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Kinetics of Coil-to-Globule Transition of Dansyl-Labeled Poly(N-isopropylacrylamide) Chains in Aqueous Solution

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The coil-to-globule transition of thermally sensitive linear poly(N-isopropylacrylamide) (PNIPAM) labeled with dansyl group is induced by 1.54 μm laser pulses (width≈10 ns). The dansyl group is used to follow the transition kinetics because its fluorescence intensity is very sensitive to its micro-environment. As the molar ratio of NIPAM monomer to dansyl group increases from 110 to 300, the effect of covalently attached dansyl fluorophores on the transition decreases. In agreement with our previous study in which we used 8-anilino-1-naphthalensulfonic acid ammonium salt free in water as a fluorescent probe, the current study reveals that the transition has two distinct stages with two characteristic times, namely, $\tau_{\rm fast}$≈0.1 ms, which can be attributed to the nucleation and formation of some "pearls" (locally contracting segments) on the chain, and $\tau_{\rm slow}$≈0.5 ms, which is related to the merging and coarsening of the "pearls". $\tau_{\rm fast}$ is independent of the PNIPAM chain length over a wide range ($M_{\rm w}$=2.8×10^6−4.2×10^7 g/mol). On the other hand, $\tau_{\rm slow}$ only slightly increases with the chain length.

Key words: Fluorescence labeling, Laser light scattering, Phase transition, Stimuli-sensitive polymer

I. INTRODUCTION

It has been predicted that the conformation of a linear flexible homopolymer chain could change from an extended random coil to a compact globule if the solvent quality switches from good to poor [1]. Although the thermodynamics of the coil-to-globule transition is well understood [2−8], the kinetics is still an open question. Theoretically, a variety of approaches have been tried to solve this problem, such as phenomenological models [9−11], Langevin models [12], and computer simulation [13, 14]. Byrne *et al.* used the Langevin-equation simulation to study the kinetics of the coil-to-globule transition of linear homopolymer chains in infinite dilution and found that small nuclei on the chain were quickly formed when the solvent quality switched from good to poor [12]. These nuclei grew into clusters at the expense of their surrounding slack segments. When these slack segments were used up, the polymer continued to collapse through coarsening until only one single-chain globule was left. Kuznetsov *et al.* studied the kinetics by both the Monte Carlo simulation and the numerical analysis on the basis of the Gaussian self-consistent approach. The Monte Carlo simulation showed that at least four different stages existed after an abrupt quench of the solution into a poor solvent [13, 14]. The first stage was characterized by the rapid formation of numerous small globules on the chain and the duration of this stage was independent of the chain length. The second stage was related to the growth of these globules. In the third stage, larger globules merged with smaller ones until only one single-chain globule was left. The Gaussian self-consistent approach also led to such three stages.

Klushin *et al.* showed that the overall characteristic collapse time (τ) was scalable to the degree of polymerization (N) as $\tau\approx N^{1.6}$ and the incorporation of hydrodynamic effects led to $\tau\approx N^{0.93}$ [10]. The conformation transition of linear homopolymer chains after a temperature quench was further studied by Halperin and Goldbart using a phenomenological model and they also found four stages [11]. The formation of smaller dense droplets ("pearls") was the fastest process. These nascent droplets then grew by accreting monomers from the neighboring connected chain segments so that these slack bridging segments became shorter, named as the "bridge-stretching" stage. The third stage involved the packing of these "pearls" before they coarsened into a single-chain globule. The characteristic transition times of these "pearling", "bridge-stretching", and "packing"

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processes were scaled to N as $\tau \approx N^0$, $\tau \approx N^{1/5}$, and $\tau \approx N^{6/5}$, respectively. Kikuchi *et al.* found that hydrodynamic interaction could speed up the transition using a hybrid mesoscale-molecular-dynamics algorithm [15]. After the temperature quenched, small pearls were rapidly formed and connected by linear slack segments and then these pearls started to absorb those remaining monomers between them, resulting in the chain contraction. They showed that even for the longest chain, the initial pearl-formation only took ∼5% of the overall transition time (τ) and τ is scaled to N as

$$\tau \approx \frac{\eta a^3}{\varepsilon} N^{4/3} \tag{1}$$

where η, a, and ε are the solvent shear viscosity, the monomer diameter, and the magnitude of the van der Waals interaction, respectively.

Recently, Wang *et al.* concluded that the collapse kinetics had four stages by dissipative particle dynamics, namely, formation of localized clusters, cluster coarsening *in situ*, coarsening into a globule and relaxation to an equilibrium globule [16]. They found that there was no chain length dependence on the characteristic time of the first stage, which was consistent with the results reported by Halperin *et al.* [11]. Though they did not obtain the exponent with reasonable accuracy, the characteristic time of the second stage increased with the polymer chain length.

Experimentally, most studies have focused on the slower stages by laser light scattering [5], temperature-jump ^{1}H NMR spectroscopy [17], and stopped-flow technique [18, 19]. Only a few experiments have been done to study the early stages of homopolymer collapse because the first and the second stages are very fast. In our previous study [20, 21], by using water-soluble 8-anilino-1-naphthalensulfonic acid ammonium salt (ANS) as a fluorescent probe free in solution we revealed that the collapse kinetics of poly(*N*-isopropylacrylamide) (PNIPAM) linear chain had two stages in dilute regime and the two characteristic transition times were less than 1 ms. When the concentration was higher than the overlap concentration, a third process appeared. Later, Tsuboi *et al.* reported phase transition dynamics of dye-labeled PNIPAM by fluorescene spectroscopy combined with the laser-induced temperature jump technique [22]. It should be mentioned that the molecular weight of PNIPAM sample used was small (∼30 kg/mol) and the fluorescent probe is relatively hydrophobic. Possibly due to the smaller molecular weight and/or the hydrophobicity of the dye, only one time constant of phase transition ($\tau \approx 0.035$ ms) was found which is independent of the sample concentrations (0.1%−1.0 %).

Note that fluorescent probes and labeling are two common methods to provide the information about local environmental microdomains in polymer systems. Different fluorescent probes and/or covalently attached dyes have been widely used to investigate the phase transition of thermally sensitive polymers and the micellization of block copolymers [23, 24]. When fluorescent probe technique is used to follow collapse kinetics, problems should be concerned such as how long it takes the probe to bind the microdomains [25]. In order to check whether the fluorescent probe ANS free in solution could response to the changes instantaneously during the PNIPAM collapse process [20, 21], the relatively hydrophilic dansyl groups have been covalently attached to PNIPAM chains because its fluorescence intensity was very sensitive to its micro-environment [26−29]. Whereas during the use of labeling method, the content of the covalently attaching dye should be as low as possible so that the dyes will not change the properties of the polymer significantly if a high enough signal-to-noise ratio is achieved. So the effect of the dye content on the phase transition of PNIPAM has been investigated by fluorescence spectroscopy and ultrasensitive differential scanning calorimetry (US-DSC), respectively. By using the laser temperature jump technique combined with a homemade fluorescene detection system, we identified the existence of a two-stage process with two characteristic transition times: the characteristic time of the first stage was identical to that reported in Ref.[20] and the second stage was a little faster than the result of our previous study [20], where the possible electrostatic repulsion between ANS molecules binding to different pearls might slow the merging and coarsening of the pearls during the second stage.

II. EXPERIMENTS

A. Materials

NIPAM from Eastman Kodak was recrystallized three times in a benzene/*n*-hexane mixture. Dansyl chloride (Fluka, 98%), *N*-hydroxyl succinimide (Sigma, >97%) and 2,2′-(ethanedioxy)diethylamine (Sigma, 98%) were used without further purification. Acryloyl chloride was purchased from Aladdin and distilled before use. Azobisisobutyronitrile (AIBN) was recrystallized twice from ethanol. Tetrahydrofuran (THF), benzene and *n*-hexane were distilled from sodium under nitrogen prior to use. Acetone was refluxed with successive small portions of potassium permanganate until the violet colour persisted, dried over anhydrous $CaSO_4$ and distilled. Triethylamine (TEA) was stirred with KOH overnight, refluxed with toluene-4-sulfonylchloride and distilled before use. Isopropylamine was dried over KOH pellets and distilled before use.

B. Synthesis

The synthesis of compound **a** was accomplished according to the published reports (Scheme 1) [30, 31]. Dansyl chloride (0.40 g, 1.49 mmol) was first dissolved

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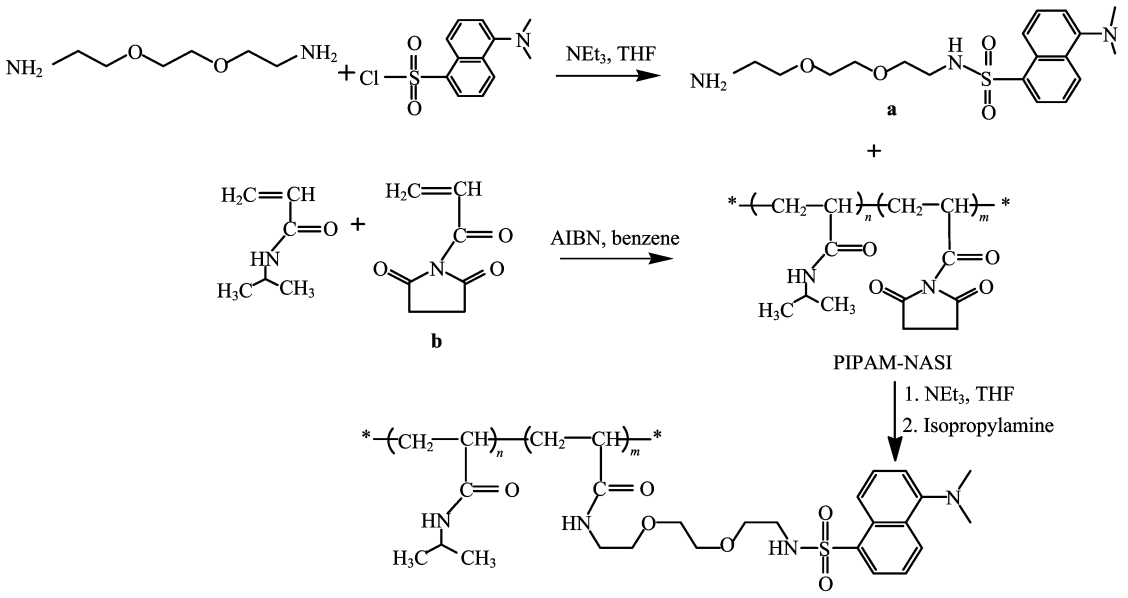

Scheme 1 Synthetic scheme for the preparation of dansyl-labeled PNIPAM.

in 5 mL of THF and then added dropwise to a solution of 2,2′-(ethanedioxy)diethylamine (11.12 mmol) in 35 mL of THF followed by triethylamine (0.19 g). After stirring overnight, the solvent was removed by rotary evaporation and the residue was acidified with 1 mol/L HCl and washed with CH_2Cl_2. The aqueous layer was basified (pH≈9) with 3 mol/L NaOH and extracted with CH_2Cl_2. Organic layers were combined, concentrated and then subjected to silica gel column chromatography (ethyl acetate:methanol=3:1) to give a highly fluorescent product. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.52 (d, 1H), 8.36 (d, 1H), 8.23 (dd, 1H), 7.58 to 7.48 (m, 2H), 7.17 (d, 1H), 3.62 to 3.50 (m, 4H), 3.48 to 3.40 (m, 4H), 3.09 to 2.96 (m, 4H), 2.88 (s, 6H).

N-Hydroxy succinimide (5.62 g) and triethylamine (5.95 g) were dissolved in 80 mL of chloroform at 0 °C. Acryloyl chloride (4.75 mL) was dissolved in 30 mL $CHCl_3$ and added dropwise over a 1 h period to the reaction mixture with stirring [32, 33]. After an additional 20 min at 0 °C, the solution was washed with 300 mL portions of ice-cold water and saturated brine, dried with $MgSO_4$ and filtered; 40 mg of 2,6-di-*tert*-butyl-4-methylphenol was added to the chloroform solution which was then condensed to a small volume using a rotary evaporator and filtered. The mixture of ethyl acetate (6 mL) and 40 mL of *n*-hexane was added slowly with stirring to the chloroform solution which was left to stand in the refrigerator for several hours. The precipitated, colorless crystals were separated by filtration and washed with an ice-cold 20 mL of *n*-hexane/ethyl acetate (4:1), then with another 20 mL of *n*-hexane/ethyl acetate (9:1), and finally with two 10 mL portions of *n*-hexane. The crystals were dried *in vacuo* at ambient temperature to constant weight. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.70 (dd, 1H, *trans*-H in CH_2), 6.33 (dd, 1H, CH), 6.16 (dd, 1H, *cis*-H, CH_2), 3.01 (s, 4H, CH_2).

C. Copolymerization of *N*-isopropylacrylamide and *N*-(acryloxy)succinimide

Purified *N*-isopropylacrylamide (5.00 g) and *N*-(acryloxy)succinimide (NASI) (1 mol%) were dissolved in 50 mL of benzene with 1% of recrystallized AIBN as initiator [2, 34]. The solution was degassed through three cycles of freezing and thawing. Polymerization was carried out by keeping the final solution at 56 °C for 30 h under vacuum. The resulting crude product was directly dissolved in acetone and then added dropwise into *n*-hexane to form white solid polymer. The obtained PNIPAM-NASI was fractionated by choosing acetone/*n*-hexane as the good/poor solvent at room temperature.

D. Reaction of fractional PNIPAM-NASI with compound a

Compound **a** and triethylamine were added successively to a solution of fractional PNIPAM-NASI in THF [35]. The mixture was stirred at room temperature for 24 h under N_2. Distilled isopropylamine was added to consume the excess succinimide groups. The resulting mixture was stirred for 6 h. The polymer was isolated by precipitation into diethyl ether before the dried polymer was purified by three precipitations, pouring the polymer aqueous solution into an equal volume of methanol [36].

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E. Instrumentation

UV-Vis spectra were measured with a Shimadzu 2401PC UV-Vis spectrometer. All proton nuclear magnetic resonance (NMR) spectra were determined on a Bruker DMX-400 instrument with $CDCl_3$ as solvent and tetramethylsilane (TMS) as internal standard. DSC measurements were performed on a NanoCal DSC from Calorimetry Sciences Corporation. The heating rate was 1.0 °C/min. The concentration of the polymers was 0.5 mg/mL. The enthalpy change ΔH during the transition was calculated by integration of the peak area. The laser light scattering measurements were carried out by using a spectrometer (ALV/DLS/SLS-5022F) equipped with a cylindrical 22 mW UNIPHASE He-Ne laser (λ_0=632.8 nm) and a multi-τ digital time correlator (ALV5000). The weight-average molar mass M_w, the radius of gyration $\langle R_g \rangle$ and the hydrodynamic radius $\langle R_h \rangle$ of PNIPAM-NASI fractions used in our experiments were determined by a combination of static and dynamic light scattering. The polydispersity index M_w/M_n was estimated from the relative width $\mu_2/\langle\Gamma\rangle^2$ of the line-width distribution $G(\Gamma)$ measured in dynamic light scattering since $M_w/M_n \approx 1+4\mu_2/\langle\Gamma\rangle^2$.

F. Fast infrared laser heating and fluorescene detection

The instrument has been described in detail elsewhere [20, 21, 37]. The fundamental output of a Nd-YAG laser (Spectra Physics, Prolab-190, repetition rate of 10 Hz) was focused into two Raman cells filled with 4 MPa CH_4. The 1.54-μm infrared laser pulse through a stimulated Raman effect was produced for our T-jump experiments. Compared to our previous instrument, a dichronic mirror with high reflectance at 1.54 μm and high transmission at 1.064 μm was placed in front of the first Raman cell in order to reflect the backward stimulated Stokes energy into the first Raman cell. Raman conversion efficiency increased by a factor of ∼3 with this configuration [38]. The light source for excitation of dansyl group was a 200 W high pressure mercury lamp (Shanghai Hualun Bulk Factory) with a transmitting filter (245−400 nm). The fluorescence with a cutoff filter of 450 nm was collected at 90° with a side-on photomultiplier tube (Hamamatsu R928) and recorded using a Tektronix oscilloscope (TDS 3054B). In a typical experiment temperature jumps of 2 °C were obtained and each datum point was normally averaged over 512 time measurements to improve the signal-to-noise ratio. The sample cell was thermally controlled at 30 °C to a precision of ±0.1 °C by a heating bath. Fluorescence spectra were recorded on a Shimadzu RF-5301PC spectrofluorophotometer by using a quartz cell with an optical path length of 2 mm and the excitation wavelength was 337 nm. All aqueous solutions were not degassed before measurements. For the emission spectra tests, the slit widths were set ranging from 3 nm to 10 nm according to the chromophore concentration. All measurements at different temperatures were made after the samples were equilibrated for 5 min at a given temperature. The temperature of the samples was monitored by an electronic thermometer with a precision of ±0.1 °C.

III. RESULTS AND DISCUSSION

A. Characterization of the dansyl-labeled PNIPAMs

Fluorescence labeling technique has been widely used in polymer science because the fluorescence spectroscopy of the labels can provide information about the micro-environment [29, 39−41]. Attention needs to be paid to the content of the used fluorescence dyes especially when the dyes are hydrophobic. The label content was as low as 0.06% when dansyl group was covalently attached to PNIPAM in the study on the phenomenon of cononsolvency as reported by Winnik *et al.* [29]. In our present study, compound **a** was used in place of 2-dansylaminoethylamine because compound **a** is more hydrophilic than the latter [42]. The succinimidyl active ester was used to introduce the dye with amino groups [43]. It can react with amines at high efficiency and produce relatively stable amide linkages. In order to get monodispersed PNIPAM-NASI fractions, the solvents used in the precipitation fractionation should be purified carefully and the obtained fractions should be used as soon as possible or kept dry at low temperature [44] because of the instability of the active ester especially when exposed to damp air. Another reason why we choose the PNIPAM-NASI as the precursor is that it has already been verified the dyes could be randomly distributed along the polymer chains [35, 45] and the random distribution of dyes would help us to follow polymer collapse kinetics. By the reaction of PNIPAM-NASI and amino dyes, a series of dansyl-labeled PNIPAM with different label contents and different molecular weights were obtained. The unreacted succinimidyl esters were quenched by treatment with an excess of isopropylamine to produce NIPAM units *in situ*. The possible free dyes in the system were removed by precipitating the aqueous polymer solution into an equal volume of methanol which was proven to be an efficient way to remove the hydrophilic small molecules [36].

The polymers were characterized by laser light scattering in THF before the introduction of the fluorescent dye. Figure 1 shows typical Zimm plot of PNIPAM-1. The extrapolation of $[KC/R_{vv}(q)]$ to $C\rightarrow 0$ and $q\rightarrow 0$ leads to M_w. Figure 2 shows the distribution of hydrodynamic radius of the four PNIPAM-NASI samples in THF, which reveals that all the PNIPAM fractions are narrowly distributed. The corresponding data are summarized in Table I. Note that the values of $\langle R_g \rangle/\langle R_h \rangle$ are all around 1.5, indicating the expanded coil state of PNIPAM chain in THF. Compound **a** was used as the

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TABLE I Characterization of PNIPAM-NASI and resultant dansyl-labeled PNIPAM samples.

Sample	$\langle M_w\rangle/(10^6$g/mol)	$\langle R_g\rangle$/nm	$\langle R_h\rangle$/nm	M_w/M_n	Dansyl-labeled PNIPAM		
					Sample	T_p[a]/°C	NIPAM:dansyl[b]
PNIPAM-1	2.8	73	51	1.3	PNIPAM-1-D	30.0	370:1
PNIPAM-2	5.6	102	75	1.3	PNIPAM-2-D	29.9	360:1
PNIPAM-3	14	152	109	1.2	PNIPAM-3-D	30.0	390:1
PNIPAM-4	42	274	185	1.3	PNIPAM-4-D	29.9	340:1

[a] Measured by fluorescence spectroscopy in water.
[b] Measured by UV-Vis spectroscopy in water.

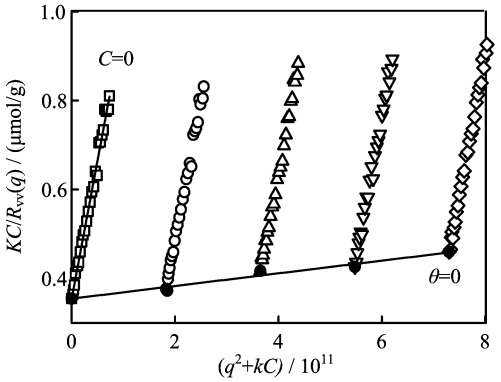

FIG. 1 Typical Zimm plot of PNIPAM-1 in THF solution at 25 °C, where C ranges from 0.05 mg/mL to 0.20 mg/mL.

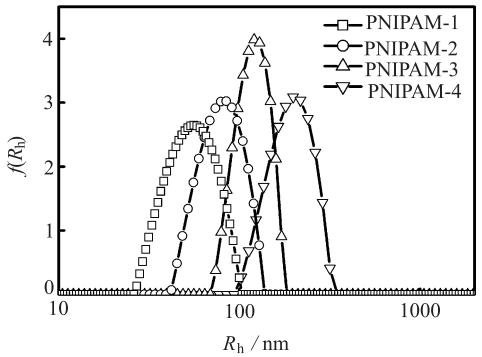

FIG. 2 Hydrodynamic radius distribution of PNIPAM-NASI fractions at 25 °C in THF solutions, C=0.05 mg/mL.

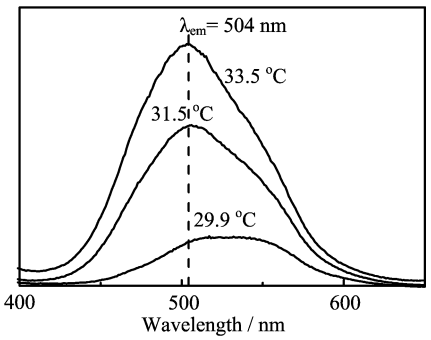

FIG. 3 Temperature dependence of static emission fluorescence spectra of dansyl group in PNIPAM-2-D solution, where C=0.5 mg/mL and λ_{ex}=337 nm.

model compound to determine the amount of dansyl group in PNIPAMs and its molar extinction coefficient in water was 4720 $(\text{mol/L})^{-1}\text{cm}^{-1}$ at its maximum absorption of 328 nm, which is close to the reported value of another dansyl compound [44]. The contents of dansyl group in the polymer chains are low and almost the same, as shown in Table I. We know that introduction of fluorescent labels into the polymer system will affect its nature more or less. This effect has been investigated in different systems [29, 46, 47]. Generally speaking, the induction of smaller amount of dyes would have less effect on the PNIPAM chains but during our experiments we also need a high enough signal-to-noise ratio which means the contents of the dyes should be as high as possible. For this reason, the effect of label content on the transition temperatures T_p has been investigated by both steady state fluorescence spectroscopy and DSC.

Figure 3 shows the fluorescence spectra of dansyl group in PNIPAM-2-D aqueous solution at three different temperatures. The wavelength of the peak shifted from 528 nm to 504 nm when the PNIPAM solution was heated, which was consistent with the results reported by Du *et al.* [48]. The temperature dependence of the fluorescence intensity of the dansyl group at 504 nm in PNIPAM-2 aqueous solutions with different dansyl contents has been shown in Fig.4. It can be concluded that although we have made modification on the fluorescent labels in order to increase the hydrophilicity of the dyes, the incorporation of large amounts of dansyl groups still affects the T_p of the polymer chains in the solution. There is no obvious influence of the dyes on the transition when the molar ratio of the monomer units to the labeled units is larger than 260. Similar results can be obtained from the DSC experiments, as shown in Fig.5(a). PNIPAM with lower content of dansyl groups has a relatively higher T_p and narrower phase transition temperature range. Also note that the enthalpy change ΔH increases with decreasing the label content of dansyl groups, as shown in Fig.5(b). It is well known that incorporation of hydrophobic comonomers decreases the polymer hydrophilicity, which leads to a

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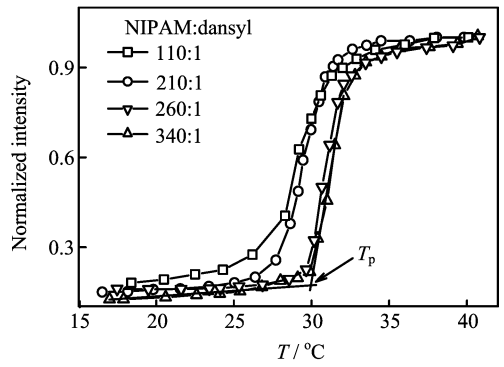

FIG. 4 Temperature dependence of fluorescence intensity (λ_{em}=504 nm) of dansyl group in PNIPAM aqueous solution using PNIPAM-2 as the precursor, where C=0.5 mg/mL and λ_{ex}=337 nm. The arrow shows how the T_p is defined in our experiments.

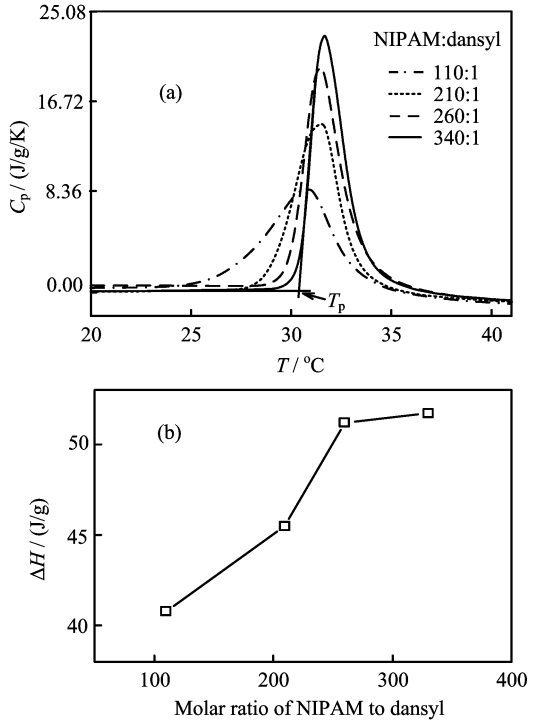

FIG. 5 (a) Temperature dependence of the specific heat capacity (C_p) of dansyl-labeled PNIPAM fractions, where the heating rate was 1.00 °C/min and C_{PNIPAM}= 0.5 mg/mL. The arrow shows how the T_p is defined in our experiments. (b) Dependence of molar ratio of NIPAM to dansyl group on the enthalpy change (ΔH).

decreased lower critical solution temperature (LCST) [49]. Endotherms become broader at higher label content of dansyl groups, which is possibly due to the inhomogeneous distribution of dyes in polymers with higher label content [50]. For polymers with lower label content of dansyl groups the endotherms are narrower, indicating random distribution of the dansyl groups in PNIPAM chains. The ΔH for the dansyl-labeled PNIPAMs with molar ratio of NIPAM to dansyl group of 110 and 340 are 40.7 and 51.7 J/K, respectively. The difference of ΔH values can be attributed to the influence of the hydrophilic CH_2CH_2O unit in the dye on the hydration of the polymer chain, that is, the binding of water molecules by the CH_2CH_2O group disturbs the hydration layer around PNIPAM [51].

TABLE II T_p of dansyl-labeled PNIPAM with different contents of dyes using PNIPAM-2 as the precursor determined by static fluorescence measurement and differential scanning calorimetry.

NIPAM:dansyl[a]	T_p/°C	
	Fluorescene	US-DSC
110:1	26.9	26.5
210:1	28.1	29.0
260:1	29.5	30.0
360:1	29.9	30.3

[a] The molar ratios of NIPAM units to dansyl group.

Table II summarized the T_p of dansyl-labeled PNIPAM with different contents of dyes using PNIPAM-2 as the precursor characterized by fluorescene measurements and DSC analysis. There is no significant difference between the values of T_p due to the high sensitivity of these two methods, and it is clearly that more fluorescent labels would decrease the T_p of PNIPAM. So we labeled all the PNIPAM fractions with small enough amounts of dansyl groups in order to decrease the effect of the fluorescene labels on the T_p of PNIPAM and provide a high enough signal-to-noise ratio for our following kinetics study. From Table I, we also know that the T_p of the four dansyl-labeled PNIPAMs are ∼30.0 °C, so we maintained the solution temperature at 30.0 °C as the starting point before the temperature jump.

B. Kinetics of dansyl-labeled PNIPAMs in water

Figure 6 shows the temperature jump-induced change in fluorescence intensity of dansyl-labeled PNIPAM-3-D with C=0.5 mg/mL. It reveals that at the very beginning the fluorescene intensity increases sharply within milliseconds and returns to its original value within 100 ms which means the polymer chain returns to its original state before the arrival of the next pulse. It indicates that there is no heat accumulation after every pulse, which makes it workable for us to increase the signal-to-noise ratio by averaging many measurements [21, 52]. From Fig.6 we also know that the temperature of the heated sample can persist at least several milliseconds which is long enough for us to observe the collapse kinetics. Successively the temperature falls to the initial value because the sample solution around is thermostated at 30 °C.

Figure 7 shows the initial time dependence of fluores-

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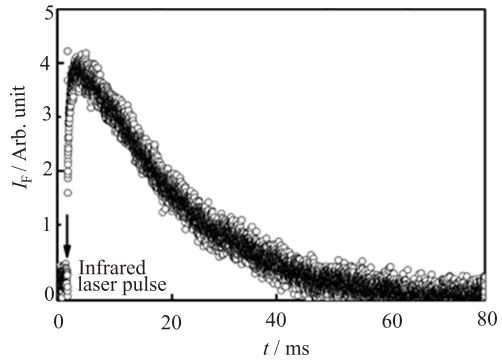

FIG. 6 Temperature jump-induced change in fluorescence intensity of dansyl-labeled PNIPAM-3-D, C=0.5 mg/mL.

cence intensity of dansyl-labeled PNIPAM-3-D, where C=0.5 mg/mL. The whole process finishes within several milliseconds which is in accord with the prediction [11, 14]. The plots are fitted with both single-exponential and double exponential functions [18]. By comparison of the residues from the two kinds of fitting, we can conclude that a double exponential fitting is more suitable to our result, especially noting the fluctuation of the fitting residue at the beginning, thereby indicating the whole phase transition process is a two-stage one. The fitting of the data in Fig.7 shows that $\tau_{\rm fast}$=0.06 ms, $\tau_{\rm slow}$=0.43 ms. It is worth noting that on the basis of Eq.(1), τ should be in the order of a few milliseconds based on the estimation that $\eta\approx$0.8 g/(m s), $a\approx$1 nm, $\varepsilon\approx2\times10^{-18}$ g m^2/s^2 and $N\approx1.2\times10^5$. As stated by Kikuchi *et al.* the characteristic transition of the pearl formation would take only a few percent of the whole transition time [10], which is consistent with our results.

In our previous study [20], using the water soluble fluorescene probe 1,8-ANS, we concluded that in PNIPAM dilute solution, two characteristic transition times exist, which were less than 1 ms, $\tau_{\rm pearls}$ ($\sim$0.1 ms) and $\tau_{\rm coarsening}$ ($\sim$0.8 ms). Both $\tau_{\rm pearls}$ and $\tau_{\rm coarsening}$ are independent of either the PNIPAM or ANS concentration, which may be due to the fast binding process that occurs within microseconds after T-jump. Regenfuss *et al.* reported that at saturation there were five ANS molecules bound to each bovine serum albumin with molecular weight of 68 kg/mol [53]. Wang *et al.* stated that each pearl contained only $\sim$5 monomer units so it was reasonable to believe the number of ANS molecules bound to each pearl is not more than one during the first stage. That is to say we do not need to consider the further binding of ANS anions on the same pearl [16]. To estimate how fast ANS molecules bind to pearls in polymer chains, first we calculate the bimolecular rate constant for a diffusion-controlled encounter of a pearl and an ANS molecule

$$k=\frac{4\pi}{1000}N_{\rm A}R_{\rm PA}D_{\rm A} \qquad (2)$$

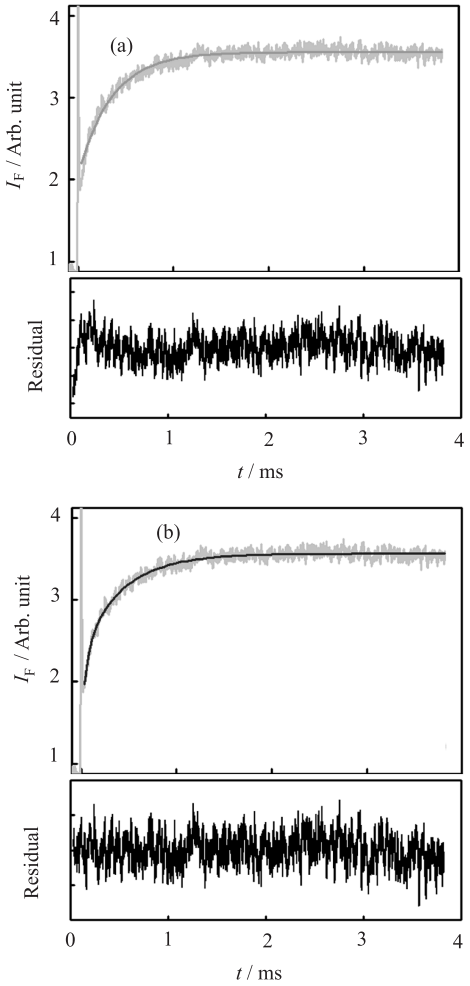

FIG. 7 An enlargement of the initial time dependence of fluorescence intensity of dansyl-labeled PNIPAM-3-D, where C=0.5 mg/mL. The solid lines represent (a) single exponential and (b) double exponential fitting curves. The corresponding residuals are shown below.

where $R_{\rm PA}$ is the sum of the molecular radii of a pearl and an ANS molecule, $N_{\rm A}$ is Avogadro's number and $D_{\rm A}$ is the diffusion constant of ANS because for simplicity the pearls in the polymer are considered to be stationary. If $D_{\rm A}\approx5\times10^{-6}$ cm^2/s [54] and $R_{\rm PA}\approx$1 nm, Eq.(2) predicts an association rate constant of 4×10^9 (mol/L)$^{-1}$ s^{-1}. In our previous study, the molar concentration of the ANS molecules is $C_A\approx$118 μmol/L. According to Wang *et al.*, the average number of clusters at the end of first stage is 10 for the polymer with 160 beads, so we can estimate that the average number of cluster (pearls) for PNIPAM with molecular weight of 5.6×10^6 g/mol is $N\approx$3000 which corresponds to a molar concentration $C_{\rm p}$ of 3 mmol/L inside an individual PNIPAM chain because

$$C_{\rm p}\approx\frac{N}{N_{\rm A}\frac{4}{3}\pi{R_{\rm h}}^3} \qquad (3)$$

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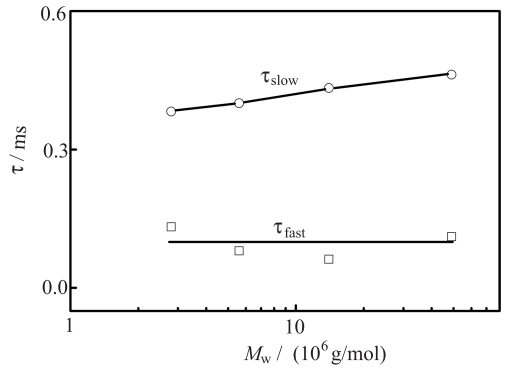

FIG. 8 PNIPAM molecular weight dependence on the two relaxation times, C_{PNIPAM}=0.5 mg/mL.

where N_{A} is Avogadro's number and R_{h} is the hydrodynamic radius of PNIPAM. Since C_{p} is much larger than C_{A}, it can be regarded as a constant during the binding process. The second-order rate equation can be reduced to a pseudo-first-order equation given as

$$C_{\mathrm{A},t} = C_{\mathrm{A}} \mathrm{e}^{-kC_{\mathrm{p}}t} \tag{4}$$

where $C_{\mathrm{A},t}$ is the molar concentration of ANS at time t, C_{A} and C_{p} are the original concentration of ANS and pearls, respectively. Assuming $C_{\mathrm{A},t}/C_A \approx 1\%$, we can estimate that t is less than 1 μs, so due to the fast binding process the change of fluorescence intensity of the probe ANS can reflect the first stage of the coil-to-globule transition.

To further answer this question whether previous experiments of using free fluorescence probes were really proper to address the problem of the coil-to-globule transition, here we used fluorescent labeling technique to investigate the collapse kinetics without any binding process. Because the fluorescent labels were randomly attached into the polymer chains, the change of fluorescence intensity of the dansyl groups should reflect the kinetics of coil-to-globule transition of PNIPAM. As suggested by Halperin *et al.* [11], Kuznetsov *et al.* [13, 14] and Wang *et al.* [16], the first stage of collapse kinetics is the formation of small "peals" on the polymer chains which is fast and should be independent of polymer chain length. Figure 8 shows the PNIPAM molecular weight dependence of the two relaxation times and τ_{fast} is independent of the PNIPAM molecular weight. Current results show that τ_{fast} (≈0.1 ms) is close to the previous value [20], which means that both the fluorescent probes and labeling methods can be used to follow the first stage of collapse kinetics. Our current result shows that the characteristic time of the second stage (∼0.5 ms) is faster than the value reported in our previous work (∼0.8 ms). If ANS free in solution is used to follow the kinetics, during the merging and coarsening of the pearls we could expect that the electrostatic repulsion between ANS anions binding to different pearls will slow the second process. Whereas

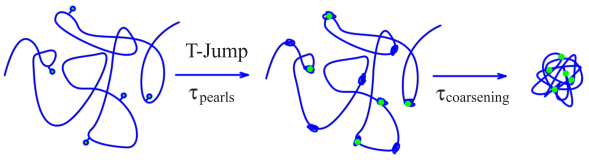

FIG. 9 Schematic diagram of the coil-to-globule transition of dansyl-labeled PNIPAM in aqueous solution.

the covalently attached dansyl groups do not contain any charges, they can move with the chain segments without any binding process and probe the microenvironment at their locations. The increase in the PNIPAM molecular weight led to an increase in τ_{slow}, consistent with previous results of Halperin *et al.* [11] and Wang *et al.* [16]. Figure 9 gives a visual description of infrared laser-heating-induced coil-to-globule transition of the dansyl-labeled PNIPAM in water.

IV. CONCLUSION

By the laser-induced temperature jump technique and fluorescent labeling method, we studied the collapse kinetics of dansyl-labeled PNIPAM in aqueous solution. The study reveals two distinct stages in the coil-to-globule transition with two characteristic times (τ_{fast}≈0.1 ms, τ_{slow}≈0.5 ms), which is consistent with our previous results. The first stage including nucleation and the formation of initial pearls is independent of the molecular weight. The second stage which has slightly chain-length dependence is attributed to the merging and coarsening of the pearls. The characteristic time of the second stage is shorter than the value obtained in our previous report in which 1,8-ANS was used as the fluorescent probe free in the PNIPAM aqueous solutions probably because of the electrostatic repulsion between ANS molecules binding to different pearls.

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