

EFFECT OF HEATING RATE ON THERMO-INDUCED AGGREGATION OF POLY(ETHYLENE OXIDE)-*b*-POLY(*N*-ISOPROPYLACRYLAMIDE) IN AQUEOUS SOLUTIONS*

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Abstract The effects of heating rate on the aggregate behavior of poly(ethylene oxide)-*b*-poly(*N*-isopropylacrylamide) in aqueous solutions were investigated in detail by laser light scattering and TEM. By employing two separate heating protocols, step-by-step heating at < 5 K/step and one-step jump, to heat the sample from 15°C to the selected temperature, we found that the heating rate only showed significant effect on the aggregates above the cloud point. The aggregate formed by step-by-step heating exhibited a much larger size and a broader size distribution than those formed by one-step jump heating. Moreover, neither of the aggregates were ideal micellar structures as indicated by the size and the R_g/R_h values. On the contrary, at temperatures below the cloud point where the block copolymer formed core-shelled micelles, the heating rate showed negligible effect on the size and size distribution of the micelles. Since the system underwent a phase separation above the cloud point, the heating rate effect could be reasonably explained by the phase separation mechanisms: the nucleation-and-growth mechanism in the metastable region and the spinodal decomposition mechanism in the unstable region.

Keywords: Heating rate; Cloud point; Nucleation-and-growth; Spinodal decomposition; PEO-*b*-PNIPAm.

INTRODUCTION

Poly(*N*-isopropylacrylamide) (PNIPAm) and copolymers containing PNIPAm segment are of great interest in recent years because of their thermo-responsive properties and thereby potential applications in drug-delivery and as intelligent materials^[1–3]. Upon heating to the transition point (typically around 32°C), the PNIPAm will undergo a phase separation^[3,4]. The copolymers containing another hydrophilic segment generally form micelles with PNIPAm as the core and the other hydrophilic block as the shell at temperatures above the critical micellization temperature (c.m.t.). The c.m.t. of such “double-hydrophilic” copolymer, depending on the property of the block other than PNIPAm, is usually close to, or higher than the cloud point of PNIPAm itself^[5–11].

Controlled or targeted drug delivery is one of the major applications of the thermo-responsive copolymers. To successfully deliver a drug, the size and the size distribution of the particles (or micelles) formed by the copolymers are two key parameters^[12,13]. The size or size distribution of the particles could be tuned by varying the preparing conditions, such as concentration^[14,15], temperature^[16,17] and ion strength of the solution^[18]. Heating rate has also been demonstrated to be able to effectively tune both the size and the size distribution of the particles formed by thermo-responsive copolymers^[19–21]. Zhu and Napper^[21] studied the temperature-induced

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aggregation behavior of PEO-*b*-PNIPAm microgels. They found that faster heating rate yielded particles with smaller size and narrower size distribution, and attributed the differences to the competition between the interparticle aggregation and the intraparticle coil-to-globule transition. In another work, Wu and coworkers^[19] found that PNIPAm-*g*-PEO formed mesoglobules with smaller size at faster heating rate. Besides the competition between the intrachain contraction and the interchain association, the viscoelastic effect was also considered to have some contribution. For block copolymers containing PNIPAm block, the similar dependence on heating rate was also observed. Hennink and coworkers^[20] investigated the effect of processing and formulation parameters on the size and size distribution of nanoparticles formed by PEO-*b*-PNIPAm in aqueous solutions at various concentrations. The heating rate was found to be a dominant factor for the size and size distribution of the nanoparticles. The faster the polymer solution was heated through the cloud point, the narrower the particle size distribution and the smaller the particle size were obtained. The similar phenomenon was also observed by quite a few other groups^[22–24].

The measurements of the cloud point at given compositions are proposed to give the binodal line in dilute solutions. However, depending on the quench rate and other experimental conditions, the measured cloud point could vary between the binodal line and the spinodal line, and the limit of it corresponded to the metastability limit^[25]. The cloud point was higher than the c.m.t. of the block copolymer with LCST behavior, *e.g.*, PEO-*b*-PNIPAm. Generally, the block copolymer becomes insoluble in the solvent above the cloud point, and it should phase-separate into two phases^[26, 27]. However, the thermo-responsive polymers, and their copolymers with hydrophilic even hydrophobic blocks formed stable particles above the cloud point^[28, 29]. No apparent macroscopic phase separation or precipitation was observed for days and even weeks. Several possible mechanisms, such as colloidal stability (mesoglobules), partial vitrification of the condensed chain, viscoelastic effect and hydration force, have been proposed to account for this observation^[28, 29].

To clarify the heating rate effect on the size and size distribution of thermo-responsive block copolymers, as well as their relationship with the cloud point and phase separation, we studied the temperature-induced aggregation process of a narrow-distributed PEO-*b*-PNIPAm copolymer below and above the cloud point in detail with laser light scattering (LLS). Since the phase separation boundary was basically determined by temperature and concentration, three different concentrations were selected. Within the temperature range studied, the solution with the highest concentration passed the cloud point, while the solution with the lowest concentration was safely below the cloud point. Two different heating protocols were employed to investigate the heating rate effect: one was a step-by-step heating with a step interval ≤ 5 K (referred to as the “slow” heating) and the other was a one-step jump (referred to as the “fast” heating) to the selected temperature. We have shown that a faster heating protocol yielded particles with considerably smaller size, narrower size distribution and denser structure when the end temperature is above the phase-separation point, *i.e.*, the cloud point. However, the presence of such heating rate dependence diminished when the temperature lay between the c.m.t. and the cloud point. Based on these findings, we proposed a general mechanism accounts for the stability as well as the size and size distribution of the formed particles.

EXPERIMENTAL

Materials

The preparation and characterization of the PEO-*b*-PNIPAm block copolymer have been described in our former work^[8]. The repeating units of PEO and PNIPAm were determined by ¹H-NMR to be 44 and 95, respectively. The sample had a narrow distribution (PDI~1.12) as determined by GPC. Milli-Q water (Millipore) with resistance higher than 18 M Ω cm was used throughout the experiments.

Sample Preparation

The stock solution of block copolymer was prepared by directly dissolving the polymer powder in water, and allowing it to stay overnight. The resulting stock solution was then diluted into desired concentration with proper amount of water. For LLS measurements, the solution was filtered through a 0.22 μ m Millipore PVDF

filter into a dust-free vial. During the experiment, the sample vial was placed in a brass holder with a precise temperature ($\pm 0.01^\circ\text{C}$) control. For a “slow” process, the PEO-*b*-PNIPAm solution was heated step by step (1–5 K/step) from 15°C , equilibrating at each temperature; while in a “fast” process, the temperature was increased from 15°C to each temperature point directly, and was equilibrated for at least an hour to ensure the equilibrium.

Light Scattering Measurements

Static light scattering (SLS) and dynamic light scattering (DLS) experiments were conducted on a commercialized spectrometer (Brookhaven Inc, Holtsville, NY) equipped with a BI-200SM goniometer and a BI-TurboCorr digital correlator. A solid-state laser polarized at the vertical direction (GXL-III, 100 mW, CNI, Changchun, China) operating at 532 nm was used as the light source. SLS measurements were carried out at scattering angles in the range of 20° – 120° . The time averaged excess scattered intensity at angle θ , also known as the Rayleigh ratio $R_{\text{vv}}(q)$, was related with the weight-averaged molar mass M_w , the Z-averaged root mean square radius R_g , the second virial coefficient A_2 and the scattering vector q as,

$$\frac{Kc}{R_{\text{vv}}(q)} \approx \frac{1}{M_w} \left(1 + \frac{1}{3} R_g^2 q^2 \right) + 2A_2 c \quad (1)$$

where $K = 4\pi^2 n^2 (\text{dn}/\text{dc})^2 / (N_A \lambda_0^4)$ and $q = (4\pi n / \lambda) \sin(\theta/2)$, with N_A , n , (dn/dc) and λ_0 being the Avogadro constant, the refractive index of the solvent, the specific refractive index increment of the solution and the wavelength of light in vacuum, respectively.

The (dn/dc) value of the PEO-*b*-PNIPAm copolymer can be estimated by the following equation,

$$\text{dn}/\text{dc} = W_{\text{PEO}}(\text{dn}/\text{dc})_{\text{PEO}} + W_{\text{PNIPAm}}(\text{dn}/\text{dc})_{\text{PNIPAm}} \quad (2)$$

where W denoting the weight percentage of a certain block. The dn/dc values of PEO and PNIPAm was adopted from literature^[17, 30].

For dynamic scattering, the intensity-intensity time autocorrelation function $G^{(2)}(t)$ was measured in the self-beating mode. The line width distribution $G(I)$ and average line width $\bar{\Gamma}$ were analyzed with a Laplace inversion program, CONTIN^[8, 19, 31]. The average line width, $\bar{\Gamma}$, was then converted into the hydrodynamic radius $R_{\text{h, app}}$ by using the Stokes-Einstein equation:

$$R_{\text{h, app}} = k_B T / 6\pi \eta D_{\text{app}} \quad (3)$$

TEM

The copper grid precoated with a thin film of Formvar was further coated with carbon. The grid was placed on a hotstage (METTLER TOLEDO, Switzerland) at 50°C . To keep the temperature constant during sample transfer, a pipette tip was first merged in the thermo-equilibrated PEO-*b*-PNIPAm solution at 50°C for 10 min. Then a few drops of solution were transferred to the thermo-equilibrated copper grid surface as fast as possible. After 2–4 min, the excess amount of solution was removed by touching the edge of the copper grid with filter paper. Finally, the grid was air-dried on the hot-stage at 50°C . The specimen was observed under a JEM-200CX transmission electron microscope, operated at 120 kV.

RESULTS AND DISCUSSION

Generally, the thermo-responsive block copolymer PEO-*b*-PNIPAm forms micelles above a critical temperature, known as the critical micelle temperature (c.m.t.). At high concentrations, the solution will become turbid with further increasing temperature to certain point, referred to as the cloud point. Figure 1 shows the temperature dependence of the excess scattered intensity of PEO-*b*-PNIPAm at different concentrations (0.3–1.0 mg/mL) during the step-by-step heating from 15°C to 50°C . The excess scattered intensity has been normalized by concentration for better comparison. The first temperature at which the excess scattered intensity started to increase sharply was taken as the c.m.t.. As shown in the inset in Fig. 1, the c.m.t.s of PEO₄₄-*b*-PNIPAm₉₅ were

42°C, 38°C and 34°C at 0.1, 0.3 and 1.0 mg/mL, respectively. The c.m.t values decreased with concentration, in consistent with the findings from literature^[16, 17]. To make a clear comparison of the heating rate effects at different concentrations, 50°C, far above the c.m.t., was selected as the final temperature. Another reason to choose 50°C was that the solution was only slightly turbid at 1.0 mg/mL, and valid light scattering data was obtainable without taking account of the multi-scattering. As shown in Fig. 1, the excess scattered intensity shot up again at 40°C at 1.0 mg/mL, indicating the occurrence of phase separation. However, the phase separation was not observed at 0.1 mg/mL.

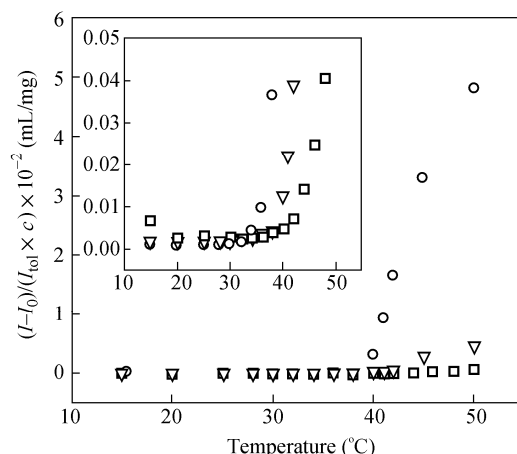


Fig. 1 Temperature dependence of the excess scattered intensity of PEO₄₄-b-PNIPAm₉₅ at different concentrations at 30°: 1.0 mg/mL (○), 0.3 mg/mL (▽), 0.1 mg/mL (□). The samples were heated step-by-step from 15°C to 50°C; The inset shows the magnification of the weak excess scattered intensity.

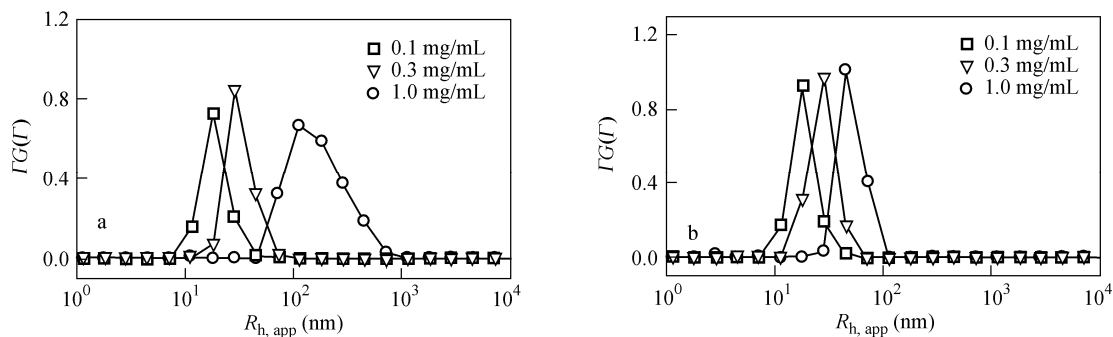


Fig. 2 $R_{h, app}$ distribution (CONTIN results) of aggregates formed by PEO₄₄-b-PNIPAm₉₅ at different concentrations (0.1–1.0 mg/mL) after (a) step-by-step heating (slow), and (b) one-step jump (fast) from 15°C to 50°C.

The scales of abscissa in both panels are the same and the scattering angle is 30°.

Figure 2 compares the size distribution of the aggregate of PEO₄₄-b-PNIPAm₉₅ at 50°C obtained by different heating protocols at various concentrations. The samples were prepared separately by one-step jump (“fast”) and step-by-step heating (“slow”) from 15°C to 50°C. As shown in Fig. 2, the heating rate exhibited strong influence on the sample at 1.0 mg/mL, where the size of the aggregate at slow heating rate was much larger than that at fast heating rate, and the size distribution was also much broader. This result agreed with those reported in literature. However, the heating rate showed almost no effect on the size and size distribution of the formed aggregate at 0.1 or 0.3 mg/mL. Note that both the states of 1.0 mg/mL sample at 50°C were stable. No prominent change in either size or size distribution was observed within a couple of hours.

The static light scattering gave similar results. Figure 3 compares the $Kc/R(\theta)-q^2$ plot of aggregate formed

by fast heating and slow heating at different concentrations. Clearly, at 1.0 mg/mL, the slow heating rate led to the formation of aggregates with much larger $M_{w, app}$ (increased at least by a factor of two) and $R_{g, app}$ (Fig. 3a). In contrast, $M_{w, app}$ of the aggregates at 0.3 mg/mL was less than 8% larger at slow heating rate (Fig. 3b), and the difference in $M_{w, app}$ of the aggregates at different heating rate could be neglected in the case of 0.1 mg/mL (Fig. 3c).

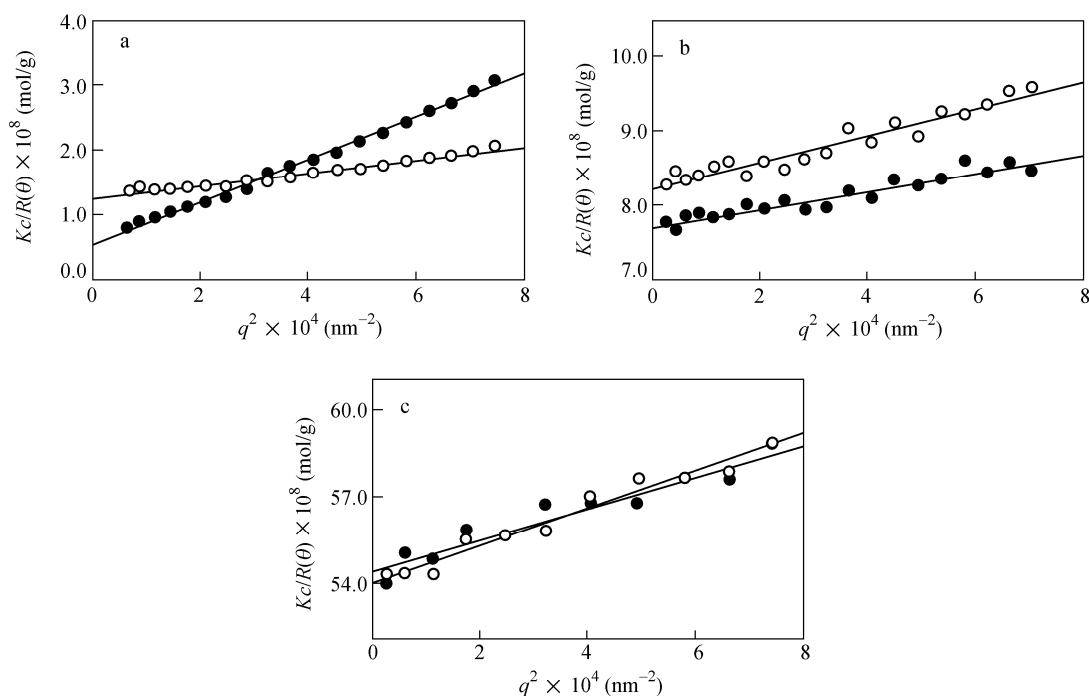


Fig. 3 $Kc/R(\theta)$ - q^2 plots of 1.0 mg/mL (a), 0.3 mg/mL (b) and 0.1 mg/mL (c) PEO₄₄-*b*-PNIPAm₉₅ solutions at 50°C at slow (●) and fast (○) heating rate. The scales of abscissa in all panels are the same.

Table 1 summarizes the results from LLS at different concentrations. The R_g/R_h ratio, which was used to evaluate the conformation of the particles, was also included in Table 1. It is well-established that the solid sphere and the random coil have the R_g/R_h ratio of 0.775 and 1.5, respectively^[32, 33]. The data in Table 1 demonstrated that the effect of the heating rate was concentration dependent. At 0.1 mg/mL, the heating rate showed no prominent effects on the size, the polydispersity and the particle structure. At 0.3 mg/mL, the size and size distribution of the particle obtained at fast heating rate were only slightly smaller than those at slow heating rate. However, a major difference was observed at 1.0 mg/mL. Not only were the size and the polydispersity decreased by a factor of two by using the faster heating, but also the $R_{g, app}/R_{h, app}$ was reduced from 1.31 to 0.94, indicating that the heating rate changed the particle structure as well.

Table 1. Comparison of PEO₄₄-*b*-PNIPAm₉₅ particle size and polydispersity at 50°C obtained by “fast” and “slow” heating protocols at 0.1, 0.3 and 1.0 mg/mL

c (mg/mL)	Heating rate	$R_{g, app}$ (nm)	$R_{h, app}$ (nm)	$R_{g, app}/R_{h, app}$	Polydispersity ^a
0.1	Fast	16.7	18.7	0.89	0.06
	Slow	15.6	17.7	0.88	0.07
0.3	Fast	23.0	28.6	0.80	0.04
	Slow	25.7	31.2	0.82	0.08
1.0	Fast	49.1	52.0	0.94	0.05
	Slow	155.0	118.2	1.31	0.30

^a Based on the polydispersity given by CONTIN analysis of the correlation functions measured at 30°

In addition, Table 1 further shows that the structure of the particle formed at 1.0 mg/mL at 50°C was different from that formed at lower concentrations, especially at slow heating rate, as indicated by a much larger particle size and higher $R_{g, app}/R_{h, app}$ value. To clarify the effect of heating rate on the particle structure, we examined the evolutionary change of the particles during the step-by-step heating process at 1.0 and 0.3 mg/mL. Figure 4 shows the variation of $R_{h, app}$ distribution at selected temperatures during the “slow” heating process. At 15°C, a temperature far below the cloud point of PNIPAm, the distribution was obviously bimodal (Fig. 4a). The small peak corresponded to the unimers, and the large peak corresponded to the associates formed by the block copolymers^[8]. As the temperature rose to 28°C, the average $R_{h, app}$ of the associate slightly decreased (Fig. 4b), merging together with the peak of unimers. At 34°C, a temperature slightly higher than the cloud point of PNIPAm homopolymer, the micelle, with PNIPAm being the core and PEO being the corona, was formed, and it coexisted with the unimers (Fig. 4c). Note 34°C is also the temperature at which the scattered intensity started to shoot up (Inset in Fig. 1). Thus it was reasonable to take this temperature as the c.m.t.. Above the c.m.t., the area ratio of the unimers decreased with temperature. At 38°C, only micelle with narrow distribution was observed (Fig. 4d). So far, the temperature-induced aggregation process (Figs. 4a–4d) of PEO-*b*-PNIPAm at 1.0 mg/mL was quite similar to that of PEO-*b*-PNIPAm at 0.1 mg/mL which has been described in our former work^[8], except that the transition point was lower in the concentrated solution. However, with further increasing temperature over the cloud point at 1.0 mg/mL, the peak corresponding to micelles sharply increased in size and also broadened in its distribution (Figs. 4e and 4f), which was not observed at 0.1 mg/mL^[8]. The temperature-induced aggregation process of PEO-*b*-PNIPAm at 0.3 mg/mL was similar to that of PEO-*b*-PNIPAm at 0.1 mg/mL, except that no apparent increase in size distribution was observed after the formation of micelle at 38°C.

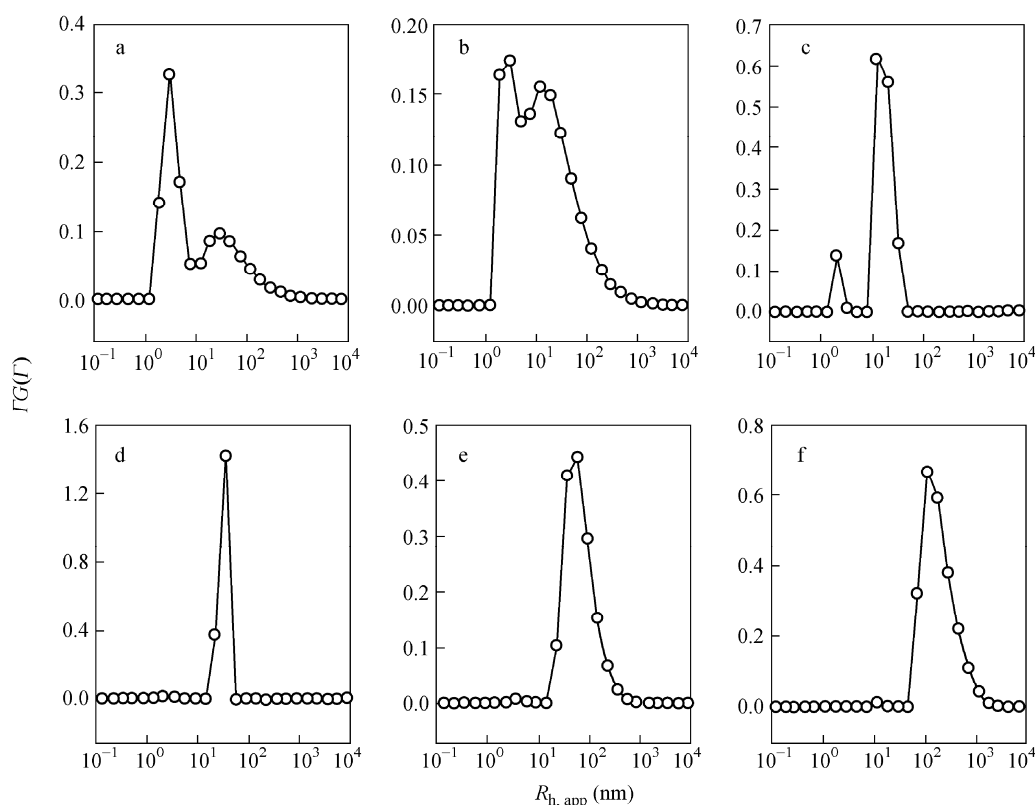


Fig. 4 Size distribution of PEO₄₄-*b*-PNIPAm₉₅ at 1.0 mg/mL during a step-by-step heating process: (a) 15°C, (b) 28°C, (c) 34°C, (d) 38°C, (e) 40°C, (f) 50°C. The scattering angle is 30°.

Figure 5 shows the sizes ($R_{h, app}$ and $R_{g, app}$) and the $R_{g, app}/R_{h, app}$ ratio of the aggregate formed by PEO₄₄-*b*-PNIPAm₉₅ at 1.0 mg/mL and at temperatures above 30°C. At temperatures lower than 38°C, $R_{g, app}$ kept almost constant around 20 nm; while $R_{h, app}$ slowly increased from 19 nm to 36 nm with temperature. Therefore, the $R_{g, app}/R_{h, app}$ slowly decreased and reached a minimum of *ca.* 0.6, a typical value for core-shell micelle^[32, 34] at 38°C. With further increasing temperature over the cloud point, a sharp increase in $R_{g, app}$ and $R_{h, app}$ occurred, and both $R_{g, app}$ and $R_{h, app}$ exceeded 100 nm at 50°C. On the contrary, the $R_{g, app}/R_{h, app}$ in this stage increased from 0.6 to 1.2, indicating that PEO₄₄-*b*-PNIPAm₉₅ underwent an aggregation process at temperatures above 38°C, which was different from the micellization process.

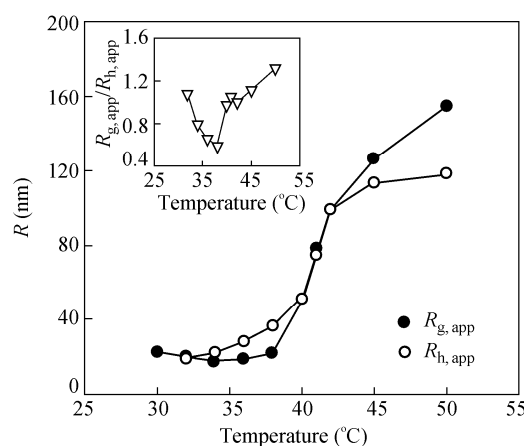


Fig. 5 Temperature dependence of hydrodynamic radius ($R_{h, app}$) and radius of gyration ($R_{g, app}$) of the aggregate formed by PEO₄₄-*b*-PNIPAm₉₅ at 1.0 mg/mL

The sample was heated step-by-step to 50°C (inset: change in $R_{g, app}/R_{h, app}$ during the heating process).

To determine the morphology of the aggregates formed at 50°C, we also carried out TEM experiments on the samples heated at different rates. As shown in Fig. 6, both morphologies were spherical, and the main difference lay in the size and size distribution. The aggregates, formed by the slow heating protocol, were spherical aggregates with diameter ranging from several tens of nanometers to several hundreds of nanometers (Fig. 6a); in contrast, the particles formed by the “fast” heating protocol yielded much more uniform aggregates with an average diameter around 100 nm (Fig. 6b), which was consistent with the light scattering results. Since 50°C is lower than the glass transition temperature of PNIPAm block, it’s believed that the morphology can be frozen once formed. Therefore, the TEM images reflect, at least partially, the morphology of aggregates in solution.

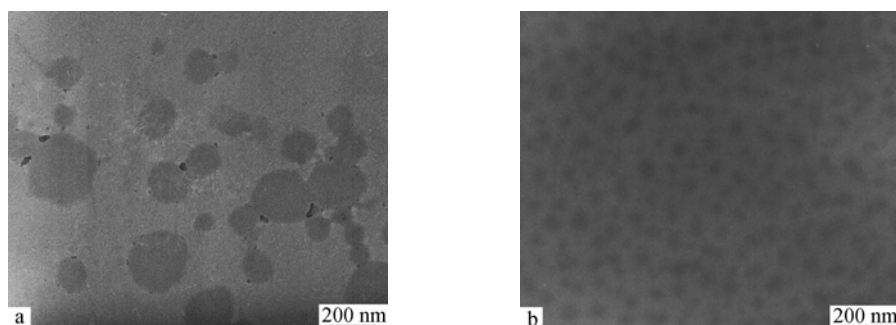


Fig. 6 TEM pictures of aggregates formed by PEO₄₄-*b*-PNIPAm₉₅ at 1.0 mg/mL after a “slow” (a) or a “fast” (b) heating process to 50°C

The heating rate dependence of the size and size distribution of the aggregates was obvious at 50°C at 1.0 mg/mL. However, such heating rate effects were very weak or negligible for solutions at lower concentrations at the same temperature. Since PEO₄₄-*b*-PNIPAm₉₅ underwent the micellization process at all the mentioned concentrations, the heating rate dependence could be caused by the phase separation because only the sample at 1.0 mg/mL passed the cloud point and the conformation of the aggregate formed therein was quite different. To verify this hypothesis, we performed a series of experiments on the sample at 1.0 mg/mL by making the temperature jump from 15°C to several selected temperatures ($\leq 50^\circ\text{C}$), separately. Figures 7 and 8 compares the $M_{w,app}$ and the size of aggregates ($R_{g,app}$ and $R_{h,app}$) obtained by these two heating protocols. Obviously, at temperatures below the cloud point (40°C), the $M_{w,app}$ and the size of aggregates obtained by heating the sample step by step were almost identical to those by heating the sample from 15°C directly to the selected temperature. The difference was only observed at temperatures above the cloud point, where the $M_{w,app}$, $R_{g,app}$ and $R_{h,app}$ of the aggregate obtained at faster heating rate were smaller than those obtained at slow heating rate. More importantly, the difference was enlarged with increasing temperature.

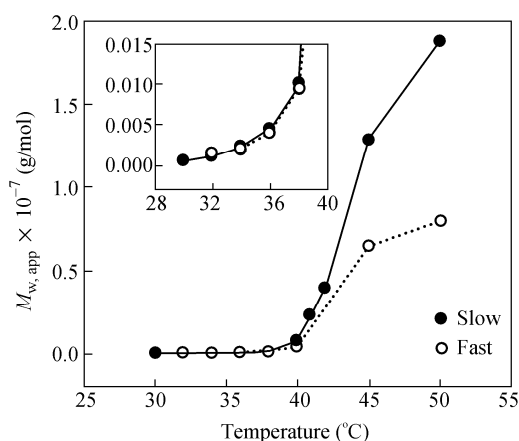


Fig. 7 $M_{w,app}$ of aggregates formed by PEO₄₄-*b*-PNIPAm₉₅ at 1.0 mg/mL after a “slow” or a “fast” heating process

The data points at fast heating process were obtained by jumping the temperature from 15°C to each selected temperatures separately; The insert magnifies the data at temperatures below 40°C.

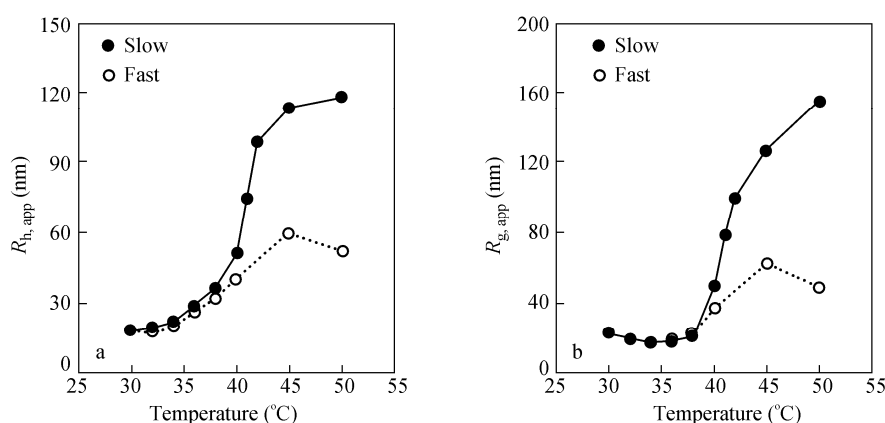


Fig. 8 Comparison of the $R_{g,app}$ and $R_{h,app}$ of the aggregate at 1.0 mg/mL obtained by “slow” and “fast” heating protocols at different temperatures

The size distribution of the aggregates formed by PEO₄₄-*b*-PNIPAm₉₅ at different heating rates shows similar results. As illustrated in Fig. 9(a), fast heating rate yielded narrower size distribution, or lower polydispersity, only at temperatures above the cloud point (40°C). The $R_{g,app}/R_{h,app}$ values of the aggregates formed by the “fast” heating and “slow” heating protocols were compared in Fig. 9(b), which showed that the difference between the “fast” heating and the “slow” heating mainly lay in the temperature range above the cloud point: in the case of “slow” heating, $R_{g,app}/R_{h,app}$ increased with temperature, and finally reached an value as high as 1.3; in contrast, during the “fast” heating process, the $R_{g,app}/R_{h,app}$ value was around 0.9, showing no obvious temperature dependence. The smaller $R_{g,app}/R_{h,app}$ value indicated that the aggregate yielded by the “fast” heating adopted a denser structure than that yielded by the “slow” heating. However, it was still much larger than a well-defined core-shell micelle.

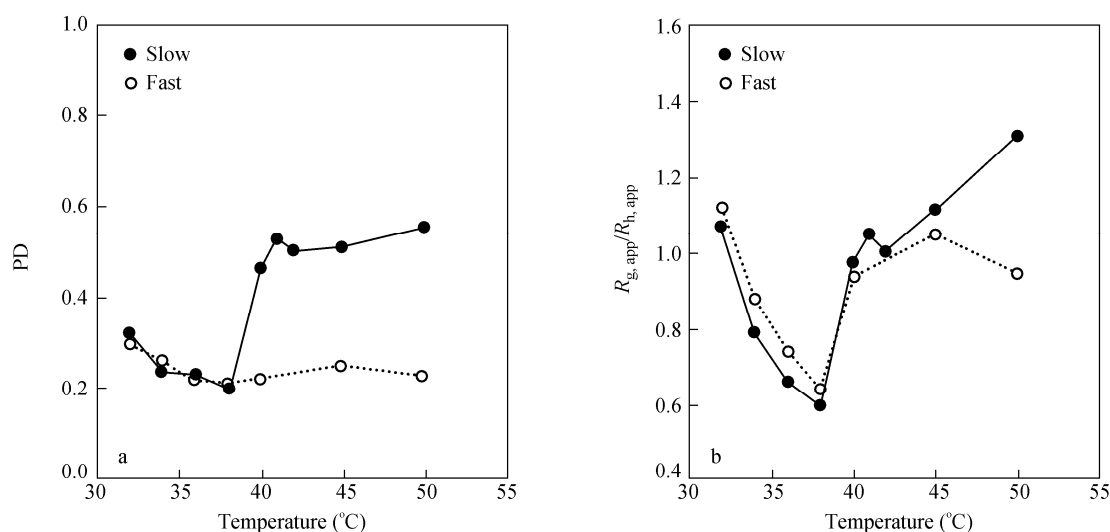


Fig. 9 Polydispersity (a) and $R_{g,app}/R_{h,app}$ (b) of the aggregates formed by PEO₄₄-*b*-PNIPAm₉₅ at 1.0 mg/mL after a “slow” or a “fast” heating process to selected temperatures

The above results demonstrated that the heating rate generated negligible effect on the size and size distribution of micelles at temperatures below the cloud point. It has been established in the literature that the micellization generally underwent two basic kinetic processes: the incorporation of unimers into micelles without reaching the thermodynamic equilibrium, and the reconstruction of micelles *via* the entry-repulsion of unimers or fusion-fission of micelles to reach the equilibrium state^[35–38]. The time scales of these processes were closely related with the polymer concentration, the addition of salt and the jump of temperature or solvent quality. Using Pluronic polymers as an example, the first process was in the order of 0.01–1 ms, and the other process was in the order of 0.2–100 ms^[35]. In our studies, the vials used for light scattering was 1.7 cm in diameter and the wall thickness was *ca.* 1 mm. It has been demonstrated by Chu and coworkers^[39] that the time scale for the solution to reach equilibrium after a temperature jump was in the order of minutes inside a similar vial, much longer than that of the micellization rate. Therefore, the heating rate showed less effect on the size and size distribution of the micelles.

At temperatures above the cloud point, phase separation occurred. Since the cloud point (40°C) of PEO₄₄-*b*-PNIPAm₉₅ at 1.0 mg/mL was far below that of PEO chains^[40–42], the phase separation was caused mainly by PNIPAm. The hydrophobicity of PNIPAm increased with temperature, and finally the short PEO chain was not strong enough to maintain the micellar structure anymore. However, even though the micellar structure was at least partially destroyed and the fine structure of the aggregates was not well-defined, it is believed that some of the PEO chains, due to their hydrophilicity, were able to dangle outside, as demonstrated by the NMR experiments done by others^[43, 44].

The heating rate effect on the size and size distribution of the aggregate above cloud point could be explained by the two corresponding phase separation mechanisms. It was known that the phase-separation followed different mechanisms in the metastable region and in the unstable region, with the former being the nucleation-and-growth and the latter being the spinodal decomposition. For a thermo-responsive diblock copolymer such as PEO-*b*-PNIPAm, the phase separation was not straightforward, mainly because of the occurrence of the micellization before the phase separation. During a step-by-step heating to the cloud point, the system first passed micelle region and metastable region; thus the phase separation followed the nucleation-and-growth mechanism and the growth was based on micelles instead of single polymer chains. Therefore, a sharp increase in $M_{w,app}$ and size was observed. The size distribution was also broad since the growth was mainly controlled by diffusion, and the diffusion for a micelle in a whole must be much slower than that for a single polymer chain. In contrast, in the case of one-step jump, the system was directly quenched into the unstable phase and underwent a spinodal decomposition, which possessed a characteristic length scale for the fastest growth^[26, 27]. Therefore, relatively narrower size distribution, smaller molecular weight and size were observed.

From the viewpoint of thermodynamics, the stability of the aggregate above cloud point was determined by the competition between the translational entropy and the surface energy of the aggregate^[45]. The translational entropy is expressed as “ $kT\ln\phi$ ” with ϕ denoting the volume fraction of polymer chains. It is large in dilute solution since ϕ is small. On the other hand, compared with homopolymers in non-solvent, the surface energy of the aggregate, expressed as γR_g^2 , is much smaller because of the nature of the amphiphilicity of the thermo-responsive (co)polymers (surface tension γ is weak) and the smaller size of the aggregates. If the translational entropy is comparable or larger than the surface energy of aggregate with certain size, the aggregates will not stick together to cause sedimentation.

CONCLUSIONS

For thermo-responsive block copolymers, the effect of heating rate on the aggregation process was temperature-dependent. Below the cloud point, the block copolymer formed micellar structures. In this stage the heating rate exhibited negligible effects on the size and size distribution of the micelles. Above the cloud point, the block copolymer would undergo a phase separation. Slow heating from the temperature below c.m.t generated the aggregates with much larger size and broader size distribution than the one-step heating. This phenomenon could be reasonably explained with the phase separation mechanism: the nucleation-and-growth initiated in the metastable region during slow heating and the spinodal decomposition in the unstable region in one-step jump heating.

The temperature-induced micellization and phase separation were reversible processes, providing great opportunity to drug encapsulation at varying temperature. The amount of drug loaded and the loading efficiency could be affected by the size, size distribution as well as the inner structure of the aggregate, all of which were shown to be temperature-dependent in the present work. It is suggested by our results that for an efficient drug delivery, a comprehensive consideration should be made on these effects. Moreover, a certain degree of hysteresis during the cooling and heating cycle might exist, bringing extra uncertainty to the drug delivery and controlled release.

REFERENCES

- 1 Gil, E.S. and Hudson, S.A., *Prog. Polym. Sci.*, 2004, 29(12): 1173
- 2 Jeong, B. and Gutowska, A., *Trends Biotechnol.*, 2002, 20(7): 305
- 3 Schild, H.G., *Prog. Polym. Sci.*, 1992, 17(2):163
- 4 Kubota, K., Fujishige, S. and Ando, I., *J. Phys. Chem.*, 1990, 94(12): 5154
- 5 Konak, C., Reschel, T., Oupicky, D. and Ulbrich, K., *Langmuir*, 2002, 18(21): 8217

- 6 Zareie, M.H., Dincer, S. and Piskin, E., *J. Colloid Interf. Sci.*, 2002, 251(2): 424
- 7 Xu, Y.L., Shi, L.Q., Ma, R.J., Zhang, W.Q., An, Y.L. and Zhu, X.X., *Polymer*, 2007, 48(6): 1711
- 8 Yan, J., Ji, W., Chen, E., Li, Z. and Liang, D., *Macromolecules*, 2008, 41(13): 4908
- 9 Virtanen, J., Holappa, S., Lemmetyinen, H. and Tenhu, H., *Macromolecules*, 2002, 35(12): 4763
- 10 Schilli, C.M., Zhang, M.F., Rizzardo, E., Thang, S.H., Chong, Y.K., Edwards, K., Karlsson, G. and Muller, A.H.E., *Macromolecules*, 2004, 37(21): 7861
- 11 Tang, T., Castelletto, V., Parras, P., Hamley, I. W., King, S.M., Roy, D., Perrier, S., Hoogenboom, R. and Schubert, U.S., *Macromol. Chem. Physic.*, 2006, 207(19): 1718
- 12 Kim, S.C., Kim, D.W., Shim, Y.H., Bang, J.S., Oh, H.S., Kim, S.W. and Seo, M.H., *J. Control. Release*, 2001, 72(1-3): 191
- 13 Maeda, H., Wu, J., Sawa, T., Matsumura, Y. and Hori, K., *J. Control. Release*, 2000, 65(1-2): 271
- 14 Tong, Z., Zeng, F., Zheng, X. and Sato, T., *Macromolecules*, 1999, 32(13): 4488
- 15 Xia, Y., Burke, N.A.D., Stover, H.D.H., *Macromolecules*, 2006, 39(6): 2275
- 16 Motokawa, R., Morishita, K., Koizumi, S., Nakahira, T. and Annaka, M., *Macromolecules*, 2005, 38(13): 5748
- 17 Zhang, W.Q., Shi, L.Q., Wu, K. and An, Y.G., *Macromolecules*, 2005, 38(13): 5743
- 18 Cho, E.C., Lee, J. and Cho, K., *Macromolecules*, 2003, 36(26): 9929
- 19 Chen, H.W., Zhang, Q.J., Li, J.F., Ding, Y.W., Zhang, G.Z. and Wu, C., *Macromolecules*, 2005, 38(19): 8045
- 20 Neradovic, D., Soga, O., Van Nostrum, C.F. and Hennink, W.E., *Biomaterials*, 2004, 25(12): 2409
- 21 Zhu, P.W. and Napper, D.H., *Langmuir*, 2000, 16(22): 8543
- 22 Soga, O., van Nostrum, C.F., Ramzi, A., Visser, T., Soulimani, F., Frederik, P.M., Bomans, P.H.H. and Hennink, W.E., *Langmuir*, 2004, 20(21): 9388
- 23 Wu, K., Shi, L.Q., Zhang, W.Q., An, Y.L. and Zhu, X.X., *J. Appl. Polym. Sci.*, 2006, 102(4): 3144
- 24 Rakhmatullina, E., Braun, T., Chami, M., Malinova, V. and Meier, W., *Langmuir*, 2007, 23(24): 12371
- 25 Toral, R. and Marro, J., *Surface Science*, 1986, 177(1): 14
- 26 Langer, J.S., Bar-on, M. and Miller, H.D., *Phys. Rev. A*, 1975, 11(4): 1417
- 27 Verma, R., Sharma, A., Banerjee, I. and Kargupta, K., *J. Colloid Interf. Sci.*, 2006, 296(1): 220
- 28 Aseyev, V., Hietala, S., Laukkanen, A., Nuopponen, M., Confortini, O., Du Prez, F.E. and Tenhu, H., *Polymer*, 2005, 46(18): 7118
- 29 Aseyev, V.O., Tenhu, H. and Winnik, F.M., *Adv. Polym. Sci.*, 2006, 196: 1
- 30 Qiu, X.P. and Wu, C., *Macromolecules*, 1997, 30(25): 7921
- 31 Zuo, J., "Application of laser Light Scattering in Polymer Science"(in Chinese), 1st ed., Henan Science and Technology Press, Zhengzhou, 1995, p.227
- 32 Burchard, W., *Adv. Polym. Sci.*, 1983, 48: 1
- 33 Wu, C. and Zhou, S.Q., *Macromolecules*, 1995, 28(24): 8381
- 34 Rubinstein, M. and Colby, R.H., "Polymer Physics", Oxford University Express, New York, 2003, p.149
- 35 Goldmints, I., Holzwarth, J.F., Smith, K.A. and Hatton, T.A., *Langmuir*, 1997, 13(23): 6130
- 36 Honda, C., Abe, Y. and Nose, T., *Macromolecules*, 1996, 29(21): 6778
- 37 Honda, C., Hasegawa, Y., Hirunuma, R. and Nose, T., *Macromolecules*, 1994, 27(26): 7660
- 38 Ye, X.D., Lu, Y.J., Liu, S.L., Zhang, G.Z. and Wu, C., *Langmuir*, 2007, 23(20): 10366
- 39 Chu, B., Ying, Q.C. and Grosberg, A.Y., *Macromolecules*, 1995, 28(1): 180
- 40 Bae, Y.C., Lambert, S.M., Soane, D.S. and Prausnitz, J.M., *Macromolecules*, 1991, 24(15): 4403
- 41 Dormidontova, E.E., *Macromolecules*, 2004, 37(20): 7747
- 42 Saeki, S., Kuwahara, N., Nakata, M. and Kaneko, M., *Polymer*, 1976, 17(8): 685
- 43 Heald, C.R., Stolnik, S., Kujawinski, K.S., De Matteis, C., Garnett, M.C., Illum, L., Davis, S.S., Purkiss, S.C., Barlow, R.J., and Gellert, P.R., *Langmuir*, 2002, 18(9): 3669
- 44 Podo, F., Ray, A. and Nemethy, G., *J. Am. Chem. Soc.*, 1973, 95(19): 6164
- 45 Rubinstein, M. and Colby, R., *Polymer Physics*, Oxford University Press, 2003