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Thermally Induced Polymeric Assemblies from the PAAc-Based Copolymer Containing Both PNIPAAm and mPEG Grafts in Water

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Graft copolymer comprising acrylic acid (AAc) units as the backbone and poly(*N*-isopropylacrylamide) (PNIPAAm) and monomethoxy poly(ethylene glycol) (mPEG) as the grafts undergoes phase transition and supramolecular assembly into colloidal particles in water upon the thermally induced hydrophobic association. The structural characteristics of the polymeric assemblies made from the graft copolymer in water are strongly dependent on the copolymer concentration and the way that the copolymer solution is subjected to heating from 25 °C to the phase transition region (occurring in the range 30∼35 °C). The resultant assemblies are characterized by forming hydrophobic PNIPAAm regions with the multicore architecture and intercore connections. Interesting enough, these colloidal systems obtained from the copolymer solutions at different concentrations (10.0 and 1.0 mg/mL) and heating methods (fast and slow heating) exhibit very different structural responses when subjected to further temperature increase (from 30∼35 to 60 °C). The mutual interactions among the components (PAAc backbone and PNIPAAm and mPEG grafts) of the copolymer were shown to play a crucial role in the evolution of the ultimate assembly structure. A molecular packing model was proposed to illustrate the mechanisms of the thermally induced structural transformation processes for the amphiphilic graft copolymer in water.

Introduction

Multiresponsive self-assemblies of amphiphilic copolymers and their supramolecular architecture are of great importance due to their potential applications in biomedical and biochemical fields including drug delivery, protein immobilization, and biosensing.[1−5] Among these subjects, the thermally induced micellization behavior of PNIPAAm-based amphiphilic copolymers in aqueous phase above the lower critical solution temperature (LCST) of PNIPAAm segments has been extensively investigated.[6−10] A number of studies were concerned particularly with structural characteristics of micelles obtained from block or graft copolymers of PNIPAAm and PEG.[11−17] In addition to the evolution of polymeric colloids via the phase transition of the primary PNIPAAm blocks or grafts at elevated temperature in aqueous phase, PEG has often been considered as the secondary block or graft due to its unique amphiphilic characteristics and the effective steric stabilization against particle aggregation.[17−19] Zhang et al. reported that micellization of a triblock copolymer, PEG-b-poly(4-vinylpyridine) (P4VP)-b-PNIPAAm, in aqueous phase can be induced by either increasing temperature at pH 2.0 or increasing pH at 25 °C.[17] The resultant core structure comprising either PNIPAAm or P4VP block strongly depends on the type of stimuli that induce the subsequent micellization.[17] It was also shown that the structure of the thermally induced micelles, for example, from PEG-b-PNIPAAm copolymers, varies with the copolymer concentration.[11] At a relatively high concentration of 2.0 mg/mL, the copolymer is thermally transformed into small and dense micelles, while large and loose micellar clusters are achieved for the copolymer solution at 0.2 mg/mL. The mesoglobular assembling of PNIPAAm-g-PEG in the continuous aqueous phase was shown to experience two different phase transitions with the onset temperatures occurring at ca. 34 and 47 °C, respectively.[15] The transition at 34 °C is closely related to the contraction of the PNIPAAm backbone segments between two grafted PEG chains, and the other at 47 °C is otherwise attributed to the stretch and collapse of the grafted PEO chains on the periphery. Dual thermo/pH-responsive micelles can also be attained from the graft copolymer of hydroxyethylcellulose-g-(PNIPAAm/PAAc).[20] In contrast to the thermally induced micelles in which the hydrophobic cores comprise exclusively PNIPAAm grafts at high pH (ca. 12), the micelles incurred at 30 °C in a low pH range (<4.6) are characterized by a core structure comprising both PAAc and PNIPAAm grafts with their complementary pairing via hydrogen bonding.

We have previously shown that the PNIPAAm and mPEG grafted copolymers with the backbone AAc residues being fully ionized are capable of undergoing thermally induced self-assembly into large aggregates (500∼800 nm in diameter) by slow heating in the temperature range 32∼35 °C.[21] The aggregates are characterized by the extensively interconnected multicore architecture in which the response of the hydrophobic

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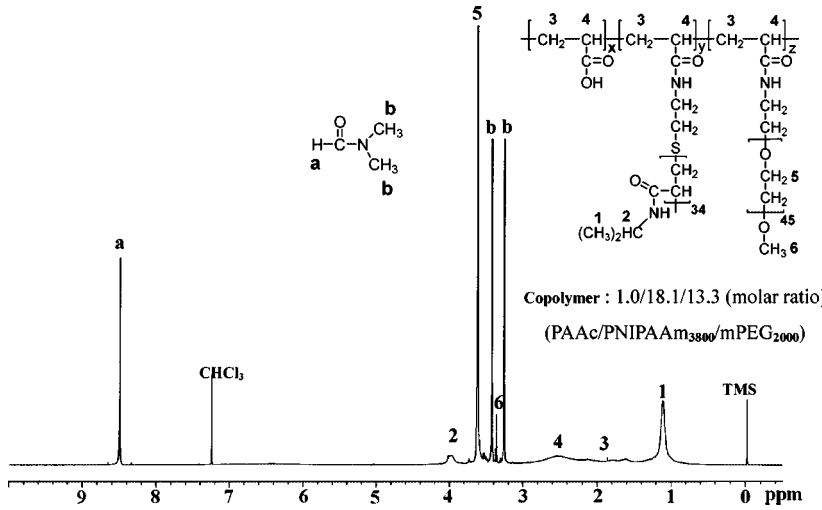


Figure 1. ^{1}H NMR spectrum of the graft copolymer in $CDCl_3$ at ambient temperature, using DMF as an external standard. The assignment of the feature signals of the copolymer and the external standard (DMF) in relation to their chemical structures is also included.

PNIPAAm cores to changes in temperature plays a key role in determining the entire assembly structure and size. The sensitivity of the PNIPAAm-rich core structure to temperature is primarily governed by the number of the grafted PNIPAAm segments along the copolymer backbone. Furthermore, the extent of the multicore structure within the polymeric assemblies can be markedly reduced by forming polymer-bound sodium n-dodecyl sulfate (SDS) micelles at ambient temperature at a critical SDS concentration 10-fold lower than its critical micelle concentration prior to the thermally driven micellization.[22] Thus, the graft copolymer can be thermally transformed into micelles with more compact and hydrophobic PNIPAAm monocore structure, accompanied by the destruction of the polymer-induced SDS micelles.

In this paper, the responses both in the interactions of varying polymer chain segments and the resultant structure of the thermally induced assemblies of the PAAc-g-(PNIPAAm/mPEG) copolymer to changes in the copolymer concentration and heating method were studied. The experimental results obtained from measurements of dynamic light scattering (DLS), transmission electronic microscopy (TEM), fluorescence analysis, variable temperature ^{1}H NMR, proton spin−lattice relaxation time (T_1), and density of the aqueous copolymer solution were correlated well with the proposed mechanisms responsible for the thermally induced structural transformation of polymeric assemblies.

Experimental Section

Materials. Synthesis and composition characterization of the graft copolymer comprising PAAc as the backbone and PNIPAAm and mPEG as the grafts were described in detail previously.[21,22] The chemical structure of the graft copolymer and its ^{1}H NMR spectrum in $CDCl_3$ at 20 °C are illustrated in Figure 1. Dry dimethylformamide (DMF) in a sealed capillary placed coaxially in the NMR tube during measurements was used as an external standard for establishing the calibration curve for mPEG with eight different concentrations in $CDCl_3$ and determining the copolymer composition. The graft copolymer was spectrometrically evaluated to be 1.0/18.1/13.3 in molar ratio of PAAc (Mw 20 000 g/mol)/PNIPAAm (3800)/mPEG (2000) and the corresponding weight-average molecular weight is 115 000 g/mol. Deionized water was produced from the Milli-Q Synthesis System (Millipore). Deuterium solvents used for the liquid ^{1}H NMR study were obtained from Cambridge Isotope (Andover, MA, U.S.A.). DMF as an external standard used in ^{1}H NMR measurements was obtained from Tedia (Fairfield, OH, U.S.A.) and distilled in vacuo before use.

Dynamic Light Scattering Measurements. Temperature/concentration-dependent hydrodynamic diameter (D_h) of polymeric assemblies in water was determined by a Brookhaven 90 plus particle size analyzer (He−Ne laser 15 mW, $\lambda = 678$ nm) at a fixed angle of 90°. The number of accumulation times was set at 50 and the data represent an average of at least three measurements. The polymer solutions were heated from 20 to 60 °C with two protocols, referred to as the fast and slow heating routes, respectively. In the latter case, the sample was heated slowly at an interval of 1 °C in the range 30∼35 °C. By contrast, the fast heating approach involves heating the polymer solution rapidly from 30 to 35 °C. In the range 35∼60 °C, the polymer solution was heated at an interval of 5 °C. The sample was equilibrated at each preset temperature for 30 min.

TEM Examinations. The sample was prepared by placing a few drops of the copolymer solution (1.0 mg/mL) at 60 °C on a preheated 300-mesh copper grid covered with carbon and allowed to stand for 20 s. Excess solution on the grid was gently removed with absorbent paper. This is followed by negative staining of the sample for 20 s by using a uranyl acetate solution (5.0 wt %). The sample was then dried at 60 °C for 2 days. The TEM image was obtained on a JEOL JEM-1200 CXII microscope operating at an accelerating voltage of 120 kV.

Variable Temperature ^{1}H NMR and Spin−Lattice Relaxation Time (T_1) Measurements. ^{1}H NMR spectra of the graft copolymer in D_2O solutions at different temperatures were obtained from a Varian Unity Inova-600 at 600 MHz without sample spinning. The pulse width of 4.9 μs with a relaxation delay of 2.0 s was utilized. Similar to the ^{1}H NMR measurement of the copolymer in $CDCl_3$, the same DMF in a sealed capillary was used repeatedly as the external standard and its signal at δ 8.45 ppm selected as reference resonance for evaluating the feature signal integrals of the graft copolymer in response to changes in temperature in comparison with those in $CDCl_3$ at ambient temperature. DHO that has been frequently used for the reference line in the D_2O liquid ^{1}H NMR measurements was not adopted in this work due to the upfield shift of its signal with increasing temperature in nature.[23,24] The spin−lattice relaxation times (T_1) of the feature protons attributed to both

PNIPAAm (methyl group) and mPEG (ethylene) grafts were determined at 600 MHz, using the standard inversion−recovery pulse sequence (180°−t−90°). In each measurement, 13−15 variable delays were employed and the waiting period was adjusted to at least 5 times larger than the expected T_1 value. T_1 was then calculated based on a nonlinear least-squares best-fitting routine of the spectrometer.

Density Measurements. Thermally induced interactions of the graft copolymer in water were quantitatively investigated in terms of the volumetric (or density) change of the copolymer solution. The density of the graft copolymer in water as a function of temperature was conducted as described in detail previously.[21] Assuming that the volume additivity is valid, the density of polymer solution ($\rho_{solution}$) evaluated experimentally at each temperature can be expressed as

$$\rho_{solution}(T) = \frac{M_{solution}}{V_{solution}(T)} = \frac{M_{polymer} + M_{H_2O}}{V_{polymer}(T) + V_{H_2O}(T)} \quad (1)$$

where $M_{solution}$, $M_{polymer}$, and M_{H_2O} are the masses of the aqueous solution, the graft copolymer, and water in the sample tube and $V_{solution}(T)$, $V_{polymer}(T)$, and $V_{H_2O}(T)$ are their corresponding volumes at each preset temperature, respectively. Rearranging eq 1 gives an explicit expression of the temperature-dependent apparent density (ρ_m) of the graft copolymer in terms of the masses of water and the copolymer and the densities of water ($\rho_{H_2O}(T)$) and the aqueous solution ($\rho_{solution}(T)$) as follows[21]

$$\rho_m(T) = \frac{\rho_{H_2O}(T)\rho_{solution}(T) \times M_{polymer}}{\rho_{H_2O}(T)(M_{polymer} + M_{H_2O}) - \rho_{solution}(T)M_{H_2O}} \quad (2)$$

Results and Discussion

I. Morphology and Size Measurements of Polymeric Particles. Because of the inherent thermoresponsive property of PNIPAAm grafts, the copolymer is capable of undergoing phase transition and supramolecular assembly into colloidal particles in aqueous phase upon thermally induced hydrophobic association. Figure 2A shows the polymeric assemblies with unimodal size distributions obtained from DLS measurements of the copolymer solutions with a concentration of 10.0 and 1.0 mg/mL, respectively, at 40 °C achieved by either fast or slow heating. Apparently, the polymeric assemblies obtained from the copolymer solution at 10.0 mg/mL and prepared by fast heating exhibit smaller hydrodynamic particle size and narrower particle size distribution than those at 1.0 mg/mL using the same heating route and those at 10.0 mg/mL prepared by slow heating. Figure 2B shows the response of the copolymer and its assemblies in terms of hydrodynamic diameters (D_h) to changes in temperature from 25 to 60 °C. Regardless of the copolymer concentration and heating method employed in this study, a remarkable increase in D_h was observed in the temperature range 30∼35 °C, indicating the phase transition occurring in a range that is consistent with the reported LCST (∼32 °C) for PNIPAAm. In spite of the appreciable differences in both size and size distribution, relatively constant hydrodynamic sizes of the polymeric assemblies were attained with the temperature being increased to 40 °C and above by fast heating for both the samples at 1.0 and 10.0 mg/mL. On the other hand, the particle size obtained from the sample at 10.0 mg/mL and prepared by slow heating first increased rapidly to a maximum at 35 °C and then decreased when temperature was further increased. These results indicate that the architecture of these supramolecular assemblies is virtually governed by the interactions of polymer segments within the assemblies and their responses to changes in temperature.

To further clarify the copolymer concentration effects on the assembly structure and justify if the selected concentrations (1.0 and 10.0 mg/mL in this work) are pertinent to the formation of different assembly structures under assessment, DLS characterization of the aqueous copolymer solutions in a concentration range 0.75∼50.0 mg/mL at 40 °C via fast heating was thus conducted, and the results are enclosed as part of the Supporting Information. The data show that a major difference in the structure of thermally induced colloidal assemblies occurs at ca. 5.0 mg/mL. Thus, the polymeric aggregates made from the copolymer solutions at 1.0 and 10.0 mg/mL adequately represent two distinct assembly models. Assemblies of similar sizes and presumably similar structures were achieved in the range 10.0∼30.0 mg/mL, and thereafter the sol−gel transition soon occurred (Supporting Information).[25,26] It is noteworthy that the aggregates produced in this work are much larger than typical polymeric micelles with a distinct core/shell morphology (ca. 40∼70 nm in D_h). It was reported that the enlarged aggregate size, in particular for those obtained from the thermally driven hydrophobic association, are caused primarily by the formation of a multicore or intermicellar structure within individual aggregates.[8,27,28] The multicore structure of assemblies are frequently stabilized by intercore connections of polymer segments (as more often observed in the graft copolymer systems) among individual hydrophobic microdomains, thereby leading to an increase in the colloidal particle size.[8,27,28] This is further confirmed by the TEM images of polymeric assemblies with varying extents of intercore connections for the copolymer solutions at 1.0 (Figure 3A) and 10.0 mg/mL (Figure 3B), respectively, at 60 °C via the fast heating mode. In contrast to the polymeric assemblies obtained from the copolymer solution at 1.0 mg/mL in which a remarkable intermicellar structure is developed, the extent of the multicore structure within the particles of the copolymer solution at 10.0 mg/mL is appreciably reduced although the same heating route was adopted. The TEM photographs also reveal that the particles of the copolymer solution at 10.0 mg/mL at 60 °C prepared by slow heating are similar to those prepared by fast heating (data not shown here). This is most likely an outcome of the transformation in size and structure as the polymeric assemblies are subjected to continual heating from 35 to 60 °C (Figure 2B).

II. Heating Mode/Concentration-Dependent Structural Characterization of Polymeric Assemblies. Representative ^{1}H NMR spectra of the graft copolymer (10.0 mg/mL) in D_2O at 25 and 50 °C prepared by fast heating are illustrated in Figure 4A. Comparing the two ^{1}H NMR spectra, a remarkable reduction in the feature signal intensities of PNIPAAm grafts at 50 °C, particularly at δ 1.1 ppm from their methyl protons, suggests the formation of polymeric micelles with hydrophobic cores comprising the solidified PNIPAAm segments to a significant extent. Such a signal intensity reduction is ascribed to a dramatic decrease in the proton spin−lattice relaxation time (T_1) upon the solidification of PNIPAAm grafts, thus rendering themselves undetectable in the liquid ^{1}H NMR measurements.[29,30] Nevertheless, this feature signal of PNIPAAm grafts at 1.1 ppm does not disappear completely at 50 °C. On the basis of the fractions detectable and undetectable, a distinct difference in the mobility of PNIPAAm segments that constitute the hydrophobic regions of polymeric assemblies was then clearly illustrated.[21,22] The

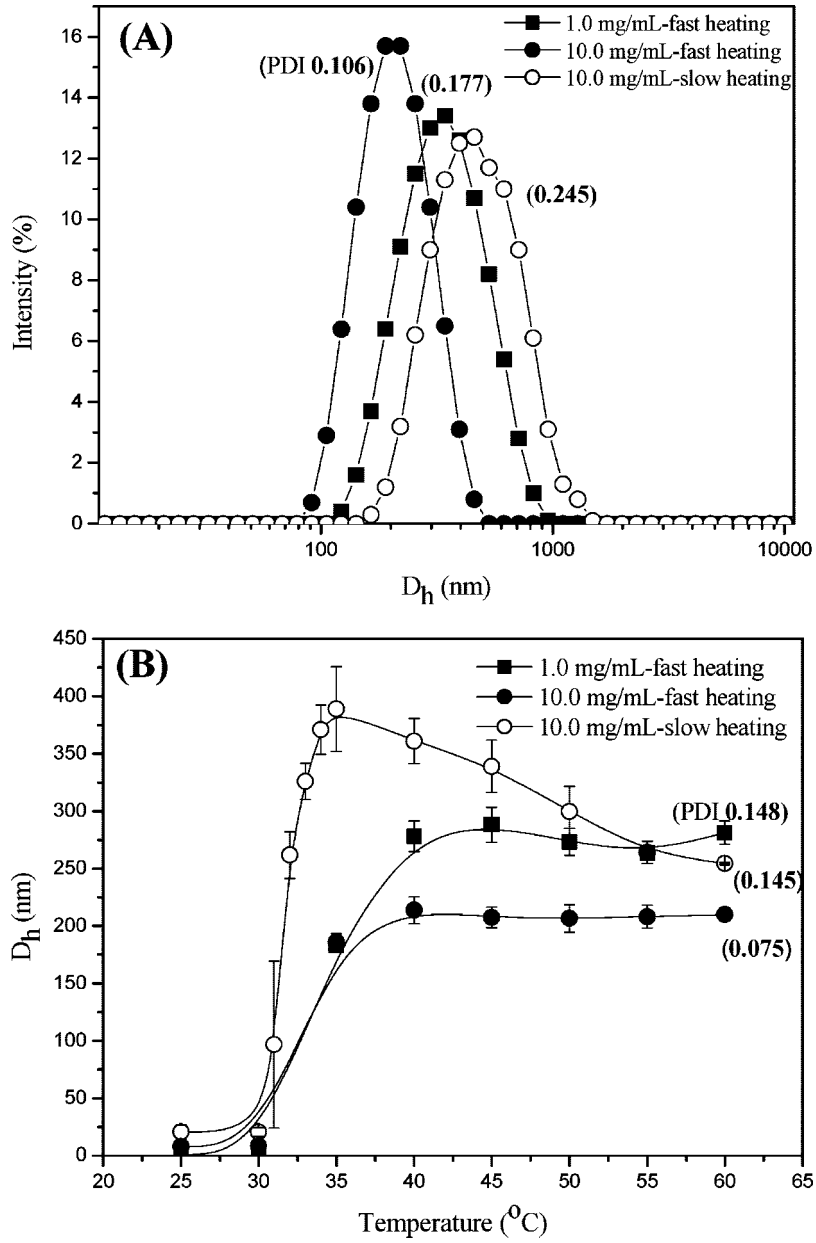


Figure 2. (A) DLS particle size distribution profiles for polymeric assemblies obtained from the graft copolymer in water at 1.0 and 10.0 mg/mL and 40 °C achieved by either fast or slow heating and (B) temperature dependence of the hydrodynamic diameters (D_h) of the graft copolymer (1.0 and 10.0 mg/mL) in water by two heating routes.

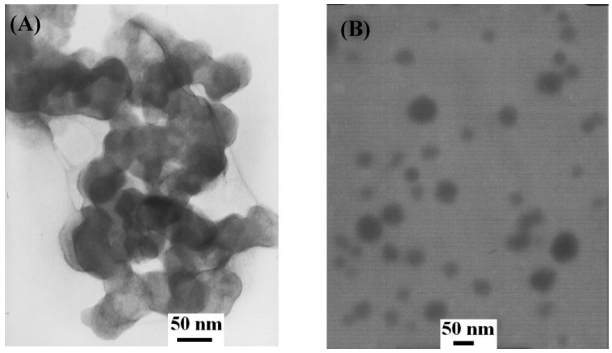


Figure 3. Representative TEM images of polymeric assemblies obtained from the graft copolymer solution with a concentration of (A) 1.0 and (B) 10.0 mg/mL at 60 °C and prepared by fast heating.

core of polymeric aggregates is thus developed in the form of both liquidlike and solidlike structures. While the solidlike inner cores comprise mainly PNIPAAm segments that are undetectable by ^{1}H NMR, those detectable PNIPAAm segments are located most likely as the liquidlike interfaces between the solidlike inner cores and hydrophilic shells.

Figure 4B illustrates the temperature dependence of the detectable fractions of PNIPAAm segments of the graft copolymer with concentrations of 1.0 and 10.0 mg/mL, respectively. At 1.0 mg/mL, the solidification of PNIPAAm grafts of the copolymer in response to phase separation and further temperature increase is rather limited (<20%), strongly implying the formation of loose and hydrated PNIPAAm-rich microdomains within the aggregates. For the copolymer solution at 10.0 mg/mL prepared by fast heating, PNIPAAm grafts were kept dehydrated and thus transformed from the liquidlike to solidlike structure beyond the phase transition region. The detected fraction of PNIPAAm grafts for the copolymer solution at 10.0 mg/mL at 35 °C achieved by slow heating was much higher than the counterpart by fast heating. This represents the enhanced hydration of PNIPAAm in the hydrophobic micro-

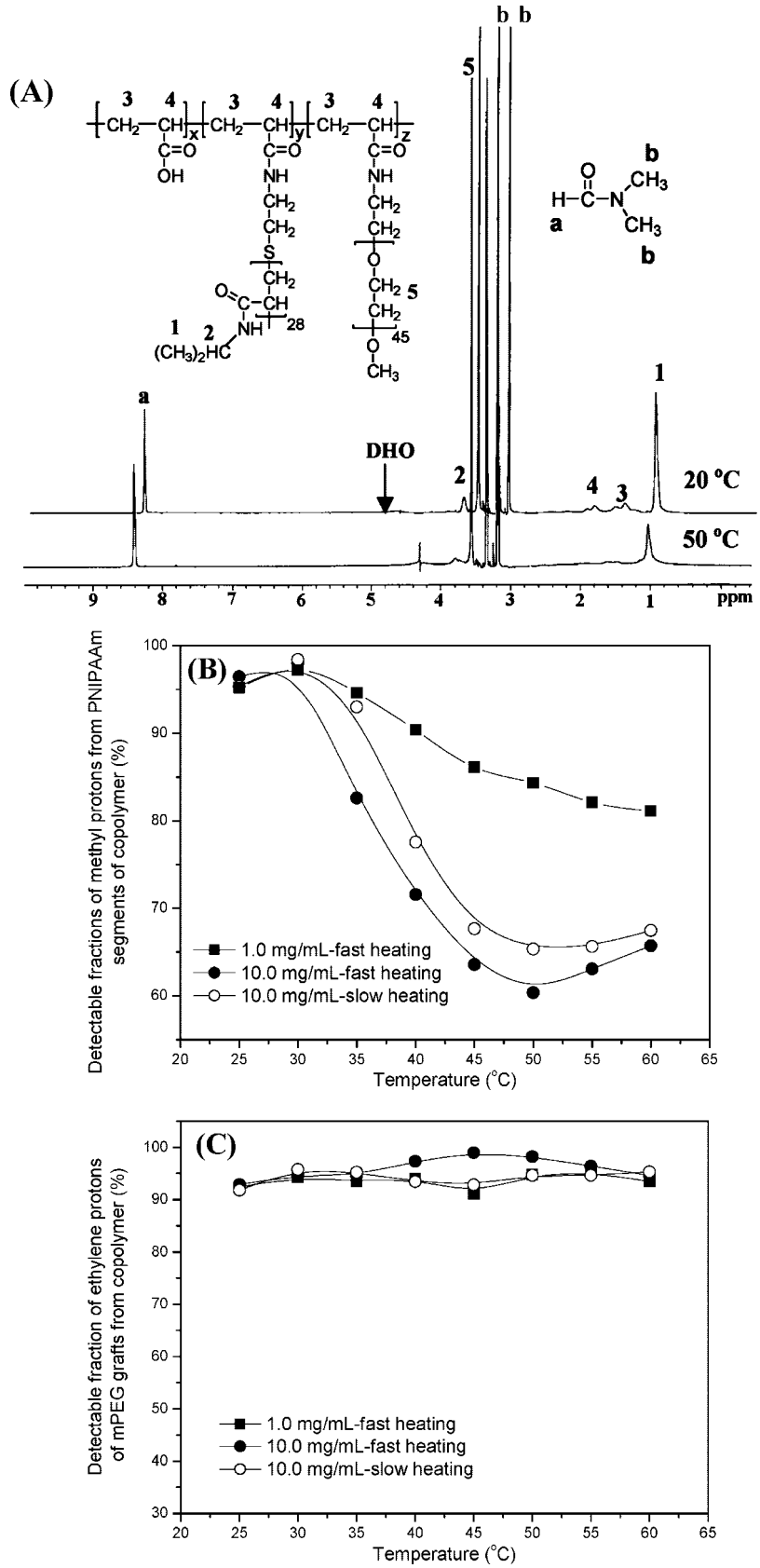
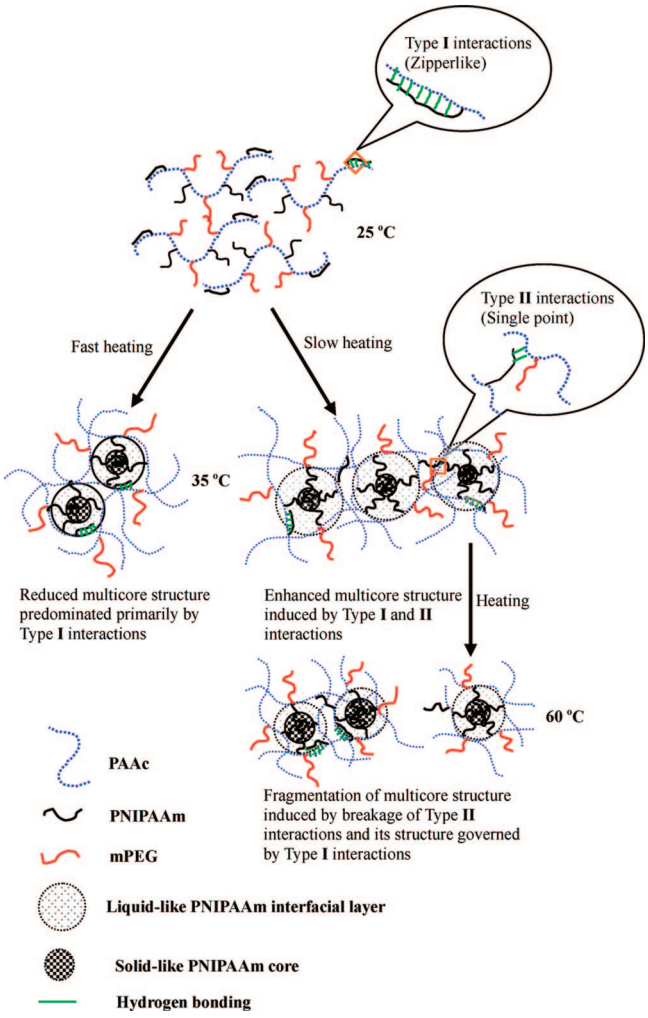


Figure 4. (A) ^{1}H NMR spectra of graft copolymer (10.0 mg/mL) in D_2O at 25 and 50 °C. Detectable fractions (%) of (B) methyl protons from PNIPAAm grafts and (C) ethylene protons from mPEG segments of the graft copolymer (1.0 and 10.0 mg/mL) in D_2O in ^{1}H NMR measurements as a function of temperature with respect to their signal integrals in $CDCl_3$ at 20 °C, using the DMF signal as a reference.

domains of polymeric assemblies (10.0 mg/mL) at 35 °C in response to switching heating from the fast to slow mode, accompanied by an increase in the colloidal particle size (see the close and open circular data points at 35 °C in Figure 2B). Further increasing the temperature, the hydrophobic PNIPAAm regions within the assemblies achieved by slow heating become

SCHEME 1: Schematic Illustration of Thermoinduced Structural Transformation of Polymeric Assemblies Obtained from Graft Copolymer in Water at 10.0 mg/mL Prepared by Either Slow or Fast Heating



more dehydrated and solidified, thereby leading to a core structure that is similar to the counterpart by fast heating at high temperature. The colloidal particle size data as a function of temperature for the copolymer solution prepared by slow heating also show a similar trend (Figure 2B). It should be noted that the feature signal intensity of mPEG segments of the copolymer at 3.6 ppm remains fully detectable with temperature being increased from 25 to 60 °C, regardless of the copolymer concentration and heating method (Figure 4C). This suggests that mPEG grafts be barely embedded in the solidlike inner core regions and, if any, interact with PNIPAAm segments only within the interfacial layers.

In agreement with the DLS and ^{1}H NMR data, the temperature dependence of the fluorescence intensity ratio (I_3/I_1) of pyrene as a nonpolar probe in the aqueous copolymer solutions confirms that the copolymer assembling process is driven by hydrophobic association in the temperature range 30∼35 °C (Supporting Information).[31] In addition, the relatively low I_3/I_1 values obtained from the copolymer solution at 1.0 mg/mL and high temperature (>45 °C) as compared to those at 10.0 mg/mL reflect the absence of fully solidified PNIPAAm regions within the aggregates. The combined results of DLS, TEM, ^{1}H NMR, and fluorescence measurements indicate that the extensive multicore structure of the polymeric aggregates obtained from the copolymer solution at 1.0 mg/mL is caused primarily by the complexation of PNIPAAm grafts with other polymer segments that concomitantly induces hydration of PNIPAAm grafts and restricts the solidlike core formation. The complexation remains intact toward further heating, thereby rendering the particle size unchanged with temperature. The difference between these two heating routes lies only in the temperature range 30∼35 °C, where the hydrophobically driven phase transition takes place. Compared to the case of 10.0 mg/mL of the copolymer solution with the fast heating mode, the multicore structure and intercore connections within the assemblies induced by slow heating in this transition temperature range are significantly enhanced (and the size is thus increased) by increased interactions among polymer segments but are reduced gradually by destruction of these labile interactions with further increasing temperature. On the other hand, the polymeric assemblies (10.0 mg/mL) produced by fast heating, retain their hydrodynamic size upon further heating beyond the phase separation region, although the liquidlike PNIPAAm interfacial layers are gradually dehydrated and solidified. Since the association of PNIPAAm with PAAc and mPEG segments occurring in the temperature range 30∼35 °C is notably reduced by fast heating, this size invariance suggests that the multicore structure and the intercore connections of the assemblies be created by segmental interactions that occur prior to phase separation and tend to resist thermal attack upon further heating to higher temperature.

The potentiometric titration of the graft copolymer solution at 0.25 mg/mL and 25 °C shows that the ionization of the backbone AAc residues with a final pH 4.7 is less than 8% (Supporting Information). It was shown that hydrogen bonds can form via the interactions of PAAc with mPEG and PNIPAAm at this pH,[20,32−34] but experimental evidence does not exist to support the significant hydrophobic association and polymer chain aggregation as observed at pH 3.0 due to the presence of ionized AAc residues.[33] This was also confirmed by DLS measurements of the graft copolymer solution (10.0 mg/mL) at 25 °C at pH 3.0 (in particulate form) and pH 4.7 (in dissolved state), respectively.[22] Although being capable of forming hydrogen bonds with both PNIPAAm and mPEG grafts, un-ionized AAc units show a strong tendency to pair with PNIPAAm.[33−35] The binding strength of hydrogen bonds obtained from the interactions between un-ionized AAc and NIPAAm units in a consecutive and complementary pairing manner[20] (referred to as type I interaction herein) can be greatly enhanced to tolerate upcoming thermal attack. However, the formation of AAc−NIPAAm hydrogen bonds is sterically restricted by the presence of PEG grafts. Thus, these zipperlike interactions only occur at sites distant from mPEG grafts and are involved in the formation of the liquidlike interfacial layers in which only those PNIPAAm grafts distributed along the same polymeric backbone can take part in the evolution of the multicore structure and intercore connections of the assemblies upon phase separation.

By contrast, with slowly heating in the range 30∼35 °C the tendency of NIPAAm residues toward forming hydrogen bonds with un-ionized AAc units is increased significantly[20,36,37] due to the thermally enhanced segmental mobility in addition to the increased inherent hydrophobic association of PNIPAAm grafts with un-ionized PAAc segments and mPEG grafts. However, due to the steric hindrance arising from neighboring mPEG chains, these additional hydrogen bonds form in an isolated single-point manner. Such interactions are referred to as the type II interactions. Note that type II interactions may further involve mPEG grafts via hydrogen bonding with un-ionized PAAc

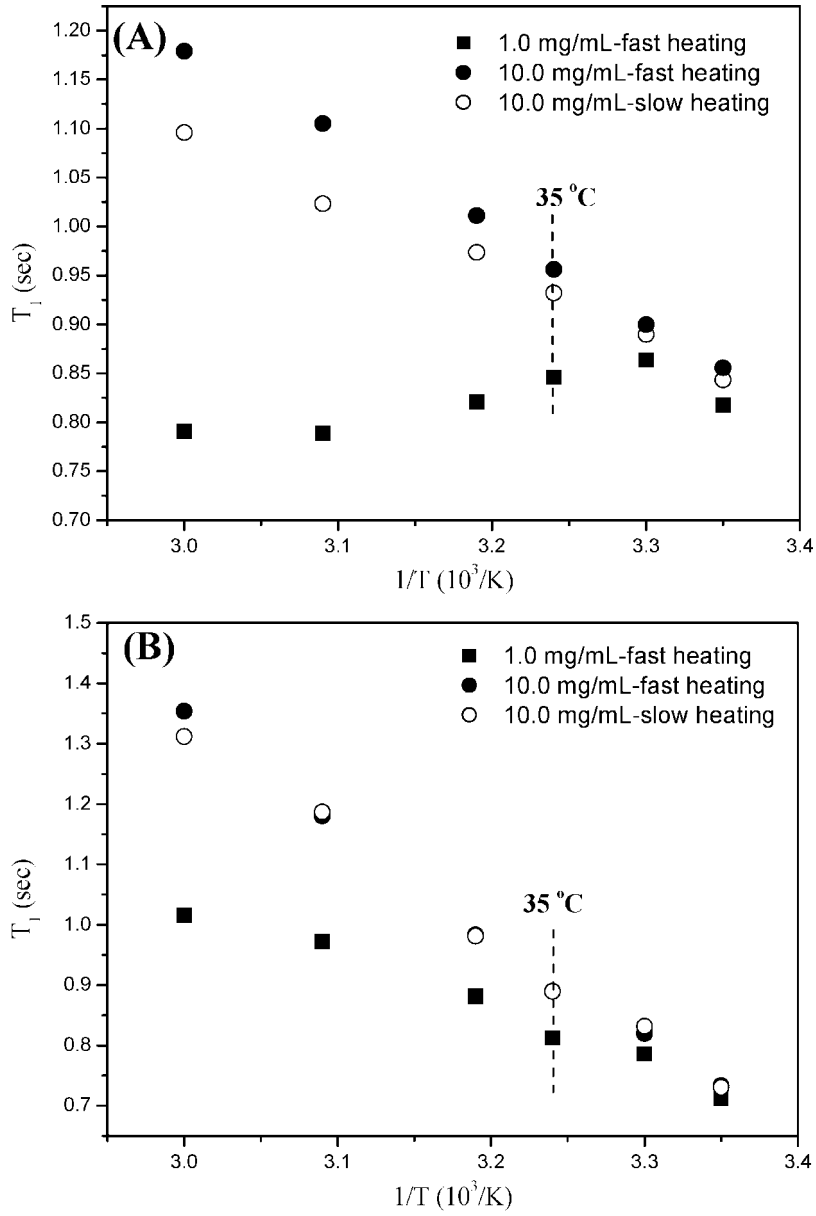


Figure 5. Temperature dependence of the spin−lattice relaxation times (T_1) of (A) the methyl protons of isopropyl groups arising from PNIPAAm grafts and (B) the ethylene protons from mPEG grafts of the graft copolymer (1.0 and 10.0 mg/mL) in D_2O prepared by either fast or slow heating.

segments simultaneously associating with PNIPAAm grafts. Type II interactions are responsible for the increase of both the colloidal particle size and the fraction of the liquidlike PNIPAAm interfacial layers (due to the embedding of mPEG grafts therein) of the assemblies obtained from the graft copolymer solution (10 mg/mL) that experiences the phase separation by slow heating. Nevertheless, the interactions are more labile to disruption by the thermally induced contraction of PNIPAAm grafts upon further heating. This will then result in a reduction in the fraction of the liquidlike interfaces, partial destruction of the intercore connections, fragmentation of the multicore structure and thus the decrease of the colloidal particle size,[28] as confirmed by DLS and ^{1}H NMR measurements in this study. When temperature is raised to 60 °C, type II interactions are markedly destroyed and the assembly structure and size become similar to those produced by the fast heating mode (Figures 2B and 4B) in which the multicore structure and its response to further temperature raise are primarily governed by type I hydrogen bonding. While PNIPAAm grafts are continually dehydrated upon further heating, type I interactions and the resultant intercore connections within the assemblies (10.0 mg/mL) by fast heating remain intact, thereby leading to a relatively constant particle size (Figure 2B).

To verify the molecular packing model proposed herein (Scheme 1), the proton spin−lattice relaxation times (T_1) of the feature groups arising from PNIPAAm and mPEG grafts of the copolymer that experienced phase separation from D_2O by both the fast and slow heating routes were investigated. The results are shown in an Arrhenius manner in Figure 5. T_1 values of the methyl protons of PNIPAAm grafts of the copolymer solution at 10.0 mg/mL increase with increasing temperature even beyond the phase separation region for both runs with different heating modes. On the basis of the relation of T_1 with the correlation time described by the BPP theory,[29,38,39] this trend represents a high thermally induced mobility of PNIPAAm grafts residing within the liquidlike interfaces since the T_1−temperature curve is primarily governed by the product of correction time and Lamor frequency while the latter was kept

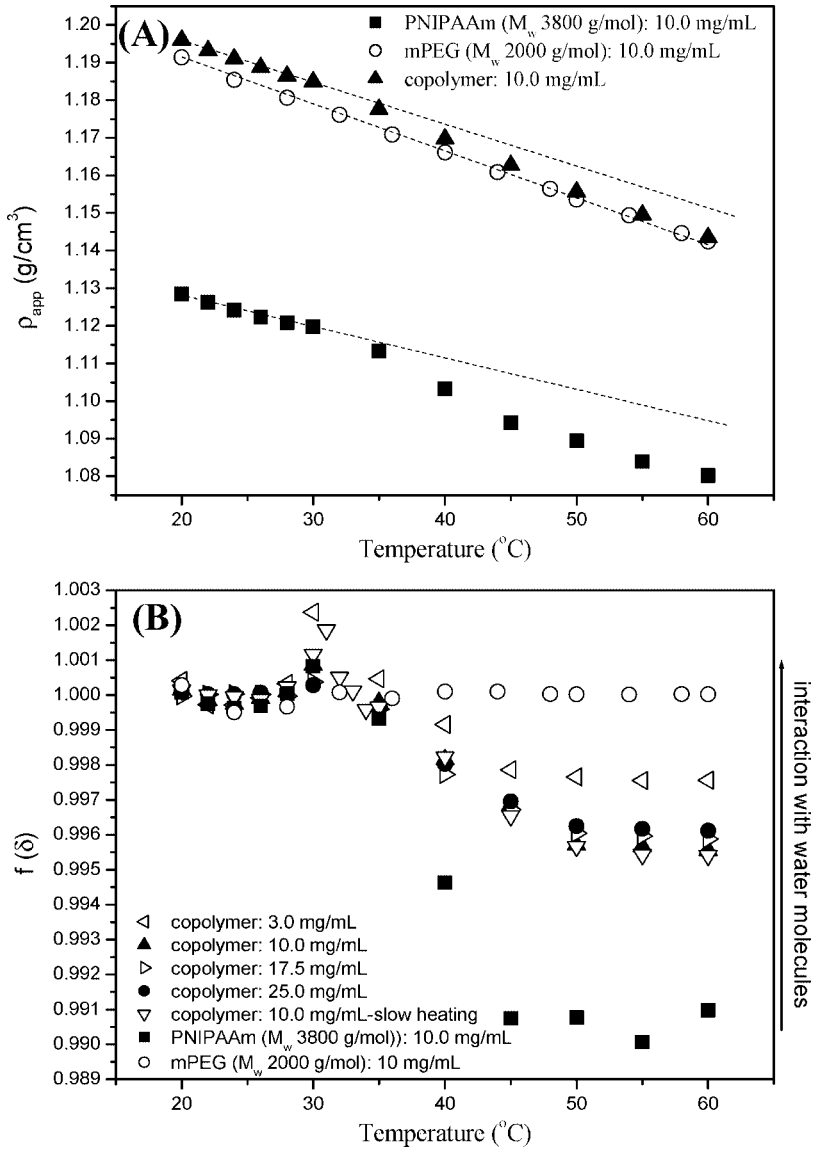


Figure 6. (A) Temperature dependence of the apparent densities of PNIPAAm, mPEG, and the graft copolymer in water (10.0 mg/mL). The dash lines represent the theoretical values extrapolated from the linear relationship established in the absence of polymer phase transition in water. (B) Temperature dependence of the function of deviation in the interaction of macromolecules with water molecules. The temperature profile was achieved by fast heating, unless stated otherwise.

constant in this study. Although T_1 values of the PNIPAAm methyl protons are abruptly reduced to such extent that these protons become undetectable by ^{1}H NMR upon transformation of the PNIPAAm segments from the liquidlike to solidlike structure as temperature is increased, the response of T_1 to temperature differs with varying copolymer concentrations and heating methods (Figure 5A). This is primarily caused by the different extents of hydration and interactions with un-ionized AAc residues and mPEG grafts. Because of the presence of type II interactions, particularly those involving mPEG grafts within the liquidlike interfacial layers of the assemblies (10.0 mg/mL) induced by slow heating, the resultant hydration of PNIPAAm segments renders their conformational freedom more restricted, and therefore the corresponding T_1 relaxation is faster than that for the counterpart prepared by fast heating.

The graft copolymer solution with a concentration of 1.0 mg/mL remains appreciably hydrated primarily as a result of the extensive interactions of PNIPAAm segments with mPEG grafts when the temperature is increased above 35 °C via the fast heating mode. This will lead to a significant increase in the detectable fraction by ^{1}H NMR (Figure 4B) and a decrease in the rate of change in T_1 with temperature (Figure 5A) as compared to those obtained from the copolymer solutions at 10.0 mg/mL. The enhanced hydration of the polymeric assemblies at 1.0 mg/mL has been confirmed by density measurements of the graft copolymer in water, as shown below. The embedding of mPEG grafts within the liquidlike interfacial layers also inevitably reduces T_1 values of mPEG by associating with the hydrophobic PNIPAAm grafts via type II interactions. For the polymeric assemblies at 1.0 mg/mL, mPEG grafts are largely involved in the association with PNIPAAm grafts, thereby leading to a significant reduction in their segmental mobility and the corresponding T_1 values (Figure 5B). As a result, the hydrophobic microdomains are more hydrated and, thus, the assemblies become larger in size (due to the intercore connections via the contribution of mPEG grafts) than those at 10.0 mg/mL. Although mPEG grafts are embedded to a larger extent within the liquidlike interfacial layers of assemblies (10.0 mg/mL) via slow heating than those prepared by fast heating, the difference in T_1 values of mPEG grafts as a function of

temperature between these two assemblies is not significant. This is mainly because most of the mPEG grafts in both colloidal systems reside within the hydrophilic shells and act as highly mobile corona surrounding the particles in interacting with water molecules.

In addition to the significant association of PNIPAAm grafts with PAAc and mPEG segments, the polymeric assemblies (1.0 mg/mL) are also characterized by enhanced hydration of the graft copolymer at high temperature as compared to those with a concentration of 10.0 mg/mL. The hydration of the graft copolymer as a function of the polymer concentration and the heating mode was evaluated in terms of changes in the specific volume (or density) of the graft copolymer in water with temperature. On the basis of eq 2, the apparent densities of mPEG, PNIPAAm, and the graft copolymer in water as a function of temperature were determined and the results are shown in Figure 6A. As expected, the apparent density of mPEG (Mw 2000 g/mol) in aqueous solution decreased linearly with increasing temperature in the entire temperature range investigated due to an increase in the partial volume of polymer primarily as a result of the thermal expansion and changes in the interactions between the polymer and water with temperature. On the other hand, an abrupt decrease in the apparent density of PNIPAAm (Mw 3800 g/mol) in water occurs as the temperature is increased to 32 °C due to a sharply reduced interaction with water upon phase transition of the polymer. While the magnitude of deviation (δ) signifies a measure of the variation between the real interaction of polymeric particles with water molecules and that presumably in the absence of phase transition, an interaction function, $f(\delta)$, that represents a quantitative parameter of the interaction of polymer with water can be easily developed as follows:

$$f(\delta) \equiv \rho_m(T)/\rho_t(T) \tag{3}$$

where $\rho_m(T)$ is the polymer density measured by densimeter and $\rho_t(T)$ is the theoretical density obtained from the extrapolation of linear relationship in the absence of phase transition as shown in Figure 6A.

Figure 6B shows the temperature dependence of $f(\delta)$ for mPEG, PNIPAAm, and the graft copolymer with varying concentrations in aqueous phase. Obviously, $f(\delta)$ approaches unity for mPEG in the entire temperature range and for PNIPAAm and the graft copolymer at low temperature (20∼28 °C) due to the absence of phase transition. The hydration of the copolymer with 3.0 mg/mL at high temperature is higher than those with a concentration of 10.0 mg/mL and higher. This is in agreement with the results obtained from ^{1}H NMR measurements. This is because PNIPAAm segments present in the current colloidal system is insufficient to induce hydrophobic association of the graft copolymer to form a distinct core/shell micelle structure. It is noteworthy that instead of 1.0 mg/mL a copolymer concentration of 3.0 mg/mL was used to improve the accuracy and preciseness of density measurements. The structure of polymeric assemblies with a concentration of 3.0 mg/mL is rather hydrated and loose that is similar to those with 1.0 mg/mL, as described previously. The hydration of the graft copolymer in the range 10.0∼25.0 mg/mL varies insignificantly due to the essentially identical architecture of those assemblies. Only a small difference in the hydration extent of the graft copolymer (10.0 mg/mL) in aqueous solutions subjected to fast and slow heating, respectively, was observed even though the heating mode-induced variance of the assembly architecture has been identified by DLS and ^{1}H NMR. It can be readily understood that the same hydration of the copolymer can be achieved by the counterbalance of changes in the hydration of different polymer segments. For example, the assemblies obtained from the copolymer solution (10.0 mg/mL) and induced by fast heating are characterized by a higher degree of hydration of mPEG grafts and a lower degree of hydration of PNIPAAm segments than those prepared by slow heating, thereby giving rise to virtually the same hydration of the entire graft copolymer, as shown in Figure 6B.

Conclusion

Aqueous polymeric assemblies obtained from a graft copolymer, PAAc-g-PNIPAAm/mPEG, with a concentration of 10.0 mg/mL and induced by fast heating are characterized by a distinct core/shell micellar structure in which the extent of the multicore architecture is insignificant due to the insufficient intercore connections. As a consequence, the microstructure and the corresponding colloidal particle size are relatively insensitive to changes in temperature beyond the phase transition region. By contrast, the multicore structure and the intercore connections of the assemblies (10.0 mg/mL) prepared by slow heating become prominent due to the greatly enhanced association of PNIPAAm grafts with mPEG and PAAc segments in the temperature range 30∼35 °C, thereby leading to the increased hydration of PNIPAAm grafts within the assemblies and an increase in the colloidal particle size. However, these intercore connections are rather labile to thermal attack and the assemblies are thus transformed via multicore fragmentation into a structure similar to that of the assemblies prepared by fast heating at high temperature (60 °C). Owing to insufficient amount of PNIPAAm in water, the copolymer solution at 1.0 mg/mL experiencing phase separation via the fast heating route favors the formation of highly loose and large aggregates in which PNIPAAm grafts are markedly hydrated and associated with PAAc and mPEG segments. This will lead to the lack of solidified hydrophobic core structure (^{1}H NMR undetectable) within the aggregates and the insensitive response in the colloidal particle size to further temperature increase.

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Supporting Information Available: The hydrodynamic diameters (D_h) of polymeric assemblies obtained from the graft copolymer in water in a concentration range 0.75∼50.0 mg/mL at 40 °C achieved by fast heating, the temperature dependence of the I_3/I_1 values from the fluorescence emission spectra of pyrene in the aqueous copolymer solutions (1.0 and 10.0 mg/mL) by either fast or slow heating and the degree of ionization (α) of the graft copolymer as a function of pH at 25 °C. This material is available free of charge via the Internet at http://pubs.acs.org.

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