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Gelation behavior of PEO–PLGA–PEO triblock copolymers in water

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Abstract

Aqueous solutions of poly(ethylene oxide-*b*-(DL-lactic acid-*co*-glycolic acid)-*b*-ethylene oxide) with two different molecular weights (550–3.4K–550 and 750–3.5K–750) show the change in turbidity as a function of temperature. The variation in turbidity is also found to be independent of block copolymer concentration. By increasing the molecular weight of the hydrophilic PEO end block with the molecular weight of the hydrophobic PLGA middle block kept almost the same, it is found that the phase boundary is shifted to a higher temperature and only soft gels are observed. Large aggregates or clusters of micelles with sizes larger than 5000 nm are observed with dynamic light scattering at the temperatures of turbid region and the size distribution of the aggregates is also found to depend only on temperature, not on concentration. The storage moduli (G') of aqueous solutions of the PEO–PLGA–PEO do not show the abrupt change at the sol-to-(hard) gel boundary, as normally seen in the case of PEO–PPO (poly(propylene oxide))–PEO block copolymers and the behavior of G' as a function of temperature is qualitatively the same and independent of polymer concentration except the absolute values of G', which are proportional to the copolymer concentration. This implies that micelle–micelle clusters are formed due to hydrophobic attraction between core phases.

Keywords: Poly(ethylene oxide)–poly(lactic acid-*co*-glycolic acid)–poly(ethylene oxide) (PEO–PLGA–PEO) triblock copolymer(s); Turbidity; Storage modulus (G')

1. Introduction

Block copolymers containing both a hydrophilic block such as poly(ethylene oxide) (PEO) and a hydrophobic block such as poly(propylene oxide) (PPO) or poly(butylene oxide) (PBO) connected through covalent bonds in a chain generally form micelles [1,2] in a selective solvent when the solution concentration exceeds the critical micelle concentration [3–5] or the solution temperature is increased above the critical micelle temperature [3,5,6] due to the self-assembling nature of the amphiphilic chains. Aqueous solutions of this kind of amphiphilic block copolymers at high concentrations typically undergo the sol-to-gel transition with increase in temperature. The gels derived from these block copolymers are the physical gels formed through non-covalent associations and those gels can also return to sols when the temperature is lowered below the gelation temperature; in other words, thermoreversible gelation [5,7]. The behavior of the sol-to-gel transition has been utilized for the delivery of labile drugs such as polypeptides and proteins because such hydrophobic drugs can be formulated in aqueous solution through a subcutaneous injection to a body [8,9]. Poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) (PEO–PPO–PEO) block copolymer series, typically known as Pluronics® (BASF) or Poloxamers® (ICI), have extensively been investigated for the drug-delivery system (DDS) and their gelation mechanism is relatively well understood by many previous studies [7,10,11]. With increasing the solution temperature and the concentration of the PEO–PPO–PEO (for example, PEO-*b*–PPO-*b*–PEO (4.5K–3.6K–4.5K), Pluronic F127), the average number of unimers per micelle (i.e. aggregation number) increases because the unimer–micelle equilibrium is further shifted toward the micellar state [11]. When the aggregation number reaches the plateau value, the micelles eventually come in contact with one another and these contacts cause entanglements among the hydrophilic corona PEO chains [11]. The gel state is thus induced as a result of the micellar packing in close-packed (cubic) array [7,10] when the micelle concentration reaches a critical volume fraction of 0.53 [11].

The PEO–PPO–PEO is, however, not biodegradable and its gel phase is found to dissolve in vivo in a short time upon

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Table 1
Characteristics of PEO–PLGA–PEO triblock copolymers used in the present study

Triblock copolymers used	^{1}H NMR		GPC		
	$\bar{M}_n$	DLLA/GA (mole ratio)	$\bar{M}_n$	$\bar{M}_w$	PDI[a]
PEO–PLGA–PEO	550–3400–550	80/20	5980	8060	1.35
	750–3500–750	80/20	6400	8400	1.31

[a] PDI (polydispersity index) = $\bar{M}_w/\bar{M}_n$

dilution [12]. Recently reported poly(ethylene oxide-*b*-(DL-lactic acid-*co*-glycolic acid)-*b*-ethylene oxide) (PEO–PLGA–PEO) triblock copolymers have properties of non-toxicity and biodegradability, and are also known to undergo the sol-to-gel transition [13,14]. Because the sol-to-gel boundary can be controlled by adjusting molecular characteristics such as molecular weight and hydrophilic (PEO)/hydrophobic (PLGA) balance, the PEO–PLGA–PEO triblock copolymers are considered as one of the promising materials for the DDS. Also, it has been found that the PEO–PLGA–PEO remains as a gel in vivo for more than 1 month [12] even with low molecular weights of the block copolymers. This implies that the gelation behavior of PEO–PLGA–PEO would be quite different from that of the PEO–PPO–PEO block copolymers. Until now, the gelation behavior and mechanism of the PEO–PLGA–PEO triblock copolymers in water have not been investigated in detail.

In the present study, phase diagrams of PEO–PLGA–PEO triblock copolymers are presented using the tube inversion method (TIM) and the turbidity measurement. The gelation behavior of aqueous solutions of PEO–PLGA–PEO triblock copolymers is examined by combining the dynamic light scattering (DLS) and rheological measurements and those results are also compared with aqueous solutions of a PEO–PPO–PEO triblock copolymer (Pluronic F127).

2. Experimental

2.1. Materials

The PEO–PLGA–PEO triblock copolymers used in this study were synthesized by ring opening polymerization. The synthesis procedure is described elsewhere [13]. The molecular weights of the PEO–PLGA–PEO triblock copolymers and the DLLA/GA ratios in the PLGA blocks are summarized in Table 1.

2.2. Phase diagrams

Vials of 4 ml with an inner diameter of 13 mm were used and the vials sealed with Teflon tape were placed in a water bath which can control temperature with an accuracy of ±0.01 °C. To ensure equilibrium, more than 20 min of equilibration time was allowed for each temperature and measurements were made every 0.5 °C from 1 to 90 °C. A change from a mobile state to an immobile one was determined by inverting the vial. *Hard gels* were defined as immobility of the solution (i.e. no movement of liquid meniscus in a vial for more than 5 min) upon inversion of the vial, while *soft gels* were defined as any detection of slow movement of the meniscus over a period of 5 min. *Sols* were defined as a state in which the solution readily flows to the bottom of the vial within 1 min upon inversion.

2.3. Turbidity measurement

A light scattering set-up was used to detect the turbidity of aqueous solutions of PEO–PLGA–PEO as a function of temperature. Temperature scans were carried out at a heating rate of 0.5 °C/min. Measurements were made in the transmission mode and the transmitted light intensity was monitored with a photomultiplier tube. A homemade light scattering apparatus is equipped with a He–Ne laser with wavelength $\lambda = 632.8$ nm. All the solutions were filtered with filters of 0.45 μm pore size to avoid any effect of impurity on the turbidity.

2.4. Dynamic light scattering

DLS measurements were made at a 90° scattering angle from the incident light path to a liquid sample holder using a Brookhaven DLS (BI-9000AT) equipped with a digital autocorrelator and a photon counter. Two light sources of a He–Ne laser with $\lambda = 632.8$ nm and a Ar laser with $\lambda = 488$ nm were used. The information on the size distribution of micelles and/or clusters was obtained by the inverse Laplace transformation of data using the CONTIN program. All the solutions were again filtered using 0.45 μm pore size filters to avoid the effect of impurity and all the filtrations were performed in sol sate (i.e. at low temperature) since it is impossible to filter the solutions in gel state due to large aggregates. All the results are obtained with the full range of delay time (i.e. from possible minimum to maximum delay time).

2.5. Rheology

Rheometer RMS-800 (Rheometrics, Inc.) in a coni-cylinder geometry (cup diameter, 52 mm; bob diameter, 50 mm; bob length, 20 mm; and bottom gap, 0.2 mm) was used to measure the dynamic viscoelastic storage modulus (G') of

PEO–PLGA–PEO solutions as a function of temperature. Temperature scans at a fixed frequency of 0.5 rad/s were carried out at a heating rate of 0.5 °C/min. Strain was fixed at 2.7%, which was small enough to ensure linear viscoelasticity.

3. Results and discussion

The phase diagram of PEO–PLGA–PEO (550–3.4K–550) in water is shown in Fig. 1(a). Within a narrow temperature window between 24 and 39 °C, hard gels are observed in concentrations ranging from 16 to 36 wt%. For copolymer concentrations ranging from 8 to 36 wt%, soft gels are also observed around the hard gel boundary, as detected by the TIM. We also note that the aqueous solutions of PEO–PLGA–PEO (550–3.4K–550) show the turbidity change upon increasing the solution temperature and one example for a 32 wt% copolymer solution is shown in Fig. 1(b). We further found that the turbidity change depends only on the temperature not on the polymer concentration as shown in Fig. 1(a), although there is a slight deviation in temperature of ±1.0 °C for each concentration. Consequently, the temperature range of the turbidity change can be classified into four distinct regions. In region I, the solution is almost transparent up to about 27 °C. In region II, turbidity increases as temperature is increased and in region III turbidity decreases upon increase in temperature. The aqueous solutions of PEO–PLGA–PEO (550–3.4K–550) show the maximum turbidity at around 31 °C. In region IV, the turbidity remains almost constant before the copolymers eventually precipitate out of water at about 40.2 °C.

The phase diagram of PEO–PLGA–PEO (750–3.5K–750) is shown in Fig. 2(a). Compared with the PEO–PLGA–PEO (550–3.4K–550), the PEO–PLGA–PEO (750–3.5K–750) triblock copolymer has almost the same molecular weight of the hydrophobic PLGA block and longer hydrophilic PEO end blocks. Similar turbidity change with four distinct regions is again observed in the aqueous solutions of PEO–PLGA–PEO (750–3.5K–750) as shown in Fig. 2(b). The relative maximum turbidity is observed at about 52 °C and the copolymer collapses in water at about 58.5 °C. In the phase diagram, however, only soft gels are observed from 14 to 35 wt% within the

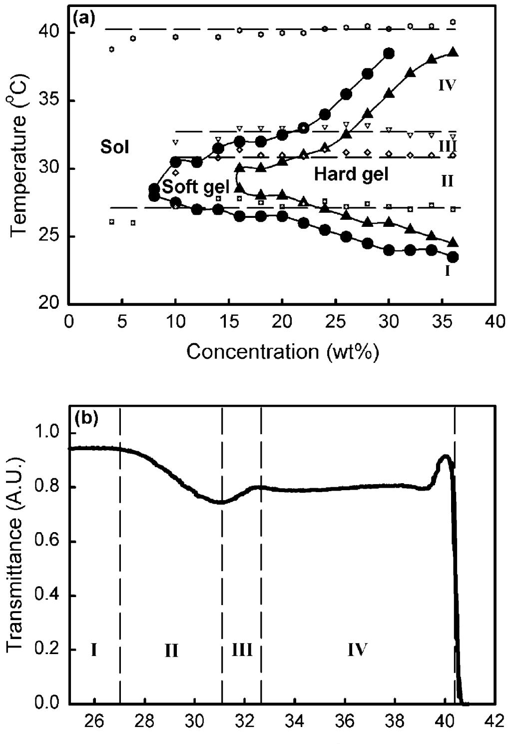

Fig. 1. (a) Phase diagram of PEO–PLGA–PEO (550–3.4 K–550) in water determined by the TIM. (▲) hard gel boundary; (●) soft gel boundary. (b) Relative transmittance of 32 wt% aqueous solution of PEO–PLGA–PEO (550–3.4K–550) as a function of temperature at a heating rate of 0.5 °C/min: (I) up to 27 °C, minimal turbidity region; (II) 27–31 °C, turbidity increase region; (III) 31–32.5 °C, turbidity decrease region; (IV) 32.5–40.2 °C, constant turbidity region before chain collapse state.

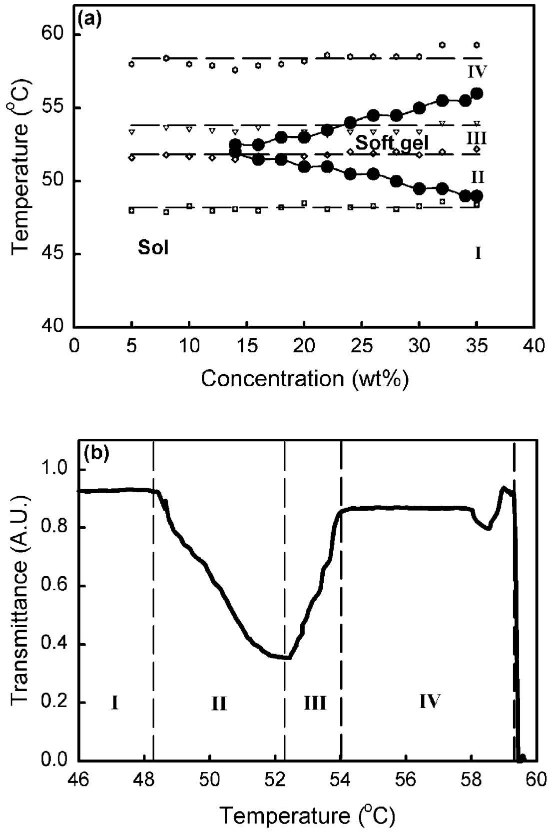

Fig. 2. (a) Phase diagram of PEO–PLGA–PEO (750–3.5K–750) in water determined by the TIM: (●) soft gel boundary. (b) Relative transmittance of 35 wt% aqueous solution of PEO–PLGA–PEO (750–3.5K–750) as a function of temperature at a heating rate of 1 °C/min: (I) up to 48 °C, minimal turbidity region; (II) 48–52 °C, turbidity increase region; (III) 52–54 °C, turbidity decrease region; (IV) 54–58.5 °C, constant turbidity region before chain collapse state.

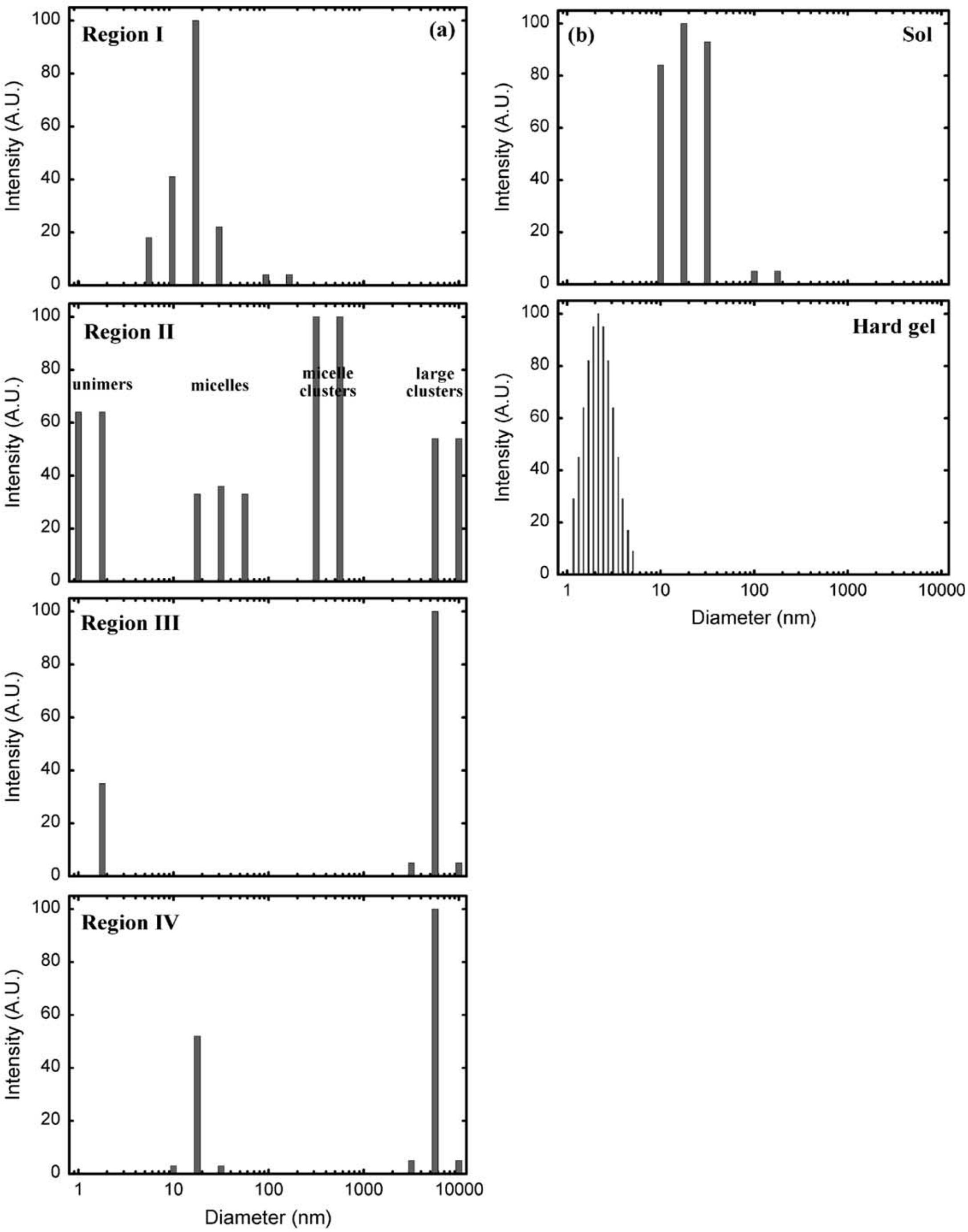

Fig. 3. (a) Size distribution of unimers, micelles and clusters for a 24 wt% aqueous solution of PEO–PLGA–PEO (550–3.4K–550) measured with DLS: region I (10 °C), region II (29 °C), region III (32 °C), and region IV (35 °C). (b) Size distribution of unimers and micelles for a 20 wt% aqueous solution of PEO–PPO–PEO (4.5K–3.6K–4.5K, Pluronic F127) measured with DLS: sol (10 °C) and hard gel (29 °C) states. Measurements were made at a scattering angle of 90° and the size distribution function was obtained by the inverse Laplace transformation of raw data using the CONTIN program. Size detection limit is about 10 000 nm.

temperature region from about 49 to 56 °C. By increasing the molecular weight of the hydrophilic PEO end blocks, it is found that both the sol-to-gel and the gel-to-sol transitions are significantly shifted to higher temperature by more than 20 °C. This implies that the transition temperature of PEO–PLGA–PEO triblock copolymers is quite sensitive to the ratio of hydrophilic to hydrophobic blocks.

Relative hydrophobicity of four different types of hydrophobic units has been placed in the order of BO (butylene oxide)/M (methylene)/L (lactide)/PO (propylene oxide) as 4/3/3/1 [15]. The middle block of the triblock copolymers used in present study is DL-lactide/glycolide with the ratio of 80/20 and this LGA hydrophobic unit is about 2.5–3 times more hydrophobic than the PO unit. In lower temperature regime, the PEO–PLGA–PEO triblock copolymers at low concentration form micelles in water with a core composed of PLGA and a corona with PEO chains [14]. The micelles of the PEO–PLGA–PEO (550–3.4K–550 and 750–3.5K–750) in aqueous solution cannot pack through the entanglement of the PEO corona chains because of the small molecular weight of the PEO chains far below the entanglement molecular weight (M_e) of about 1600 [16]. The initial micelle structure is believed to be disrupted mainly due to the massive liquid–liquid phase separation upon heating the solution. We believe that the macroscopic liquid–liquid phase separation causing the turbidity change in solution is driven by the attractive hydrophobic interaction of the PLGA cores upon heating since the hydrophobic attraction is known to be entropy driven [17]. It is also reasonable to think that the relatively long PEO end blocks somewhat suppress the entropy driven hydrophobic attraction by providing steric layers, and this argument can explain the elevated temperature of sol-to-gel and gel-to-sol transitions as well as the detection of only soft gels for PEO–PLGA–PEO (750–3.5K–750) as shown in Fig. 2(a).

As the concentration of the block copolymer is increased, the micelle clusters induced by the hydrophobic attraction form more extensively in water phase and finally reach a point where all the clusters are connected in solution, namely, the percolation threshold. This percolation of the micelle clusters is believed to induce the macroscopic gelation. This hypothesis for the gelation with PEO–PLGA–PEO triblock copolymers is verified by the observation of large clusters with size above 5000 nm using a DLS in both soft gel and hard gel states. Fig. 3(a) shows the DLS measurements of a 24 wt% PEO–PLGA–PEO (550–3.4K–550) triblock copolymer in water. The diameter of unimers is known to be around 1–2 nm, while micelles are with sizes around 10–50 nm. At a temperature in region I (i.e. 10 °C) showing the minimal turbidity, micelles are dominant. At a temperature of 29 °C in the region II representing the turbidity increase, the motion of large clusters as well as the motion of three modes (unimers, micelles, clusters of micelles) is detected. Moreover, as the solution goes through the sol-to-gel transition around 25 °C, it is also noted that unimers coexist with micelles and clusters (see region II in Fig. 3(a)). This implies that micelle structure is not well maintained while going through the gelation and it is quite sensitive to temperature. At a temperature of 32 °C in the region III representing the turbidity decrease and a temperature of 35 °C in the region IV showing the constant turbidity, the fraction of the large aggregates with sizes larger than 5000 nm appears to increase with increase in temperature. It is interesting to note here that for aqueous solutions of PEO–PLGA–PEO (550–3.4K–550 and 750–3.5K–750) the size distribution of unimers, micelles, and clusters is closely related with turbidity rather than the apparent sol or gel state.

In contrast, it was reported that for a 35 wt% aqueous solution of Pluronic P-94 (PEO_{28}–PPO_{48}–PEO_{28}) at a temperature of 40 °C in the hard gel region, only the motion of micelles is detected with DLS since higher polymer concentration and temperature favor the micelle formation [4,18]. For a 20 wt% aqueous solution of Pluronic F-127 (PEO_{100}–PPO_{65}–PEO_{100}) having longer hydrophilic PEO end blocks at a temperature of 10 °C in the sol state, as shown in Fig. 3(b), micelles with sizes ranging from 10 to 30 nm are dominant. When the F127 solution crosses the sol-to-hard gel transition around 25 °C only unimer motion is detected due to the strong entanglement among the PEO corona chains causing the frozen state of micelles. Moreover, at a temperature in upper sol, the PEO chains shrink and disentangle themselves since water acts as a poor solvent and consequently the size of aggregates decreases (data not shown). All the DLS data obtained so far point to the fact that the hard gels formed from PEO–PLGA–PEO (550–3.4K–550) are random aggregates of copolymers due to the hydrophobic attraction and cannot form the close-packed array as seen in the aqueous PEO–PPO–PEO solution.

In rheological measurements, the abrupt changes in shear moduli (G' and G'') have been generally defined as the sol-to-hard gel transition and this criterion is in excellent agreement with the results obtained with TIM in aqueous solutions of PEO–PPO–PEO (Pluronics®) [17,19]. In aqueous solutions of PEO–PLGA–PEO (550–3.4K–550), however, the storage modulus (G') did not show such an abrupt transition at the hard gel transition temperature as shown in Fig. 4.

It is also interesting to note that the G' behavior as a function of temperature is all similar regardless of the copolymer concentration and that only the absolute G' value is proportional to the concentration of the copolymer. Even in

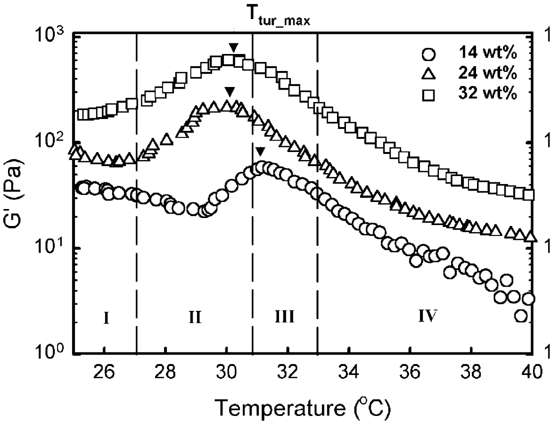

Fig. 4. Change of storage modulus (G') as a function of temperature in aqueous solutions of PEO–PLGA–PEO (550–3.4K–550). Frequency = 0.5 rad/s, strain = 2.7%, and a heating rate of 0.5 °C/min is used. Block copolymer concentrations are indicated in the figure.

the hard gel state with 32 wt% aqueous solution in the temperature range between 26 and 37 °C, a relatively low value of the maximum G' ($<10^3$ Pa) was observed, while the PEO–PPO–PEO triblock copolymer yields a fairly high value of G' ($\approx 10^4$ Pa) in the hard gel state. G' gradually decreases after reaching the local maximum in G' upon heating and it is worthwhile to note that the temperature showing the local maximum G' corresponds to the temperature showing the maximum turbidity at about 31 °C for PEO–PLGA–PEO (550–3.4K–550) and at about 52 °C for PEO–PLGA–PEO (750–3.5K–750) (data not shown), respectively. The local maximum in G' is thus believed to be closely related to the extensive clustering of micelles and these clusters of large size have a significant effect on the increase of G'.

4. Conclusions

We investigated the gelation behavior of aqueous solutions of PEO–PLGA–PEO triblock copolymers. In the phase diagrams constructed by the TIM, both the sol-to-gel and the gel-to-sol transitions were located and we also found that the turbidity behavior depends only on the temperature not on the copolymer concentration. Large aggregates or clusters of micelles with sizes larger than 5000 nm are detected with DLS in the turbid solution region and the fraction of the large aggregates appears to increase with increase in temperature. No abrupt change in G' was observed at the sol-to-hard gel transition temperature for the PEO–PLGA–PEO (550–3.4K–550). The general behavior of G' as a function of temperature was qualitatively the same and independent of the concentration while the absolute value of G' was proportional to the copolymer concentration. These observations lead us to conclude that while the PEO–PPO–PEO block copolymer such as Pluronic F127 undergoes the sol-to-hard gel transition by simple micellar interactions, the PEO–PLGA–PEO (550–3.4K–550 and 750–3.5 K–750) undergoes the sol-to-gel transition through the macroscopic liquid–liquid phase separation induced by the hydrophobic attraction among LGA core blocks upon heating the solution. This different gelation behavior makes the PEO–PLGA–PEO block copolymers more promising and attractive materials for applications such as the DDS.

Acknowledgements

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