Effect of Intermolecular and Intramolecular Forces on Hydrodynamic Diameters of Poly(*N*-isopropylacrylamide) Copolymers in Aqueous Solutions

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ABSTRACT: A novel copolymer, poly(*N*-isopropylacrylamide-*co*-hydroxypropyl methacrylate-*co*-3-trimethoxysilypropyl methacrylate) has been synthesized and the hydrodynamic diameters in various aqueous solutions under different temperatures are determined by dynamic light scattering. The results show that the hydrodynamic diameters of copolymers have no obvious change in each working solution below lower critical solution temperature (LCST); across LCST, the diameters increased suddenly at different initial temperature in various aqueous solutions; above LCST, they decreased slightly as the temperature increased in UHQ water, and increased continuously with increasing temperature or salt concentration in saline solutions, and reduced with the rising of pH value in pH buffer. These are attributed to different intermolecular and intramolecular forces leading to disparity in dimension, conformation, and LCST of copolymers. The hydrogen bonding between water molecules and copolymer chains could maintain size and conformation of copolymer single chain; the hydrogen bonding between amide linkages and hydrophobic interactions between isopropyl groups result in intramolecular collapse and intermolecular aggregation; the electrostatic repulsion weakens aggregation extent of copolymers. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2012

INTRODUCTION

Poly(*N*-isopropylacrylamide) (PNIPAAm), a temperature-responsive polymer, shows remarkable hydrated and dehydrated changes in response to temperature shift, and displays lower critical solution temperature (LCST) in aqueous solution.[1] The behavior is associated with the formation and deformation of hydrogen bonding between water molecules and polymer chains. PNIPAAm forms hydrous and unfolded structure below LCST, while exhibits dehydrated and compact configuration above LCST. Since the integrated structure and surface wettability of the polymers could be tuneable by changing temperature, PNIPAAm and its derivatives have been currently applied in drug delivery,[2,3] immobilization of (bio)macromolecules and ions[4,5] as well as smart culture substrates and artificial extracellular matrix for tissue engineering.[6–9]

The hydrodynamic diameters variation with temperature is commonly regarded as a criterion for the thermo-sensitivity of PNIPAAm-containing materials, and furthermore the LCST can be also obtained from dynamic light scattering (DLS)

results.[10–13] There have been two distinct external performances on the temperature dependence across LCST in ultra high quality (UHQ) water. Across LCST, the hydrodynamic diameters (or particle size) of temperature-responsive products show an abrupt change from large to small or generate a sharp transition from small to large. The former is often found in macrogels or microgels[14–16] presenting swollen-to-collapsed changes, and polymers with long linear or long branched chains[17–19] showing coil-to-globule transitions. The latter can be observed in linear or block or micelle polymers with short chains[11,12,20,21] exhibiting segment-to-cluster conversions. Although there are two opposite expressions, both collapse and aggregation are caused by the loss of hydrogen bonding between water molecules and amide linkages, and the formation of hydrophobic interactions between isopropyl groups and hydrogen bonding between amide linkages. When the environmental temperature is higher or lower than LCST, there presents the internal structural adjustment between molecules but the hydrodynamic diameters are not changed obviously.

The PNIPAAm and its derivatives are more widely used in various solutions with different concentrations of salt and other species, different pH value, or temperature in practical application. If the PNIPAAm-containing materials are placed in salt solutions or pH buffers, the hydrodynamic diameters happen to change. The addition of salts weakens the hydrogen bonds between amide linkages of PNIPAAm and water molecules, resulting in the greater shrinkage of polymers with long chains and macro/microgels, or the larger aggregation of polymers with short chains in aqueous solutions at the temperature above LCST. Furthermore, the degree of shrinkage or aggregation depends on the valence of the counter ion and the salt concentration.[14,22,23] The presence of other monomers in PNIPAAm-based copolymers may even more affect the configuration changes and temperature sensitivity in water and salt solutions.[24] PNIPAAm are not pH-sensitive polymer, but if introduced the acidic or alkaline compositions (for example, acrylic acid, propylacrylic acid) to PNIPAAm backbone during the synthesis, the final copolymers display dual thermo- and pH-responsive properties. The copolymer compositions and solution parameters such as pH value and ionic strength have great influence on the hydrated/dehydrated degrees and LCST of copolymers.[15,25–27]

Although previous studies revealed the fact that the intermolecular and intramolecular forces including hydrogen bonding, hydrophobic interactions, and electrostatic repulsion induce the changes in dimension, conformation, and LCST of PNIPAAm-based materials, systematical studies are limited to analyze the size and configuration changes of temperature-responsive polymers in various experimental conditions from the point of bearing force. This study systematically investigates the dimensional and conformational variety of PNIPAAm-based copolymers by analyzing the intermolecular and intramolecular forces acting on copolymers in various aqueous solutions including UHQ water, salt solutions, and pH buffers under different working temperatures from 15°C to 45°C, based on the variance of hydrodynamic diameters determined by DLS. And use it as the basis to put forward a viewpoint that the formation and extent of aggregation (collapse) or disassociation (swelling) of PNIPAAm-mediated materials depend on which intermolecular and intramolecular forces play dominant roles. The proposed conclusion could be served as a guide to analyze and predict the dimension and conformation of PNIPAAm-containing materials under different conditions.

EXPERIMENTAL

Materials

N-isopropylacrylamide (NIPAAm) with purity of 98% was purchased from Wingch (China) and freshly recrystallized from *n*-hexane and freeze-dried. Hydroxypropyl methacrylate (HPM) and 3-trimethoxysilylpropyl methacrylate (TMSPM) were bought from Aldrich (Milwaukee, WI). 2,2-Azobisisobutyronitrile (AIBN) was provided by BDH (UK) and refined via recrystallization in ethanol followed by freeze-drying. The others including HCl, NaOH, NaCl, and $CaCl_2$ were purchased from Aldrich. The sample cuvettes for DLS experiments were cleaned by soaking in 5% (v/v) Decon90 solution (Decon Laboratories

Limited, E. Sussex, UK), then immersed into piranha solution (H_2O_2: H_2SO_4 = 1 : 3 by volume) for 30 min, followed by abundantly rinsing with tap water and UHQ water, and dried before use.

Polymer Synthesis and Characterization

The synthesis and characterization of PNIPAAm-based copolymers was described in our previous study.[28] Briefly, PNIPAAm copolymers were synthesized by incorporating NIPAAm, HPM, and TMSPM (initial molar ratio = 18 : 1 : 1) initiated by AIBN (1% total mol of reactants) via free radical polymerization at 60°C for 12 h under nitrogen, and then precipitated and dried at −60°C under vacuum for 24 h. The pH value of PNIPAAm copolymer solution with 1.0 mg mL^{-1} was 5.9 at 20°C in UHQ water, reflecting that the novel copolymer was a substance with weak acidity. The Fourier transform infrared spectroscopy showed that chemical structure of PNIPAAm copolymer and the ^{1}H nuclear magnetic resonance results confirmed the monomer molar ratio of final product with 18 : 1 : 1. Furthermore, the gel permeation chromatography indicated the molecular weight of copolymers with M_w of 192 kD, M_n of 119 kD, and polydispersity index M_w/M_n of 1.6. Figure 1 schematically illustrates the synthesis of PNIPAAm copolymers and its FTIR spectrum.

Dynamic Light Scattering

The DLS was used to measure the hydrodynamic diameters of PNIPAAm copolymers in aqueous solutions, accordingly proving their thermo-sensitivity and determining the LCST. The DLS experiments were performed according to the reported protocols[11] with some modifications. The PNIPAAm copolymers were dissolved in various aqueous solutions and stirred at room temperature for above 12 h. These mixtures were filtered with 0.2-μm filter membranes, respectively, to remove impurities prior to adding 1 mL solution into the sample cuvette. The cuvette was then placed in the refractive-index-matched bath of Malvern Nanosizer (Nano-S-ZEN1600, Malvern Instruments Ltd, UK) with scattering angle fixed at 173°. The experimental temperature was changed controllably between 15°C and 45°C, and the temperature fluctuation was kept within 0.1°C. The same concentration of 1.0 mg mL^{-1} PNIPAAm copolymers was used in all DLS experiments and the same heating and cooling rates were applied between parallel measurements. After temperature changing each time, the data were not captured until the solution had reached the desired temperature and the copolymers had been in the equilibrium state (ca., 10 min). The data were analyzed using Malvern Instruments Dispersion Technology Software v4.20. Each correlation curve was accumulated 30–50 times to eliminate noises.

Measurements were performed in UHQ water, and also conducted in salt solutions and pH buffers. The procedures were basically identical in various aqueous solutions, expect for different solvents and some special steps listed as followed. At least three replicates were applied in each evaluation.

Salt Solutions. To investigate the dependence on salt type and salt concentration, 10 m*M*, 50 m*M*, 100 m*M* NaCl solutions and 10 m*M* $CaCl_2$ solution were used for solvents, respectively.
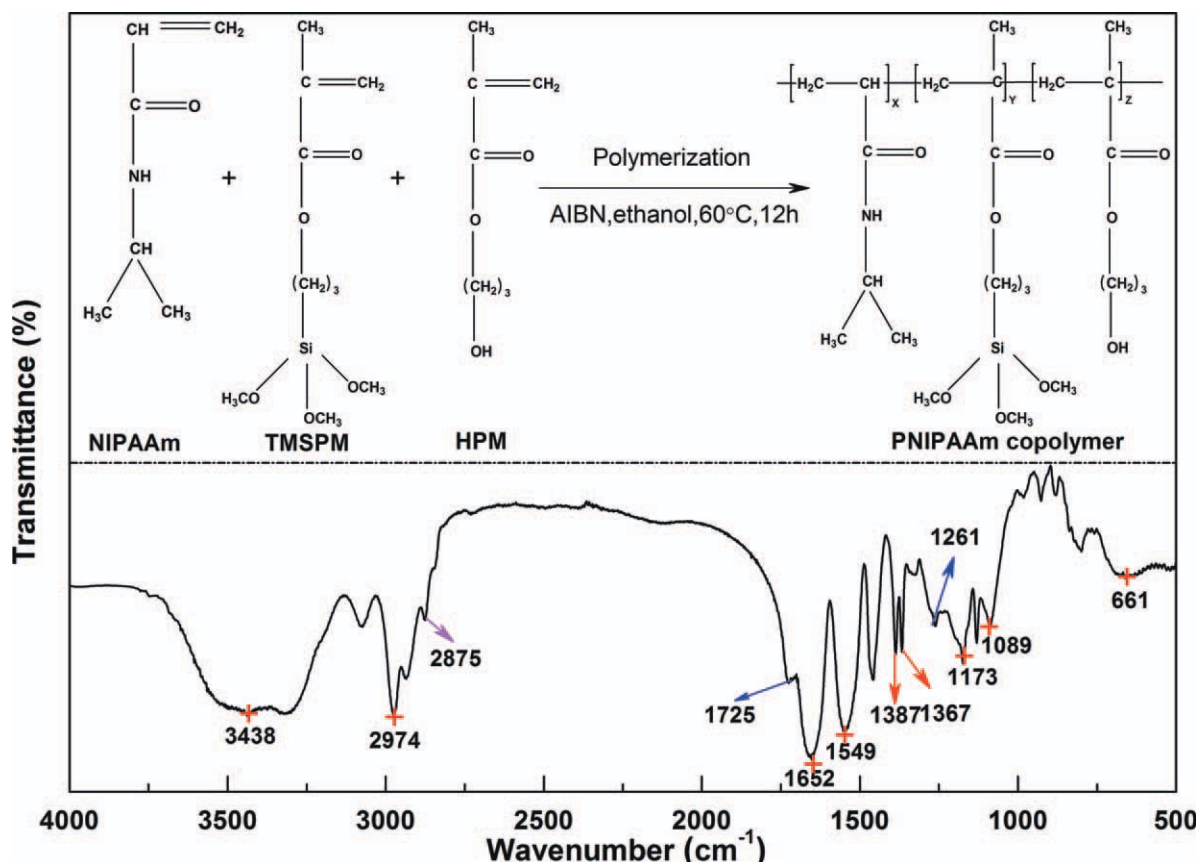
Figure 1. Schematic illustration for the synthesis of PNIPAAm copolymer and its FTIR spectrum. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

pH Buffers. The solution was prepared using UHQ water or 10 mM NaCl solution as solvent, and then adjusted pH value with NaOH or HCl, determined by pH meter (HANNA Instruments, UK). To avoid generating unnecessary salt caused by acid–base neutralization, the untreated solution was divided into two groups. One group was adjusted by gradually adding HCl to decrease the pH value, and the other was modulated by gradually adding 1M NaOH solution to increase the pH value.

Statistics

The experimental data were analyzed by OriginPro 8.0 software (OriginLab Corporation). The average hydrodynamic diameters of PNIPAAm copolymers at the given temperature or pH value were expressed as Mean $\pm$ SD (standard deviation) values derived from size distributions by volume of the parallel groups.

RESULTS AND DISCUSSION

The fundamental size distributions generated by DLS is based on the intensity of scattered light. According to the Rayleigh approximation, the intensity of scattered light is proportional to the 6th power of particle diameter. Thus, particle size rather than number is the decisive factor of the intensity distribution. The intensity distribution can be converted into volume or number distribution via Mie theory. The number distributions are seldom used because small errors in data acquisition can

lead to the huge errors. Thus all the DLS results in this study were expressed in volume distribution.

PNIPAAm Copolymers in UHQ Water

Figure 2 shows typical scattering profiles from DLS. There were four types of size distributions by volume. Below LCST, the hydrodynamic diameters of the PNIPAAm copolymers were distributed from 5 nm to 68 nm, with the peak centered at 10 nm. The polydispersity index (PDI) width had the values of 6 nm at 15°C and 23°C, respectively, indicating that the copolymers were soluble in water and no obvious formation of aggregates appeared. The molecular size and distribution of the copolymers did not change much with temperature below LCST, indicating that the strong hydrophilic shells were formed by the hydrogen bonding between water molecules and hydrophilic groups within copolymers including amide linkages, hydroxyl, and siloxane groups. The transition occurred above 29°C. At 30°C, the peak shifted to 122 nm was increased significantly and the PDI width of 49 nm was wider obviously. This observation demonstrated that the dehydration of copolymer chains was led to the release of water molecules, and the increase in particle size implied the increasing intermolecular hydrophobic interaction between isopropyl groups and hydrogen bonding between amide linkages. At 31°C, the results indicated that the PNIPAAm-based copolymers had special characteristic of bimodal size distribution. The first size distribution was shown between
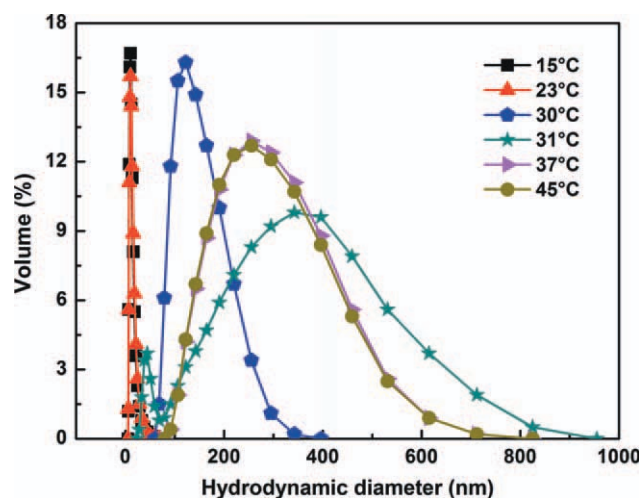
Figure 2. Typical hydrodynamic diameter distributions by volume of PNIPAAm copolymers in UHQ water measured by DLS at different temperatures. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

24 nm and 68 nm, with the peak at around 44 nm, accounting for 14% of overall. The lowest hydrodynamic diameter observed at 31°C was smaller than minimum value at 30°C. This was because a small part of copolymer clusters or single molecule chains with relatively larger size had begun to form shrinkage structure by further adjustments of chain segment under the intermolecular and intramolecular hydrophobic interaction coupled with possible hydrogen bonds. The second size distribution was between 68 nm and 955 nm, with the peak in the vicinity of 342 nm, occupying overall 86% about. This suggested that most of copolymers continued to aggregate intensively to form even larger aggregates induced by the increasing intermolecular hydrophobic interaction and anhydrous hydrogen bonding. The average hydrodynamic diameter at 31°C was estimated to be around 280 nm. Further increase in temperature led to larger aggregation, with the size distribution becoming narrow again. Above LCST, the hydrodynamic diameters of the copolymers were in the broader region of 79 nm to 825 nm with maximum peak of 255 nm and larger PDI width of 109 nm, presenting a cloudy solution as a result of nanoparticle precipitation. The size distribution at 37°C was similar to that at 45°C, showing that the PNIPAAm copolymers underwent a gradual adjustment of molecular conformations under the combined action of hydrogen bonds and hydrophobic interactions. Thus, the copolymers remained as hydrated molecules below LCST, whilst above it they formed nanoaggregates with a wide size distribution. Despite this, the PNIPAAm copolymers had relatively stable states either below or above LCST.

The temperature responses of the PNIPAAm copolymers can be visualized by plotting the average hydrodynamic diameters against temperature change, accompanied with real-time photos at different temperature, as shown in Figure 3. Figure 4 illustrates the dominant forces acting on the PNIPAAm copolymers in UHQ water. Below 29°C, the diameters of the scattering objects were around 12 nm with a slight downtrend, indicating that the fully hydrous copolymers existed in UHQ water and

the small adjustment of molecular chains were caused by intramolecular forces.[11,18] Below LCST, three kinds of hydrogen bonds, such as hydrogen bonding between water molecules and amide linkages (HB_{wa}), hydrogen bonding between water molecules and hydroxyl groups (HB_{wh}), hydrogen bonding between water molecules and siloxane groups (HB_{ws}), are dominant forces preventing nanoparticles from intramolecular collapse and intermolecular aggregation. Heating solution led to the start of structural cluster and dehydration above 29°C. In the transition range of 29–31°C, the diameters increased about 20 times owing to the formation of intermolecular aggregates with the hydrophobic interactions between isopropyl groups (HI_{ii}) and hydrogen bonding between amide linkages (HB_{aa}), implying that the LCST of PNIPAAm copolymers in UHQ water was around 29°C. Furthermore, the incorporation of HPM and TMSPM monomers reduced the LCST of PNIPAAm homopolymer by 2–3°C. Above 31°C, the slight decrease of hydrodynamic diameter was not due to the decline in the numbers of hydrated hydrogen bonds, but because the PNIPAAm-based nanoparticles undertook a gradual adjustment of molecular conformations under the main force of hydrophobic interaction coupled with residual hydrogen bonds until they reached a relative stress balance and formed the self-sustaining structure. When the temperature is above LCST, HB_{wa} are partially substituted by HB_{aa} and HI_{ii}, but there still exist HB_{wa}, HB_{wh}, and HB_{ws} in UHQ water. The maximum diameter of PNIPAAm copolymers is formed by the combined hydrophobic interactions and four kinds of hydrogen bonds. In the cooling process, the copolymer aggregates could dissociate into individual chains. The behavior of the copolymers during cooling treatment displayed almost overlapping profiles observed in the heating process, whereas the average hydrodynamic diameters in cool-down stage were smaller than those in heat-up stage at the same temperature, inferring that the transitions of PNIPAAm copolymer nanoparticles were revertible but not entirely.
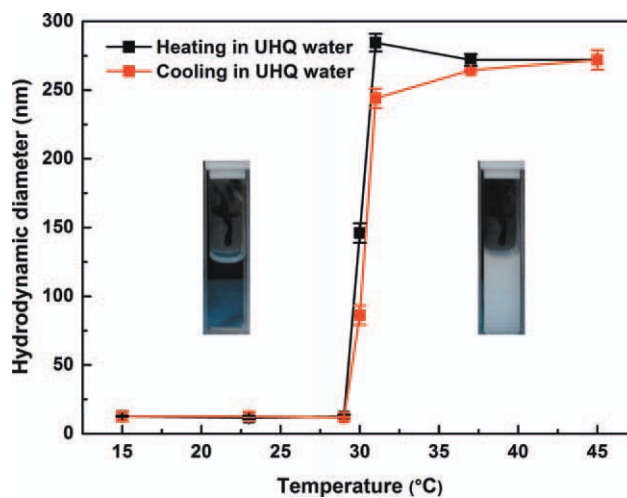

Figure 3. Changes in the average hydrodynamic diameters of the PNIPAAm copolymers in UHQ water as plotted with temperature during heating and cooling processes, accompanied with real-time photos below or above LCST. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
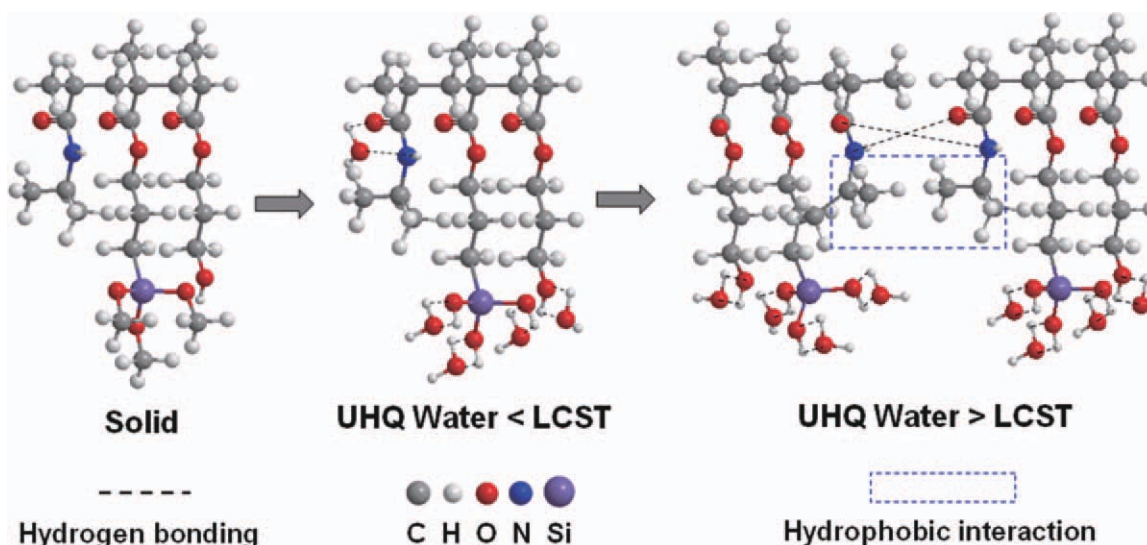
Figure 4. The dominant forces acting on the PNIPAAm copolymers in UHQ water below or above LCST. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

PNIPAAm Copolymers in Salt Solutions

The temperature dependence of the PNIPAAm copolymers in 10, 50, and 100 mM NaCl solutions are shown in Figure 5. In general, the average hydrodynamic diameters of about 13 nm in different concentrations of NaCl solutions below LCST were similar to those in UHQ water; however, the diameters increased continuously with increasing temperature or NaCl concentration above LCST, differing from those in UHQ water.

At low temperature below LCST, the hydrophilic amide linkages, hydroxyl, and siloxane groups within the PNIPAAm copolymers were connected with water molecules through hydrogen bonding even in the presence of salts. Upon heating to above LCST, like in UHQ water, the hydrogen bonding between water molecules and hydrophilic groups were partially substituted by nonaqueous hydrogen bonding and hydrophobic interactions, which promoted concordantly the formation of intramolecular collapse and intermolecular aggregate. However, the remaining hydrogen bonds between water molecules and copolymer chains including amide linkages, hydroxyl, and siloxane groups limited the aggregation of PNIPAAm copolymers in UHQ water, therefore the size of nanoparticles developed to a relative maximum value at 31°C. Nevertheless, the interactions between solvent and aggregates were changed due to the addition of salt molecules,[14,16,23] as Figure 6 displayed. The NaCl are ionized completely in water to form hydrated cation and hydrated anion, suggesting that there would be a possible competition on the water between NaCl and PNIPAAm copolymers. The addition of NaCl intensifies the destruction of the hydrogen bonding between water molecules and copolymers, therefore the nonaqueous hydrogen bonds and hydrophobic interactions play a dominant role above LCST in NaCl solution. The destructive effect of NaCl on hydrated hydrogen bonding increased with the rise of the temperature and NaCl concentration, leading to greater hydrodynamic diameters of nanoaggregates. This explains why the maximum average hydrodynamic diameters of nanoaggregates in UHQ water were only 280 nm, whereas the sizes could reach more than 4000 nm in NaCl solutions above LCST. But the fixed copolymer concentration (1 mg mL^{-1}) limited the unrestricted increase of aggregate size, so when it was up to some degree, the hydrodynamic diameters of PNIPAAm copolymers slightly decreased. This is also the result of molecular conformation adjustment under different intermolecular and intramolecular forces acting on copolymers. From the inset graph in Figure 5, it could be seen that the LCST decreased as the salt concentration increased, showing respectively 29°C, 28°C, and 27°C in 10, 50, and 100 mM NaCl solutions. Just as the explanation mentioned above, the higher salt concentration could improve the formation of PNIPAAm copolymer aggregates at lower temperature.
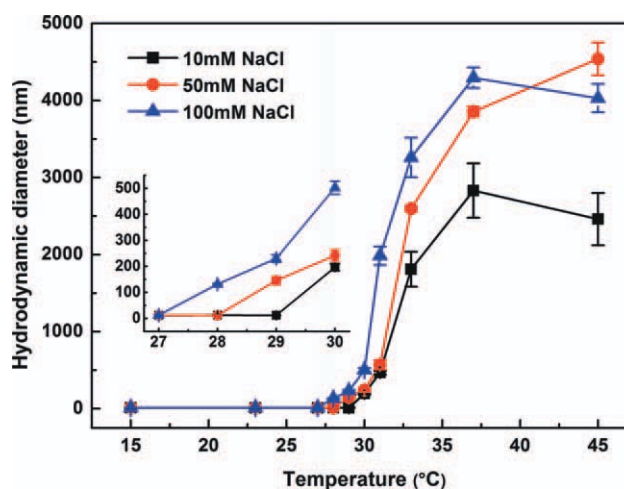

Figure 5. Changes in the average hydrodynamic diameters of the PNIPAAm copolymers in 10, 50, and 100 mM NaCl solutions as plotted with the rising temperatures. The inset graph showed the sharp size change initiated at different temperature. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
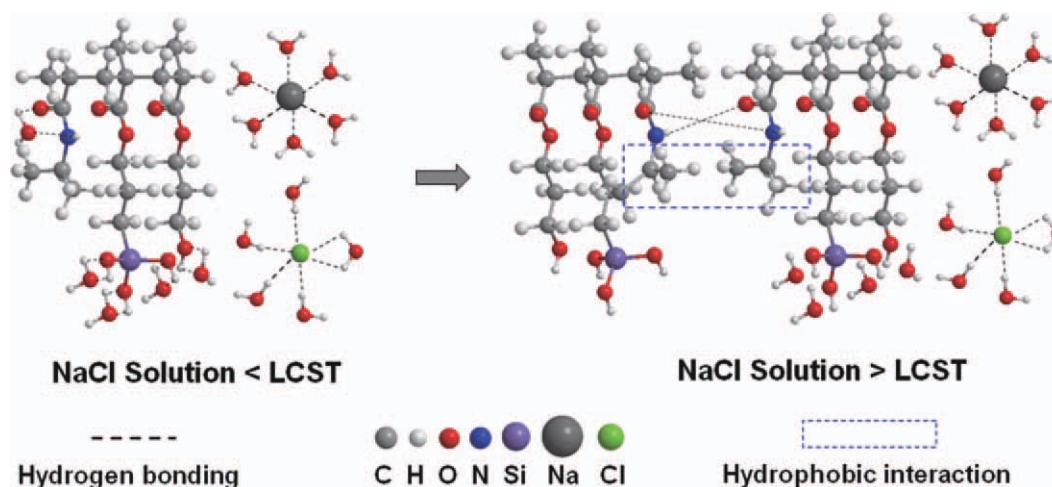
Figure 6. The dominant forces acting on the PNIPAAm copolymers in NaCl solution below or above LCST. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

It can be clearly seen from Figure 7 that the hydrodynamic diameters of PNIPAAm copolymers vary with temperature in NaCl and CaCl$_2$ solution with the same concentration of 10 mM. The ion strength of CaCl$_2$ is three times more than that of NaCl at the same concentration, indicating that it is easy for Ca^{2+} to form hydrated ions compared with Na^{+}. Therefore, the LCST of PNIPAAm copolymers in 10 mM CaCl$_2$ solution was lower than that in 10 mM NaCl solution. But above respective LCST, the hydrodynamic diameters of aggregates in CaCl$_2$ solution were smaller than those in NaCl solution. It is most probably caused by weak alkalinity of CaCl$_2$ solution. The measured pH values of PNIPAAm copolymers in 10 mM CaCl$_2$ and 10 mM NaCl solutions were 6.7 and 5.6, respectively, at 20°C. Compared to the pH value 5.9 at 20°C of PNIPAAm copolymers in UHQ water, the addition of alkaline CaCl$_2$ clearly increased the pH value. There was ionization equilibrium when the PNIPAAm copolymers were dissolved in aqueous solutions. Thus, the acidic solvents enhance the reverse reaction to form uncharged nanoparticles, whereas the alkaline solvents have an impact on ionization in favor of the positive reaction to produce charged nanoparticles, and accordingly the electrostatic repulsion weakens the aggregation degree of copolymers, causing a decrease of nanoparticle size. In this study, the use of weak alkaline CaCl$_2$ aqueous solution (pH$_{20°C,10 \text{ m}M}$ ≈ 7.8) and near neutral NaCl medium as the solvents for PNIPAAm copolymers facilitated the generation of phenomenon that the aggregate size in CaCl$_2$ solution was smaller than those in NaCl solution above respective LCST. It is concluded that the solution concentration or ionic strength can not be used as independent criteria for the effect of salts on hydrodynamic diameters of PNIPAAm-based materials. The reactions between polymers and solutes and the binding capability to polymers of various ions should also be considered.[22]

PNIPAAm Copolymers in pH Buffers

As mentioned earlier, the pH values of solutions were, respectively, 5.9 and 5.6 at 20°C when the PNIPAAm copolymers were dissolved in UHQ water and in 10 mM NaCl solution. Therefore, the copolymers could not be easily ionized in acid solvents (uncharged nanoparticles), whereas they can be ionized in neutral or alkaline solvents (charged nanoparticles). It could be observed in Figure 8 that the hydrodynamic diameters of copolymers in either UHQ water or NaCl solution decreased with the rising of pH value at 37°C, whereas they had no obvious change at 23°C. As expected below LCST (at 23°C), no aggregation or precipitation occurred over the whole pH range. The hydrogen bonding between water molecules and hydrophilic groups within the copolymers (like HB$_{wa}$, HB$_{wh}$, and HB$_{ws}$) still played an important role in maintaining the size and conformation of copolymers chains. Above LCST (at 37°C) at the lower pH (<5.9 or 5.6), the action mode of HCl to PNIPAAm copolymers was the same as that of NaCl. Therefore the hydrodynamic diameters of the copolymers increased with the increase in H^{+} concentration. However, above LCST at the higher pH (=5.9 or 5.6), the copolymers were deprotonated to anionic form, and the aggregates would be smaller due to the enhanced electrostatic repulsion (ER) between charged sites
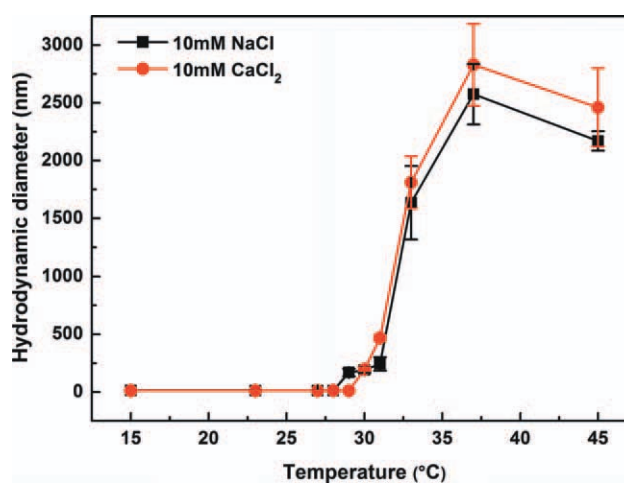

Figure 7. Changes in the average hydrodynamic diameters of the PNIPAAm copolymers in 10 mM NaCl and CaCl$_2$ solutions as plotted with the rising temperatures. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

along the backbone with the increasing OH$^-$ concentration.[15,25,29] Under the presence of NaCl, the diameters of nanoparticles were obviously larger than those in UHQ water at the same pH value above LCST.

In general, the novel PNIPAAm copolymers in aqueous solutions are under control of actions based on the various intermolecular and intramolecular forces in different conditions, such as HB$_{wa}$, HB$_{wh}$, HB$_{ws}$, HB$_{aa}$, HI$_{ii}$, and ER. The dimension and conformation of aggregates and the LCST of copolymers depend on which forces play dominant roles in working conditions, as summarized in Table I. Below LCST, adding salts or changing pH value in solution could not alter the size and conformation of nanoparticles, since HB$_{wa}$, HB$_{wh}$, and HB$_{ws}$ are dominant forces preventing nanoparticles from intramolecular collapse and intermolecular aggregation. However, it is completely different above LCST. When the temperature is raised to and above LCST, HB$_{wa}$ are partially substituted by HB$_{aa}$ and HI$_{ii}$, but there still exist HB$_{wa}$, HB$_{wh}$, and HB$_{ws}$ in water. In UHQ water, the maximum diameter of PNIPAAm copolymers is formed by the combined hydrophobic interactions and four kinds of hydrogen bonds. The usage of salts and acidic solvents leads to the intensive destruction of HB$_{wa}$, HB$_{wh}$, and HB$_{ws}$, while HB$_{aa}$ and HI$_{ii}$ become absolute dominant forces and accordingly larger aggregates are produced. Another strong force of electrostatic repulsion is introduced by the addition of alkaline solvents. Under the action of electrostatic repulsion, the aggregation extent of PNIPAAm copolymers is inhibited.

For PNIPAAm copolymer with short chains prepared in this study, HB$_{wa}$, HB$_{wh}$, and HB$_{ws}$ could maintain the size and conformation of single chains, HB$_{aa}$ and HI$_{ii}$ result in weak intramolecular collapse and strong intermolecular aggregation, and ER weakens the aggregation extent of copolymers. The number of amide linkages and isopropyl groups within PNIPAAm copolymers increases with the increasing molecular weights of copolymers. Therefore, when the temperature is below LCST, the amount of the hydrogen bonds between water molecules and

Figure 8. Changes in the average hydrodynamic diameters of the PNIPAAm copolymers in UHQ water and 10 m*M* NaCl solution under different pH value at 23 and 37°C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table I. The Dominant Forces Acting on the PNIPAAm Copolymers Under Different Conditions

Solvent	Temperature	
	Below LCST	Above LCST
UHQ water	HB$_{wa}$, HB$_{wh}$, HB$_{ws}$	HB$_{wa}$, HB$_{wh}$, HB$_{ws}$, HB$_{aa}$, HI$_{ii}$
NaCl solution	HB$_{wa}$, HB$_{wh}$, HB$_{ws}$	HB$_{aa}$, HI$_{ii}$
CaCl$_2$ solution (alkalescence)	HB$_{wa}$, HB$_{wh}$, HB$_{ws}$	HB$_{aa}$, HI$_{ii}$, ER
HCl (acid medium)	HB$_{wa}$, HB$_{wh}$, HB$_{ws}$	HB$_{aa}$, HI$_{ii}$
NaOH (alkaline medium)	HB$_{wa}$, HB$_{wh}$, HB$_{ws}$	HB$_{aa}$, HI$_{ii}$, ER

HB$_{wa}$ means hydrogen bonding between water molecules and amide linkages; HB$_{wh}$ means hydrogen bonding between water molecules and hydroxyl groups; HB$_{ws}$ means hydrogen bonding between water molecules and siloxane groups; HB$_{aa}$ means hydrogen bonding between amide linkages; HI$_{ii}$ means hydrophobic interactions between isopropyl groups; ER means electrostatic repulsion.

hydrophilic groups within copolymers grow accordingly, and strong hydrophilic shells still maintain unfolded "coil" structure of molecular chains. Across LCST, the effect of HB$_{aa}$ and HI$_{ii}$ on copolymers also has been enhanced for large copolymers. If the copolymer concentration in aqueous solution is lower, shrinkage is more likely to occur within the copolymers with larger molecule weights, thus forming much smaller "globule"; when the copolymer concentration was higher than the critical value, although each molecular chain shrinks as intramolecular interactions, copolymers aggregate to produce larger "globule" caused by intermolecular HB$_{aa}$ and HI$_{ii}$. Above LCST, the PNIPAAm-based nanoparticles undertake a gradual adjustment of molecular conformations under the intramolecular or intermolecular HI$_{ii}$ coupled with possible hydrogen bonds until they achieve a relative stress balance and form self-sustaining structure.

For other types of PNIPAAm-based materials, the conformational transitions across and above LCST in various aqueous solutions can be also analyzed using the above conclusion, i.e., HB$_{wa}$ and other hydrated hydrogen bonds could keep their original dimension and configuration, HB$_{aa}$ and HI$_{ii}$ lead to large intermolecular aggregation or intramolecular collapse, and ER inhibited intermolecular agglomeration or intramolecular compression. The analysis can be used for predicting the dimension and conformation of PNIPAAm-containing materials under different conditions.

CONCLUSIONS

The novel temperature-responsive PNIPAAm copolymers can be synthesized by free-radical polymerization with weak acidity. The average hydrodynamic diameters of PNIPAAm copolymers had no obvious change in each working solutions below LCST. Across LCST, the diameters increased suddenly at different initial temperature in UHQ water, solutions with salts or pH value. Above LCST, they decreased slightly as the temperature increased in UHQ water, and increased continuously with increasing temperature or salt concentration, and reduced with the rising of pH
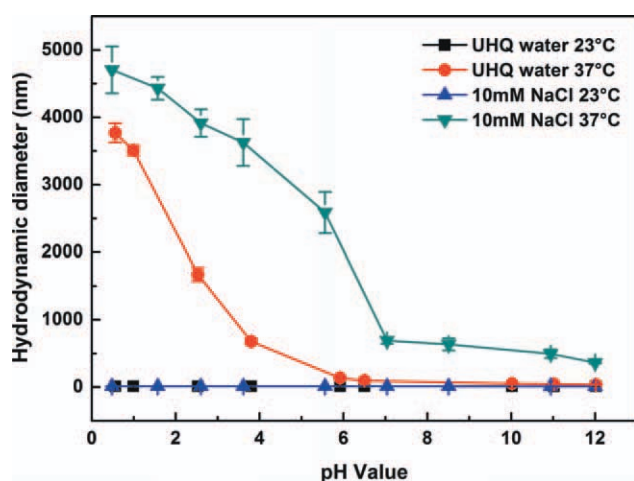
value in pH buffers. Furthermore, the solution concentration or ionic strength cannot be used as independent criteria for the effect of salts upon polymer sizes. The different intermolecular and intra-molecular forces lead to the disparity in dimension, conformation, and LCST of copolymers. The strong hydrated hydrogen bonds maintain the original dimension and configuration of PNIPAAm-based materials, while anhydrous hydrogen bonds and hydrophobic interactions result in intermolecular aggregation or intramolecular collapse, and the electrostatic repulsion weakens the extent of aggregation or shrinkage.

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