measurements were performed on a strain-controlled rheometer (ARES, Rheolometric Scientific). The rheometer was equipped with two force transducers for torque measurements ranging from 0.02 to 2000 g·cm. Samples were transferred from bottles to a Couette cell (Couette diameter, 34 mm; bob diameter, 32 mm; bob height, 33.3 mm; bob gap, 2 mm) before measurements. The narrow gap ensured a virtually constant shear rate. The outer Couette was in a thermostat bath linked to an environment controller (Neslab, RTε130) with an accuracy of ±0.05 °C. The surface was overlaid with a thin layer of low-viscosity silicone oil to minimize solvent evaporation. In the oscillatory shear mode, the storage modulus G' and loss modulus G'' were collected as a function of temperature from 5 to 40 °C with heating and cooling rates of 0.5 °C/min and an angular frequency ω of 1 rad/s. The strain amplitude was set at a suitable value determined by preliminary experiments to ensure the linearity of viscoelasticity depending on the temperature (usually 200% and 10% before and after gelation, respectively).

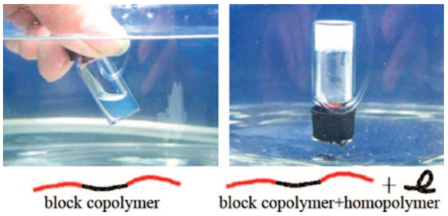

Figure 1. Photographs showing the sol and gel states of PLGA−PEG−PLGA triblock copolymer (Copolymer-1) in water. The left image refers to the block copolymer with 15% weight concentration and the right image to the same block copolymer, but with addition of 2 wt % of PEG 6K. $T = 17$ °C.

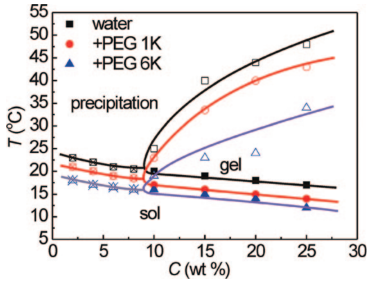

Figure 2. State diagrams of PLGA−PEG−PLGA triblock copolymer (Copolymer-1)/water systems before and after addition of 2 wt % PEG homopolymers with MWs indicated in the legend. The sol−gel and gel−precipitation transition boundaries (solid and open symbols, respectively) were determined by the tube inverting approach. The sol−precipitation transition (cross symbols) was determined by measurements of turbid temperatures.

Turbid Point Measurements. The measurements of turbid points followed the way in the literature.[32] The temperature dependence of transparency was used to determine the turbid temperature T_{turbid}. Samples were kept in a water bath. The temperature was raised with a step of 1 °C from 10 to 25 °C. At each temperature, 10 min was kept to ensure equilibrium. Then the optical density (OD) at 650 nm was recorded. T_{turbid} was determined by the crossover point of two extrapolation lines in the OD−temperature plot.

Results

Phase Diagrams Determined by the Tube Inverting Approach. Most of the presented data resulted from Copolymer-1, while similar behaviors were observed for Copolymer-2 and Copolymer-3. Figure 1 illustrates the typical optical images of the sol and gel states of PLGA−PEG−PLGA triblock copolymer (Copolymer-1) in water. The left image was 15 wt % triblock copolymer in water, exhibiting a typical sol behavior. In contrast, it became a gel after the addition of PEG homopolymer, as shown in the right image. This implies that the addition of PEG homopolymers might cause the sol−gel transition to a lower temperature.

The state diagrams with and without PEG homopolymers are illustrated in Figure 2. Each state diagram is made of three regions (sol, gel, and precipitation) divided by three transition boundaries (a sol−gel transition, a gel−precipitation transition, and a sol−precipitation transition). The addition of PEG homopolymers shifted all three boundaries to lower tempera-

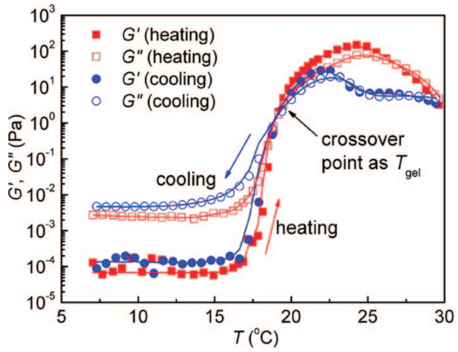

Figure 3. Storage modulus G' and loss modulus G'' as a function of temperature T of the PLGA−PEG−PLGA triblock copolymer (Copolymer-1) aqueous system. C = 10 wt %. Oscillatory frequency ω = 1 rad/s. Shear strain amplitudes were 200% and 10% before and after T_{gel}, respectively. Heating and cooling rates: 0.5 °C/min. The solid lines are just for guidance of eyes.

tures. Two of the boundaries (sol−precipitation and gel−precipitation) were relatively more sensitive to MW of PEG homopolymers. According to our previous reports, the gel region could further be divided into two subregions corresponding to transparent gels and opaque gels.[23] In the presence of PEG homopolymers, this phenomenon was also observed and the region of transparent gels was narrower. We also found that the critical gelation concentration (CGC) remained almost unchanged after the addition of PEG homopolymers.

Rheological Measurements. The storage modulus G' and loss modulus G'' reflect the energy stored and that dissipated during a cyclic deformation, respectively. The results obtained in both heating and cooling processes of Copolymer-1 are shown in Figure 3. In the sol state, G'' was much larger than G', exhibiting a typical liquid behavior. At about 16 °C near the sol−gel transition, both moduli G' and G'' increased abruptly until G' exceeded G'', and thus the energy stored in the system was larger than that lost. In polymer physics, this crossover point denotes the sol−gel transition temperature (T_{gel}).[34] In Figure 3, T_{gel} as defined above was about 19.1 °C for a 10 wt % triblock copolymer solution without any addition of PEG homopolymers and close to the gelling temperature we got from the tube inverting approach, 20 °C. After G' and G'' reached the maximum values in heating (139 and 84 Pa at about 24 °C, respectively), they soon decreased with a trend to the gel−precipitation transition. Figure 3 also indicates a bit hysteresis upon cooling following heating. Nevertheless, the reheating coincided with the first heating very well if the cooled sample was stored overnight (data not shown). Such a physical gelation is thus thermoreversible.

The concentration effect of PEG homopolymers on the rheological properties of Copolymer-1 aqueous solutions is illustrated in Figure 4. With the increase of PEG concentration, the T_{gel} was decreased. The increase of the loss modulus and thus the viscosity at the sol state should also be kept in mind, when the injectability of such a mixture material was evaluated in potential medical applications.

Molecular Weight Effects of PEG Homopolymers. The above experiments not only confirm the existent results in the literature[19] but also show some new features in this ternary system, which will be discussed later. To further understand the mechanism of the PEG homopolymer effects, a series of PEG homopolymers with MW ranging from 200 to 8000 as well as the associated small molecular counterpart, ethylene glycol (EG), were, for the first time, investigated by both the tube inverting approach and more quantitative rheological measurements. Six data sets of dynamic rheological tests of Copolymer-1 aqueous samples are shown in Figure 5. Similar to PEG homopolymers, the small molecular additive EG lowered T_{gel} although the effect was not so significant than their larger molecular counterparts. Another important feature in this figure is the dashed line which connects the crossover points of G' and G'' (T_{gel}). When the additives changed from EG to PEG 400, T_{gel} decreased from about 18 °C to about 17 °C. T_{gel} was around 16.3 °C when the MW of PEG homopolymer was larger than 800. This was valid from PEG 1K to PEG 10K investigated

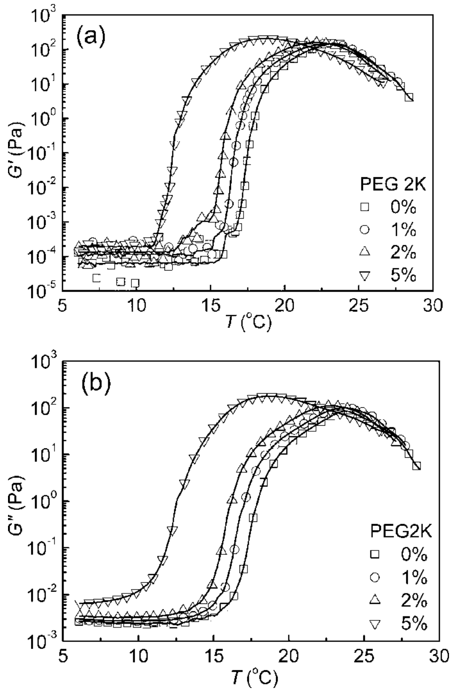

Figure 4. Storage modulus G' (a) and loss modulus G'' (b) as a function of temperature T of the aqueous systems of PLGA−PEG−PLGA triblock copolymer (Copolymer-1) with and without addition of PEG 2K. The block copolymer concentration: C = 10 wt %. Oscillatory frequency: ω = 1 rad/s. Shear strain amplitudes were 200% and 10% before and after T_{gel}, respectively. Heating rate: 0.5 °C/min. Legends are the indication of weight concentrations of the PEG 2K. The solid lines are just for guidance of eyes.

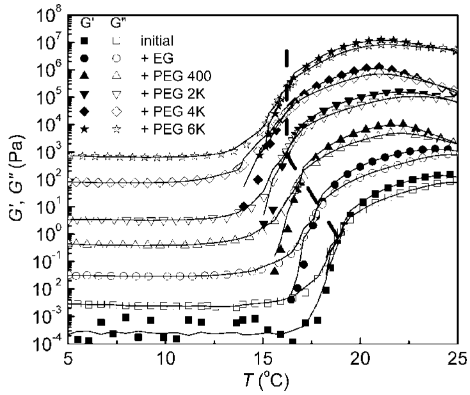

Figure 5. Molecular weight effect of PEG homopolymers added (2 wt %) on gelation temperature T_{gel} of the PLGA−PEG−PLGA triblock copolymer (Copolymer-1) aqueous systems (C = 10 wt %). T_{gel} here was determined as the crossover point of storage modulus G' (solid symbols) and loss modulus G'' (open symbols). The dashed line connects those crossover points. Oscillatory frequency: ω = 1 rad/s. Shear strain amplitudes were 200% and 10% before and after T_{gel}, respectively. Heating rate: 0.5 °C/min. Data were vertically shifted by a factor b_T to ensure clarify and avoid overlap (water: $b_T = 10^0$; EG: $b_T = 10^1$; PEG 400: $b_T = 10^2$; PEG 2K: $b_T = 10^3$; PEG 4K: $b_T = 10^4$; PEG 6K: $b_T = 10^5$). Some data sets of G' at low temperatures were omitted to keep clarity. The solid and dashed lines are just for guidance of eyes.

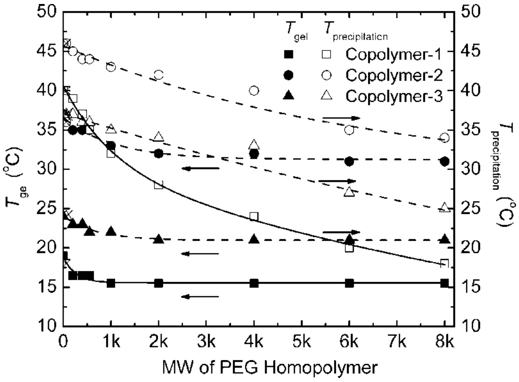

Figure 6. Sol−gel transition temperatures T_{gel} and gel−precipitation temperature $T_{precipitation}$ of the PLGA−PEG−PLGA triblock copolymers (Copolymer-1 and Copolymer-2) and MPEG−PLGA diblock copolymer (Copolymer-3) aqueous systems as a function of MW of PEG homopolymers. The cross symbols in the plot were from the small molecular EG. The data corresponding to the zero MW of PEG homopolymer come from the samples without addition of any PEG homopolymer or EG. The concentration of block copolymer $C = 15$ wt %, while $C = 2$ wt % for all the additives, either EG or PEG homopolymers. All data were determined by the tube inverting approach. The solid and dashed lines are just for guidance of eyes.

in our experiments. The transition temperatures determined via the tube inverting approach are basically consistent with the rheological measurements.

We also determined gel−precipitation transition temperatures $T_{precipitation}$ under different MWs of PEG homopolymers. As shown in Figure 6, $T_{precipitation}$ was lowered with the increase of MW of PEG homopolymer while T_{gel} was changed less significantly in the case of relatively high-MW homopolymers, and thus the associated gel window became narrower —$T_{precipitation} - T_{gel}$ varied, for instance, from about 20 °C without PEG homopolymers to 3 °C with the addition of PEG 8K (2 wt %) for Copolymer-1. This was more significant when the concentration of PEG homopolymers increased (1−5 wt % examined in this work, data not shown). For some samples with high concentration and large MW of PEG homopolymers, the sol−gel transition was even immediately followed by the gel−precipitation transition. It illustrates that the addition of high-MW PEG homopolymers would cause macroscopic phase separation in this system.

Now we turn to examine the nongelling region with concentrations of triblock copolymer lower than its CGC. These samples varied from clear transparent solutions to turbid viscous suspensions upon heating. The turbid temperature T_{turbid} was used to characterize the sol−precipitation transition boundary. Figure 7 illustrates the effect of MW of PEG homopolymers (2 wt %) on the turbid temperature for 8 wt % aqueous systems of PLGA−PEG−PLGA triblock copolymer (Copolymer-1). T_{turbid} with copolymer concentrations lower than CGC had a similar trend of MW dependence to $T_{precipitation}$ with copolymer concentrations higher than CGC. The former was considered to be related to the aggregation or association of micelles to form large clusters with size comparable with the wavelength of visible light. Thus, no matter whether the concentration of triblock copolymer is higher or lower than CGC, PEG homopolymers seem to promote micelle aggregation.

Effects of PEG Homopolymers on Thermogelling of PEG/PLGA Block Copolymers with Altered Composition. Now we extended our observations to block copolymers with a bit different molecular weight and chain architecture. Coplymer-2 and Copolymer-3 with appropriate compositions to make thermogelling available in the normal temperature range were examined. As shown in Table 1, Copolymer-2 has a larger MW as compared to Copolymer-1, and Copolymer-3 is a diblock copolymer with one hydroxyl end group replaced by a methoxyl group. Figure 8 illustrates the phase diagrams of Copolymer-2 and Copolymer-3 with and without the addition of PEG homopolymers. The addition of a PEG homopolymer shifted both sol−gel and gel−precipitation transition boundaries to lower temperatures for both Copolymer-2 and Copolymer-3 (Figure 8), similar to the trend for Copolymer-1 (Figure 2). Again, the CGCs remain unchanged, which reinforces our above observations about CGC in Copolymer-1 system. The MW effects of PEG homopolymers on Copolymer-2 and Copolymer-3 are shown in Figure 6. A similar change trend was again observed among the cases of three block copolymers with an only exception that a crossing between the curves of $T_{precipitation}$ vs MW of PEG homopolymer in the cases of Copolymer-1 and Copolymer-3 was observed in this figure. Nevertheless, we found, basically, similar effects of addition of PEG homopolymers on the thermogelling of PEG/PLGA block copolymer aqueous systems examined so far, although the chain architecture, MW, and end group of the block copolymers influence their gelling behaviors to a certain extent.

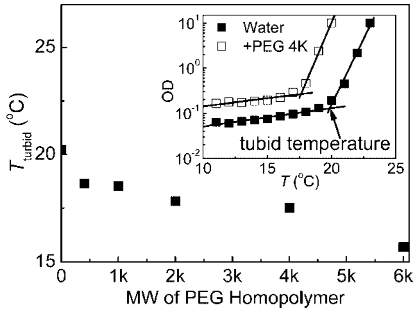

Figure 7. MW effect of PEG homopolymers (2 wt %) on the turbid temperature T_{turbid} of the Copolymer-1 aqueous systems below critical gelation concentration (CGC). The concentration of copolymer $C = 8$ wt %. The inset is two typical plots of the measurement of T_{turbid} from the plot of optical density at 650 nm vs temperature for the initial sample without the addition of any PEG homopolymer and a sample added with PEG 4K.

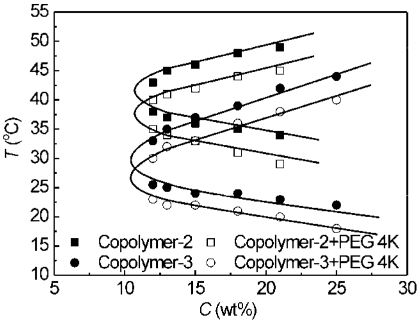

Figure 8. State diagrams of PLGA−PEG−PLGA triblock copolymer (Copolymer-2)/water system and MPEG−PLGA diblock copolymer (Copolymer-3)/water system before (solid symbols) and after (open symbols) addition of 2 wt % PEG 4K homopolymer. The sol−gel and gel−precipitation transition boundaries were determined by the tube inverting approach.

Molecular Weight Effect of PEG Homopolymers on the Sol−Gel Transition of Pluronic Aqueous Systems. A comparative investigation of a Pluronic F127/PEG homopolymer/water system was also performed. In contrast to the report that the addition of PEG homopolymers increased gelling temperatures of Pluronic F127,[16] our systematic investigations revealed that the influence of PEG homopolymers and EG on T_{gel} of Pluronic F127 was rather complex. While PEG homopolymers with relatively large MWs (≥4K) could greatly increase T_{gel}

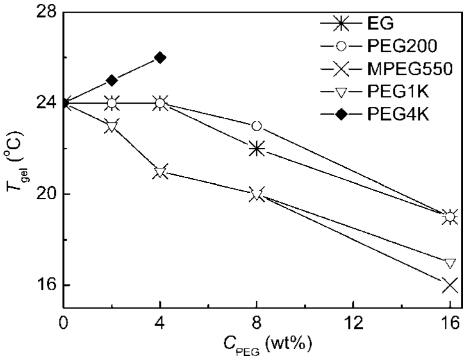

Figure 9. Sol−gel transition temperature T_{gel} of a PEG−PPG−PEG triblock copolymer (F127, C = 20 wt %) as a function of the concentration of EG, PEG 200, MPEG 550, PEG 1K, and PEG 4K. The sol−gel transition behavior did not occur in some samples with high concentrations of PEG 4K (>4 wt %), and thus the associated T_{gel} data points were not available.

and even no gelation occurred for some samples with higher concentrations of PEG 4K (≥8 wt %), EG and oligo(ethylene glycol) with small MWs of PEG homopolymers (≤1K) lowered T_{gel}. A similar result was seen for PEG 400 on T_{gel} of F127.[35] Figure 9 also demonstrates the significant effect of homopolymer concentrations.

Discussion

Our discussion is focused upon the sol−gel transition temperature T_{gel} since this transition temperature is the most important for an injectable biomaterial. The decrease of sol−gel transition temperature by the addition of PEG homopolymers was found in PEG−PLGA−PEG triblock copolymers by Jeong et al.,[19] in PLGA/PEG copolymers (either triblock copolymer or diblock copolymer with different MWs), and also in some cases of PEG−PPG−PEG (Pluronic F127) by us in this paper. Thus, the detail molecular characteristics such as composition and block sequence do not seem to be the essence of the PEG homopolymer effect. It might be possible for giving a general explanation for the observed changes. In the following paragraphs, we would like to list the candidate mechanisms, most of which will soon be ruled out.

Medium Viscosity. One of the most trivial reasons might be the enhancement of medium viscosity to a certain extent so that it takes more time for the liquid−air surface in the vial to flow when a vial is turned vertically in the tube inverting measurement, causing a misjudging of T_{gel}. It seems reasonable since the addition of high-MW polymer must increase medium viscosity significantly. However, the same effects of small molecular PEGs and even EG were observed by us (Figures 5 and 6), which disapproved this assumption. Our quantitative dynamic rheological measurements directly rule out such an interpretation even for the addition of high-MW PEG homopolymers (Figures 4 and 5).

Water State. In principle, one could also anticipate that the effect of PEG homopolymers on the early gelling of PLGA−PEG−PLGA triblock copolymer is due to the increase of effective water polarity in the presence of PEG homopolymers. Then, the micelle formation might be more favored since the driving force of micellization is the poor solvency conditions for the PLGA block in water, and thus T_{gel} might be lowered. However, this potential explanation is not justified. Investigations about solute−solvent interactions in the PEG/water system by solvatochromic probes have found a decrease, instead of an increase, of solvent polarity in this binary system for all of the PEG molecular weight studied, varying from PEG 200 to PEG 6K.[36]

Chain Bridging. To date, the public interpretation of earlier thermogelling of block copolymers of PLGA and PEG after addition of PEG homopolymer was proposed by Jeong et al.[19] They ascribed the decrease of T_{gel} to the "bridge effect". It was anticipated that PEG homopolymers could interact with the partially exposed PLGA cores intermixed with PEG coronas, and thus the intermicellar interaction was enhanced by PEG homopolymers acting as bridges between different micelles, resulting in earlier physical gelling.[19]

We doubt this interpretation as follows. First, there is no physical basis of the driving force for a PEG homopolymer chain attached to either PLGA cores or PEG coronas. Second, even though the topological entanglement between PEG homopolymers and the PEG blocks in the corona might serve as a driving force, the effects of EG and low-molecular-weight PEGs (Figures 5−7) can never be interpreted by the entanglement bridging. The entanglement, if existing for a relatively long PEG homopolymer, might be sensitive to the molar concentration of the PEG additive. However, the different molar concentrations upon a constant weight concentration have not led to significantly different gelling temperatures, as shown in Figures 5 and 6. Last but not least, if the "bridge effect" was justified, the critical gelation concentration must be lowered in the presence of PEG homopolymers because fewer micelles were then required to form a percolated network. According to our observations, CGC was unchanged upon addition of PEG homopolymers (Figures 2 and 8). A more convincing experiment was designed by us. We had ever prepared a series of samples with a given concentration (8 wt %) of Copolymer-1 just a bit below CGC but varying the concentrations of PEG homopolymers from 1 to 20 wt %. In the tube inverting experiment, none of the samples formed hydrogels upon heating. On the basis of these arguments, the "bridge effect" might be of minor importance.

An Immiscibility Mechanism. We suggest that the immiscibility between the PEG homopolymers and the hydrophobic blocks in amphiphilic block copolymer chains might account for the decrease of T_{gel}. A direct experimental evidence of such a toward-immiscibility driving force comes from the phenomenon of precipitation upon an excessive addition of PEG homopolymers into block copolymer aqueous systems and the further analysis of chemical composition of precipitates and the solid content in the supernatant. When 10 wt % PEG 4K was added into a 12 wt % Copolymer-1 aqueous solution, precipitation occurred at all of examined temperatures. After freeze-drying, the solute in the supernatant became powder-like (PEG crystals), while the precipitation was semisolid (PLGA−PEG−PLGA). The ^{1}H NMR measurements revealed that the powder contained mainly PEG 4K, while the semisolid just contained mainly the copolymer and a few PEG homopolymer (data not shown). The macroscopic phase separation demonstrates an apparent repulsive interaction between PEG homopolymer and PLGA−PEG−PLGA copolymer chains in water. So, we put forward an immiscibility mechanism to account for the effect of PEG homopolymers to enhance physical gelling of block copolymers composed of polyester and polyether, as schematically presented in Figure 10.

The above mechanism is partially based upon our previous research of the hierarchical mechanism of thermogelling of PLGA−PEG−PLGA block copolymers in water[23] as well as those investigations of physical gelling of polyester and polyether amphiphilic block copolymers by others.[13,37] We suppose that such a physical gelling results basically from the formation of a percolated micelle network. Figure 10a describes a state in which micelles are well dispersed. After PEG homopolymers are added, block copolymer micelles tend to aggregate to form larger clusters to avoid these PEG homopolymers due to the

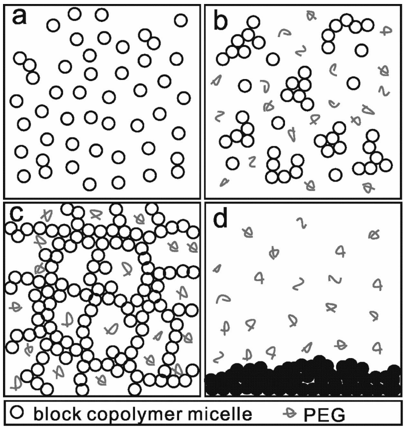

Figure 10. Schematic presentation of the "immiscibility mechanism" to account for the effect of the addition of hydrophilic homopolymers represented by PEG on the physical gelling behaviors of the aqueous system of amphiphilic block copolymers represented by PLGA−PEG−PLGA (Copolymer-1 and Copolymer-2) or MPEG−PLGA (Copolymer-3). (a) Block copolymer micelles are formed in water. (b) The addition of hydrophilic homopolymers promotes micellar aggregation to a certain extent due to the immiscibility between homopolymer and amphiphilic block copolymer chains in water. (c) Micellar aggregates are further percolated to form a three-dimensional micelle network with a random fractal. (d) Too many homopolymers or higher temperatures cause precipitation due to macroscopic phase separation between the block copolymers and the homopolymer aqueous solution.

immiscibility between homopolymers and copolymers in water (Figure 10b). This hypothesis was partially confirmed by our dynamic light scattering experiments: the addition of PEG 6K greatly shifted the hydrodynamic sizes of micellar aggregates in PLGA−PEG−PLGA triblock aqueous system to larger values (data not shown, and the measurements were just for a relatively dilute suspension because a concentrated system was too scattered to be detected in our laser scattering equipment). The secondary evidence was found in the measurements of T_{turbid}: the optical density at 650 nm was significantly higher in the presence of PEG homopolymers than that in pure water at the same temperature (see the inset of Figure 7), which also meant larger micellar aggregates. With a further increase of temperature or the concentration of PEG homopolymers, the augmented aggregates were percolated to form a random-fractal-like three-dimensional micelle network resulting in the physical gelling (Figure 10c). Hence, the associated T_{gel} is lowered with the addition of PEG homopolymers. If too many PEG homopolymers are added or temperature is sufficiently increased, a macroscopic phase separation happens between amphiphilic block copolymers and the PEG aqueous solution. As we indicated just now, samples of precipitates and the solid content in the supernatant were collected and analyzed by ^{1}H NMR, which confirmed the illustration of Figure 10d.

In our opinion, the onset of thermogelling is determined by the percolation point of micelles instead of PEG homopolymers, so the addition of PEG does not alter CGC significantly (Figures 2 and 8). A larger coil size of PEG homopolymer certainly enhances the immiscibility between the copolymer and homopolymer and thus greatly lowered the gel−precipitation boundary as well as the sol−precipitation boundary (Figures 6 and 7). Obviously, an increase of homopolymer concentration must also lead to an earlier thermogelling (Figure 4).

Osmotic Pressure. Another possible mechanism might be related to the internal osmotic pressure gradient induced by the inhomogeneous distribution of the PEG homopolymer molecules in the amphiphilic copolymer aqueous system if the copolymer micelles are regarded as semipermeable regions, unattainable for homopolymers. Malmsten and Lindman anticipated that the osmotic pressure gradient could increase the CMC of PEG−PPG−PEG triblock copolymers in water which in turn led to a higher gelling temperature.[16] Even if this elegant mechanism might serve as a possible mechanism to account for the homopolymer effects on physical gelling of a Pluronic aqueous system, it does not work for our PEG/PLGA block copolymer aqueous system since the addition of PEG homopolymers led to, however, earlier thermogelling according to our observations. Considering that this candidate mechanism is in a dilemma in interpreting PEG/PPG and PEG/PLGA aqueous systems, we also do not think that the osmotic pressure gradient is responsible for the late thermogelling of Pluronic F127 solutions.

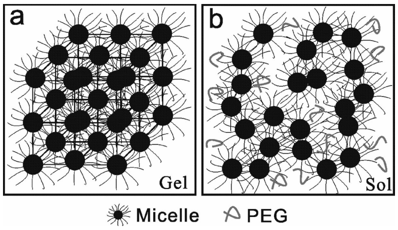

Figure 11. Schematic presentation of the order-interfering mechanism to account for the effect of addition of hydrophilic homopolymers represented by PEG of a relative large MW (MW ≥ 4000) to depress the physical gelling of an aqueous system of amphiphilic block copolymers represented by PEG−PPG−PEG with an inherent ordered gel structure. (a) Block copolymer micelles in an aqueous solution are associated into a long-range ordered structure upon gel formation. (b) The addition of long hydrophilic homopolymer coils disturbs the superlattices and thus depresses the gel formation to a certain extent leading to a higher T_{gel}.

An Order-Interfering Mechanism. Now we should interpret in which way the effect of addition of PEG homopolymers on physical gelling of PEG−PPG−PEG in water is so different from that of block copolymers of PEG and PLGA. Figure 11 schematically presents an order-interfering mechanism. In the Pluronic hydrogels, some ordered structures have been found in the gel state (e.g., a cubic lattice for F127[17,38]). We think that PEG homopolymer coils interfere the packing of Pluronic micelles into the superlattices (comparison between parts a and b of Figure 11), and thus a critical gelling temperature has to be increased, even if thermogelling can still happen.

It should be indicated that the system of PEG−PPG−PEG/PEG/water might also embody the immiscibility mechanism to a certain extent. A study of PEG/PPG/water ternary systems has already revealed a weak repulsive interaction between PEG and PPG in a wide temperature range.[39] We think that both the immiscibility mechanism and the order-interfering mechanism underlie in the PEG−PPG−PEG/PEG/water system, and it is their competition that complexes the MW dependence of PEG homopolymers on the sol−gel transition temperatures of Pluronic F127 (Figure 9). If the MW of PEG homopolymer is large, the order-interfering mechanism is dominant, and an increase of T_{gel} is expected. The "immiscibility mechanism" is predominant when oligo(ethylene glycol)s are added, and thus T_{gel} is decreased, just similar to the effect of PEG homopolymers to PLGA−PEG−PLGA gelling systems. So, the contradictory roles of PEG homopolymers on physical gellings of two kinds of amphiphilic block copolymers are accounted for in a harmonious way by the two mechanisms suggested by us.

Our interpretation of the PEG homopolymer effects on the PLGA−PEG−PLGA aqueous system implies that the internal structure of the associated physical gelation is a random fractal instead of a regularly ordered packing. To date, the detailed structure of physical hydrogels of various block copolymers composed of polyester and polyether is still an open problem, but recent small-angle neutron scattering[13] and ultrasmall-angle scattering[15] investigations of the hydrogels formed by similar triblock copolymers did reveal the random aggregation of micelles rather than the long-range ordered packing of micelles. Thus, the preclusion of micelles away from superlattices by PEG homopolymers should be taken into minor consideration in the case of block copolymers composed of polyester and polyether so far examined.

It should be mentioned that PEG homopolymers lowered $T_{\mathrm{precipitation}}$ in the PLGA−PEG−PLGA/PEG/water system (Figures 2 and 6) and also lowered melting temperatures of gels in the PEG−PPG−PEG/PEG/water systems,[16] although the effects on T_{gel} might be different in the two systems. In both systems, the transitions associated with the upper boundaries in the state diagrams could be interpreted by the immiscibility between the amphiphilic block copolymers and hydrophilic homopolymers.

Conclusions

The gelling behaviors of PLGA−PEG−PLGA triblock copolymers and MPEG−PLGA diblock copolymer aqueous systems with specific composition in the presence of PEG homopolymers with different chain lengths and concentrations have been examined by the tube inverting approach and dynamic rheological measurements, etc. All of the observed transition temperatures (including sol−gel transition temperature T_{gel} and gel−precipitation transition temperature $T_{\mathrm{precipitation}}$ for the samples above CGC, and the sol−precipitation transition temperatures T_{turbid} for the samples below CGC) have been found to be decreased with the addition of PEG homopolymers. The misjudging of gel points due to the increase of the medium viscosity in the tube inverting approach, the change of the water polarity, the osmotic pressure gradient, and the chain bridging have been ruled out as the main mechanism to account for the effect of PEG homopolymers. We have further put forward the immiscibility mechanism to interpret the effect of addition of PEG homopolymers to trigger earlier thermogelling of amphiphilic block copolymers of PEG and PLGA in water. Such an effect is also responsible for a similar change in the case of physical gelling of PEG−PPG−PEG aqueous systems with addition of EG or oligo(ethylene glycol). The conventional late gelling of Pluronic solutions upon addition of PEG is accounted for by the order-interfering mechanism, and it is emphasized that such a mechanism is predominant upon the addition of long PEG coils only. Our work also indirectly support that the surpermolecular structure of the hydrogels of the block copolymers of PEG and PLGA with the examined composition is more like a randomly percolated micelle network than an ordered packing of block copolymer micelles. The combination of the immiscibility between homopolymers and block copolymers and the ordered or random network structure account for all of the experimental phenomena of these polymeric aqueous systems so far observed.

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