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Thermal Properties of Poly(*N*-isopropylacrylamide)-*g*-poly(ethylene oxide) in Aqueous Solutions: Influence of the Number and Distribution of the Grafts

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ABSTRACT: Thermal properties of copolymers, poly(*N*-isopropylacrylamide) (PNIPA) grafted with different amounts of poly(ethylene oxide) (PEO), were studied in aqueous solutions. A functional backbone copolymer with M_w 1.8 × 10^5 was grafted by amino-terminated PEO (M_w 6000) in water at two different temperatures, 15 and 29 °C. The average number of PEO side chains increased from 6 to 7 with increasing reaction temperature. Above the lower critical solution temperature (LCST), the polymers form stable spherical aggregates observable by light scattering. In a viscous flow, however, the formation of aggregates during the collapse of the PNIPA chains was prevented, and small polymer spheres were detected. EPR experiments showed that with increasing temperature an amphiphilic spin probe, 5-doxylstearic acid, diffused more easily out from the copolymer with more PEO grafts, this indicating that the hydrophilic side chains affect the size of the hydrophobic core in a collapsed copolymer. A polymer grafted in dioxane, which contained 10 PEO grafts, was used as a reference. A comparison of all the three graft copolymers suggests that the conformation of PNIPA during the grafting affects the properties of the product polymers.

Introduction

Amphiphilic copolymers containing poly(ethylene oxide) grafts have been synthesized and studied widely over the recent years.[1−5] The property of amphiphilic and block copolymers to self-assemble to a core−shell structure in solution has attracted much of the attention.[6,7] From the medical point of view these polymeric micelles are potential carriers in drug delivery systems.[8] In biochemical applications, the surface properties and thus the biocompatibility of a polymer can be modified utilizing the physicochemical properties of PEO, like low protein and cell adhesion in aqueous systems.[9]

Responsive polymers that respond to external chemical and physical stimuli like temperature, pH, ionic strengh, or solvent composition have recently attracted noticeable attention.[10−14] Poly(*N*-isopropylacrylamide) (PNIPA) is one of the most studied responsive polymers which exhibits a lower critical solution temperature (LCST) in water around 32 °C. This article describes the properties of linear graft copolymers, for which PNIPA was chosen as the main chain because of its thermosensitivity. For a hydrophilic graft, poly(ethylene oxide) was chosen because of its high solubility in water in a wide temperature range, also above the LCST of PNIPA.

The synthetic methods for the preparation of PEO graft and block copolymers have been studied intensively. Qiu et al. prepared successfully a high molecular weight copolymer by free radical polymerization using a PEO macromonomer as a comonomer.[15] Bo et al. prepared copolymers of a PEO macromonomer and various hydrophobic monomers by radical copolymerization.[7] The authors studied the polymerization conditions in detail to avoid cross-linking reactions. In the present case the aim was to synthesize well-defined graft copolymers. A functional backbone copolymer was prepared into which amino-terminated PEO chains were grafted. This method has been used successfully in various syntheses by other authors.[16−18] For instance, Wesslen used a large selection of functional copolymers for the preparation of PEO-grafted polymers.[5] In the present work, the graft copolymers were prepared by reacting PEO with poly(*N*-isopropylacrylamide-*co*-glycidyl methacrylate), PNIPA-*co*-GMA, in water at 15 and 29 °C. With increasing temperature the hydrodynamic volume of PNIPA decreases. This was expected to have an effect on the localization of PEO grafts on the backbone. A reference polymer was prepared by conducting the grafting reaction in dioxane.

Recently, we have studied the influence of the amount of PEO on the shrinking and especially on the aggregation of PNIPA-*g*-PEOs.[19] In that study, the number of PEO grafts (M_w 6000 g/mol) in a PNIPA chain with M_w 180 000 g/mol was varied in a wide range, from 6 to 79. It turned out that the copolymers grafted in water at 15 and 29 °C behaved differently. The most obvious reason for the different properties of the polymers was concluded to be a slight difference in the number of PEO grafts. Recent theoretical investigations suggest that polymers consisting of structural units with varying solubilities may remember the conformation in which they have been synthesized.[20,21] A possibility remains that the PNIPA copolymers grafted with PEO show different characteristics also owing to the variation in the localization of the side chains. Detailed knowledge of the properties of the graft copolymers with slightly differing amount of PEO grafts is a prerequisite for evaluating the effect which the conformation of the parent polymer during the grafting reaction may have on the behavior of the product copolymers.

Experimental Section

Materials. The monomer *N*-isopropylacrylamide (NIPA, Polysciences, Ins.) was used as received. Glycidyl methacrylate (GMA, Aldrich) was distilled in vacuo and stored in a freezer. A commercial amino-terminated poly(ethylene oxide) (NH_2-PEO, Shearwater Polymers) with a methoxy group as

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Table 1. Summary of Reaction Conditions and Compositions of Linear and Graft Polymers

					comonomer (mol%) PEO (mol%)					
sample	T (°C)	t (h)	AIBN (mg)	concn (g/L)/ a solvent	in feed	in polymer	no. of PEO grafts	mass % of PEO (wt %)	M_w (g/mol)	M_w/M_n^{e}
PNIPA-*co*-GMA	70	7	10	176/dioxane	5.0	1.5^a			$1.78\times 10^{5\ c}$	3.42
PNIPA-*g*-PEO-6/15	15	120		10/water	2.3	0.38^b	6	17	$2.14\times 10^{5\ d}$	
PNIPA-*g*-PEO-7/29	29	120		10/water	2.3	0.44^b	7	19	$2.20\times 10^{5\ d}$	
PNIPA-*g*-PEO-10	reflux	72		126/dioxane	3.0	0.6^b	10	25	$2.40\times 10^{5\ d}$	

[a] Determined by ^{1}H NMR. [b] Determined by ^{1}H NMR and ^{13}C NMR. [c] Measured by static laser light scattering. [d] Calculated by M_w(backbone polymer) + number of PEO grafts × M_w(PEO). [e] Measured by GPC (polystyrene standards in $CHCl_3$, room temperature).

the other terminus and with M_w 6000 g/mol was used as received. Azobis(isobutyronitrile) (AIBN, Fluka) was recrystallized twice from methanol. Dioxane (Lab-Scan, Analytical Sc.) was distilled. The water used for all the measurements was purified and deionized in an Elgastat UHQ-PS purification system. A spin probe, 5-doxylstearic acid, was purchased from Sigma.

Syntheses (Table 1). a. PNIPA-*co*-GMA. NIPA was dissolved in dry dioxane and flushed with nitrogen for 30 min. AIBN was added when the mixture reached 70 °C. GMA was added dropwise in a period of the first 2 h of polymerization. In this way a random distribution of GMA on the copolymer was expected to be obtained. The copolymer was precipitated twice into diethyl ether and dried in vacuo for 24 h.

b. PNIPA-*g*-PEOs were synthesized by grafting PNIPA-*co*-GMA in aqueous solutions in a double-wall reaction vessel at two different temperatures, 15 and 29 °C. The stability of temperatures was ensured for 48 h before the actual reactions. NH_2-PEO was added after dissolving PNIPA-*co*-GMA in pure water, and the mixture was stirred for 5 days. The polymer which has been used as a reference was grafted by refluxing a dioxane solution of PNIPA-*co*-GMA and NH_2-PEO for 3 days.

After the reaction, the unreacted epoxides were not quenched. In an acidic or basic environment the epoxide group hydrolyses through a ring opening which affects the water solubility of the polymer. To check the stability of the epoxy function, PNIPA-*co*-GMA was dissolved in deionized water (pH 5.8) and monitored for 2 weeks by NMR. No evidence of the ring opening was detected.

The graft copolymers were purified in dialysis and dried in vacuo. The copolymers were stored in a freezer at −20 °C.

Instrumentation and Characterization. a. NMR Spectroscopy. ^{1}H and ^{13}C NMR spectra of the synthesized polymers were measured at ambient temperature with a 200 MHz Varian Gemini 2000 spectrometer using $CDCl_3$ as a solvent. The composition of the copolymer PNIPA-*co*-GMA was determined by ^{1}H NMR from the characteristic peaks of the monomers. PNIPA-*co*-GMA: ^{1}H NMR ($CDCl_3$) [200 MHz] δ ppm: 3.95 (br, 1 H, $-NH-CH-Me_2$) for NIPA, 3.25 (br, 1 H, $-O-CH_2-CH-$Epox) for GMA. The compositions of the graft copolymers were calculated using the characteristic peaks of NIPA and PEO in both the ^{1}H NMR and ^{13}C NMR spectra. PNIPA-*g*-PEO: ^{1}H NMR ($CDCl_3$) [200 MHz] δ ppm: 4.0 (br, 1 H, $-NH-CH-Me_2$) for NIPA, 3.65 (s, CH_2-CH_2-O, EO−homosequence). ^{13}C NMR ($CDCl_3$) [200 MHz] δ ppm: 22.7 (CH_3-) for NIPA, 70.6 ($-CH_2-$, EO−homosequence) for PEO (see ref 19).

b. Laser Light Scattering. Static light scattering (SLS) and dynamic light scattering (DLS) measurements were conducted with a Brookhaven Instruments BI-200SM goniometer and a BI-9000AT digital correlator. The light source was Spectra Physics model 127 helium/neon laser (633 nm, 35 mW). In SLS, the angular dependence of the time-averaged scattering intensity (R_θ) was measured. When the polymer concentration approaches zero and the scattering angle, θ, is small, the weight-average molar mass, M_w, is proportional to R_θ/Kc:

$$\left[\frac{Kc}{R_\theta}\right]_{c=0} = \frac{1}{M_w}\left(1 + \frac{16\pi^2}{3\lambda^2}\langle R_g^{\,2}\rangle_z \sin^2\!\left(\frac{\theta}{2}\right)\right) \qquad (1)$$

Here, $K = 4\pi^2 n^2(dn/dc)^2/(N_A\lambda_0^{\,4})$ in which n, dn/dc, N_A, and λ_0 are the refractive index of the solvent, the specific refractive index increment, Avogadro's constant, and the wavelength of light in a vacuum, respectively. The wavelength in a given medium is defined as $\lambda = \lambda_0/n$. The time correlation functions measured by DLS were analyzed with a Laplace inversion program (CONTIN). Experiments were carried out in a temperature range from 20 to 45 °C, at the scattering angle of 90°. At each temperature the samples were equilibrated from 20 to 60 min.

c. Electron Paramagnetic Resonance. EPR spectra were recorded with a Varian E4 spectrometer. Aqueous samples of PNIPA-*g*-PEO were prepared where the polymer concentration was 1.0 g/L. The concentration of the spin probe 5-doxylstearic acid was kept low, in the range 10^{-4}−10^{-3} M. The spin probe was first dissolved in ethanol in 1 mm o.d. glass capillaries. Ethanol was evaporated, after which the polymer solution was added and the probe was slowly solubilized in the solution. The samples were diluted with the polymer solutions until no sign of a broad singlet line due to the insolubilized probe could be detected. In other words, the observation of a motionally narrowed three-line spectrum was used as a criterion of the total dissolution of the probe. The registration of spectra, microwave power 1 mW, and modulation amplitude 1 G were used. The samples were allowed to equilibrate at each temperature at least 15 min, after which two or three spectra were registered with intervals of 10 min.

d. Viscosimetry. Reduced viscosities of the 1.0 g/L aqueous polymer solutions were measured with a modified Ubbelohde viscometer. Temperature ranged from 20 to 45 °C. At each temperature the samples were equilibrated for 20−60 min. The measurements were conducted twice for both solutions.

e. Microcalorimetry. Calorimetric measurements were conducted with a NANO differential scanning calorimeter (Calorimetric Sciences Corp.) with a heating rate 1 °C/min. Polymer concentration was 1.0 g/L.

Results and Discussion

Grafting of PNIPA-*co*-GMA with PEO. Table 1 summarizes the reaction conditions and the compositions of the copolymers. The number of the PEO grafts was determined by ^{1}H and ^{13}C NMR, which both gave identical results. It turned out that in the polymers grafted in water the average number of PEO chains per one PNIPA varied from 6 to 7, depending on the reaction temperature. The polymer grafted at the vicinity of the LCST of PNIPA-*co*-GMA contained more PEO than the one grafted at $T \ll$ LCST. For clarity, the polymers will be hereafter denoted as PNIPA-*g*-PEO-6/15 and PNIPA-*g*-PEO-7/29 to indicate the number of grafts/reaction temperature. The reference polymer grafted in boiling dioxane contained 10 PEO grafts per one chain and will be denoted as PNIPA-*g*-PEO-10.

The conformation of the PNIPA-*co*-GMA chain during the grafting is expected to have an influence on the distribution of the PEO chains along the backbone. In an aqueous solution, depending on the reaction temperature the attachment sites for PEO are suggested to be located either on the surface of the shrunken PNIPA-*co*-GMA chain ($T \sim$ LCST) or randomly through-

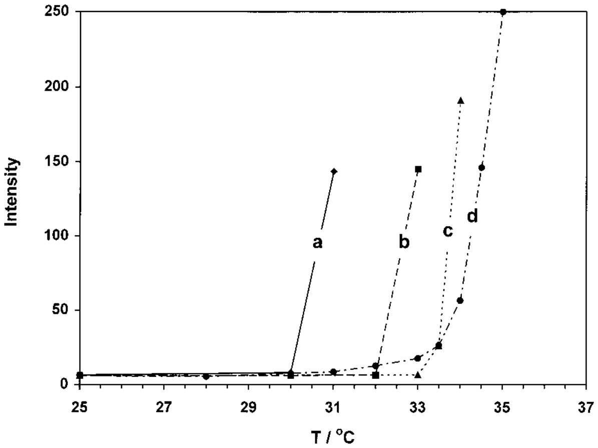

Figure 1. Dependence of the scattered light intensity of the aqueous copolymer solution on temperature. Polymer concentration = 1.0 g/L. (a) PNIPA-*co*-GMA; (b) PNIPA-*g*-PEO-6/15; (c) PNIPA-*g*-PEO-7/29; (d) PNIPA-*g*-PEO-10.

out the polymer coil ($T \ll$ LCST). It should be noted that the degree of grafting is low compared to the number of reactive groups in PNIPA-*co*-GMA. In three copolymers, 25−40% of the epoxy groups had reacted with NH_2-PEO (see Table 1). Thus, different distributions of the substituents in principle are possible. Even small differences in the distributions may be important since we have observed[19] that the PEO grafts tend to turn outward into the aqueous phase at temperatures below and above the LCST of PNIPA.

Laser Light Scattering. In Figure 1, the intensity of light scattered from the aqueous polymer solutions with $c = 1.0$ g/L is plotted against temperature. A sharp increase in intensity indicates the formation of aggregates. At very low concentrations it is possible to observe the coil-to-globule transition of single PNIPA chains of very high molecular weight.[15] In the concentration range used in this work, however, aggregates formed by several chains are observed. The LCSTs are 31, 32.5, 33.5, and 34 °C for the PNIPA-*co*-GMA, PNIPA-*g*-PEO-6/15, PNIPA-*g*-PEO-7/29, and PNIPA-*g*-PEO-10, respectively. The differences in the critical temperatures were small but clearly observable and reproducible. Partial mixing of PNIPA and PEO during the collapse is seen to increase the LCST. The difference in the LCST of PNIPA-*co*-GMA and PNIPA-*g*-PEO-6/15 is of the same order of magnitude as that between PNIPA-*g*-PEO-6/15 and PNIPA-*g*-PEO-7/29. A further increase of the number of grafts does not practically change the LCST.[19] In general, the changes in the LCST are fairly small. For in vivo applications the transition temperature should be higher; this may be achieved for example by copolymerizing NIPA with polar monomers.[22]

The number of grafts in the PNIPA-*g*-PEO copolymers used in this investigation is low although the mass fraction of PEO is fairly high, ∼0.2. Kawaguchi et al.[1] studied poly(*n*-butyl methacrylate) particles grafted with PEO macromonomers. It was shown that when a small amount of PEO is added to the particle, the PEO chains lie widely on the particle surface. With increasing packing density, the chains extend far into the continuous medium. This process is known as a pancake-to-brush transition.[23] This model may be applied also in the present case. Above the critical temperature PNIPA chains form aggregates sterically stabilized by the outer shell of PEO. At this state, PEO chains are expected to be stretched out to the aqueous phase in the direction perpendicular to the surface of the PNIPA core.

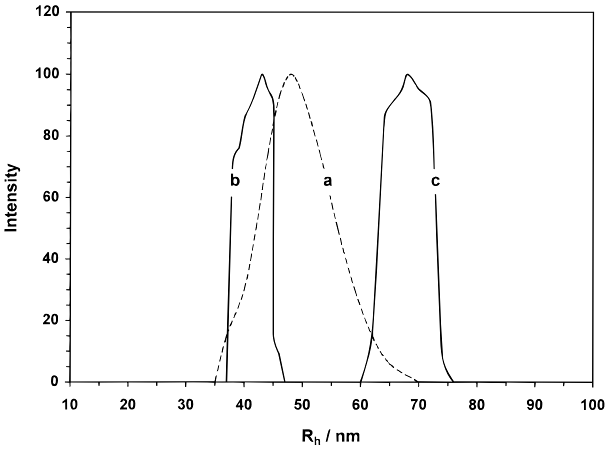

Figure 2. Size distribution of the aggregates at 45 °C. Polymer concentration = 1.0 g/L. (a) PNIPA-*g*-PEO-6/15; (b) PNIPA-*g*-PEO-7/29; (c) PNIPA-*g*-PEO-10.

The distribution of the hydrodynamic radius, R_h, of the aggregates of PNIPA-*g*-PEO-6/15, PNIPA-*g*-PEO-7/29, and PNIPA-*g*-PEO-10 with $c = 1.0$ g/L was measured at 45 °C. The samples were equilibrated at least 2 h before each measurement to ensure the stability of the aggregates. The distributions are shown in Figure 2. The result deserves two comments. First, of the three polymers studied the reference polymer with the highest number of PEO grafts, the one prepared in an organic solvent forms the largest aggregates. This is contrary to expectation because PEO should prevent the aggregation. Second, the size of the aggregates is not directly proportional to the number of the PEO grafts. PNIPA-*g*-PEO-7/29 forms the smallest aggregates, and the size distribution of these particles is narrower than those for the other two polymers. These observations support the hypothesis that the distribution of PEO chains along the PNIPA chain is different in every sample. Evidently, the PEO grafts are most effectively concentrated on the surface of the collapsed PNIPA in PNIPA-*g*-PEO-7/29. Further, in the polymers grafted in water the surface stabilization by PEO is more effective than in PNIPA-*g*-PEO-10. Here it is assumed that an increase of the number of PEO chains on the surface of a single collapsed PNIPA chain decreases the size of the aggregate. If this assumption is valid, the observed differences in the aggregate sizes indicate that the polymers tend to readopt the conformation in which they have been synthesized.

The scattered light intensity from a PNIPA-*g*-PEO-10 solution at 45 °C was 25% lower than that from a solution of PNIPA-*g*-PEO-6/15. This indicates that the density of the aggregate particles formed by the polymer with 10 PEO grafts is lower than the density of a less substituted polymer.

As already mentioned, in the concentration range studied in this work single chains (unimers) were not observed. The PEO side chains do not prevent the aggregation of the polymers but affect strongly the size of the aggregates. An increase in polymer concentration was observed to favor the aggregate formation. The average hydrodynamic radii and apparent weight-average molar masses, M_w, of the aggregates measured at 45 °C are plotted against polymer concentration in Figure 3. The results of the light scattering measurements are summarized in Table 2. The values of $\langle R_g\rangle$/

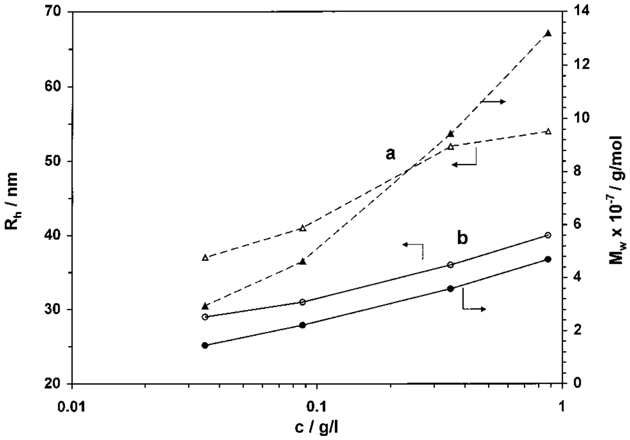

Figure 3. Concentration dependence of the average hydrodynamic radius (R_h) and the weight-average molecular weight (M_w) of (a) PNIPA-*g*-PEO-6/15 and (b) PNIPA-*g*-PEO-7/29 at 45 °C.

$\langle R_h \rangle$ in Table 2 are close to that of a hard sphere, 0.778.[24] The ratio $\langle R_g \rangle / \langle R_h \rangle$ decreases as the polymer concentration decreases, indicating that the dilution leads to more compact aggregates. Wu et al. studied single PNIPA chains in an extremely dilute solution (~5 μg/mL) and stated that the low value for the $\langle R_g \rangle / \langle R_h \rangle$ equal to 0.62 was due to a higher chain density at the center than near the surface.[25] This is an obvious conclusion also in the present case, at low polymer concentrations.

Viscosimetric Measurements. Figure 4 shows the reduced viscosities of the graft copolymer solutions against increasing temperature. Heating induces the collapse of the copolymers, and the viscosities of the polymer solutions approach the viscosity of water. η_{sp}/c approaches zero when the graft copolymers are in their fully collapsed, compact state and flow freely with water through the capillary. A growth of the aggregates is observed in the course of increasing temperature above the LCST. The difference in the LCSTs of the graft copolymers is parallel with that measured by light scattering. However, dynamic flow tends to break the aggregates which is observed as an upward shift of the viscosimetrically determined LCST compared to that measured by light scattering. The collapse transition observed viscosimetrically is more gradual than that observed by light scattering. In the viscosity measurements one first observes the shrinking of the invidual chains and later, at higher temperatures, also the aggregate formation. Whereas light scattering from a static sample detects mainly the aggregation, the aggregate formation is perturbed in a flowing solution. At low temperatures the reduced viscosity of the PNIPA-*g*-PEO-6/15 is much higher than that of the PNIPA-*g*-PEO-7/29. The result suggests that in a flow field the latter polymer adopts a more compact conformation than the one with fewer PEO grafts. The most obvious reason for this is a different distribution of the grafts (inside/outside the coil). This will be a subject for further scattering studies.

Electron Paramagnetic Resonance. Nitroxide spin probes derived from fatty acids have traditionally been used in studies of organized structures such as lipid bilayer membranes or synthetic liquid crystals.[27] In the present case it was of interest to see whether the capability of the graft copolymers to solubilize the probes changes upon the collapse of the PNIPA main chain. Various small pyrrolidine-1-oxyl derivatives were tested as probes, but owing to their high water solubility the changes in the spectra with increasing temperature were too small to be accurately analyzed. Even 4-benzoxy-2,2,6,6-tetramethylpiperidine-1-oxyl which has been successfully used previously in studies of PNIPA gels[28] could not detect the collapse of the PNIPA-*g*-PEO polymers. Thus, 5-doxylstearic acid was used because of its capability of dissolving in hydrophobic structures. 5-Doxylstearic acid is practically nonsoluble in cold water but sparingly soluble when heated. It was, however, soluble in the polymer solutions.

All the measured EPR spectra were motionally narrowed ones consisting of three sharp lines, regardless of temperature. Contrary to expectation, the contraction of the PNIPA main chain of the copolymers did not lead to any detectable binding of the probe into the hydrophobic core of the collapsed polymer. The water solubility of the probe increases with temperature, and upon the collapse of the PNIPA chain, an increase in the rate of rotational motion of the probe was detected. Because the nitrogen hyperfine coupling constant did not change during the collapse, it may be concluded that the polar head of the probe including the nitroxide moiety resided outside the hydrophobic part of the polymer, in a polar surrounding composed of PEO chains and water.

The changes in the shapes of the spectra with increasing temperature were small but reproducible. The rotational correlation times of the probes were estimated using Kivelson formalism[29] where the rotational diffusion is expected to be isotropic. The logarithmic correlation times are plotted against inverse temperature in Figure 5. As may be seen from the figure, the correlation times first increase with temperature, then suddenly decrease close to the critical temperature, and start to increase again above the LCST. An evident interpretation of the data is as follows. Hydrophobic interactions increase in strength when the temperature is raised; the binding of the probe to the hydrophobic parts of the polymer explains the slowing down of the rotational motion of the label with increasing temperature when temperature is well below or above the critical. Owing to the increasing water solubility of the probe with increasing temperature, the nitroxides diffuse out from the polymer core during the collapse of the PNIPA chain, this leading to an abrupt decrease of the correlation time. Usually, from the slope of a straight line in an Arrhenius plot one can estimate the activation energy of the rotational motion of a spin probe. In the present case we assume that the activation energies calculated from the data in the temperature range where the PNIPA chain collapses may be used as indicators of the activation energies of the diffusion of the probe out from the polymer core.

Two important conclusions may be drawn from the data in Figure 5. First, the collapse of PNIPA-*g*-PEO-7/29 occurs in a wider temperature range than that of PNIPA-*g*-PEO-6/15. This is well in accordance with the results from light scattering measurements which show that increasing the number of PEO grafts makes the conformational change more gradual.[19] The data in general show the same tendency as the viscosity data in Figure 4. Second, the activation energies E_a of the probe diffusion are 45 and 115 J/mol for PNIPA-*g*-PEO-7/29 and PNIPA-*g*-PEO-6/15, respectively. That the E_a for the polymer with seven PEO grafts is smaller than that for the polymer with six grafts is important, showing that the amphiphilic probe diffuses more easily

Table 2. Light-Scattering Characterization of the PNIPA-*g*-PEO-6/15 and PNIPA-*g*-PEO-7/29 in Water at 45 °C

	$M_{\mathrm{w,aggregate}}$ (g/mol)		N_{polymer}[c]		$\langle R_g \rangle$ (nm)		$\langle R_g \rangle / \langle R_h \rangle$		$\langle \rho \rangle_{\mathrm{aggregate}}$ (g/cm^3)	
c (g/L)	**a**	**b**	**a**	**b**	**a**	**b**	**a**	**b**	**a**	**b**
3.50×10^{-2}	2.94×10^{7}	1.45×10^{7}	136	65	20	21	0.54	0.75	0.235	0.229
8.75×10^{-2}	4.62×10^{7}	2.21×10^{7}	214	99	25	26	0.62	0.87	0.261	0.284
3.50×10^{-1}	9.44×10^{7}	3.58×10^{7}	437	161	36	33	0.69	0.92	0.274	0.295
8.75×10^{-1}	13.2×10^{7}	4.69×10^{7}	611	211	43	38	0.80	0.95	0.337	0.289

[a] PNIPA-*g*-PEO-6/15. [b] PNIPA-*g*-PEO-7/29. [c] Aggregate number.

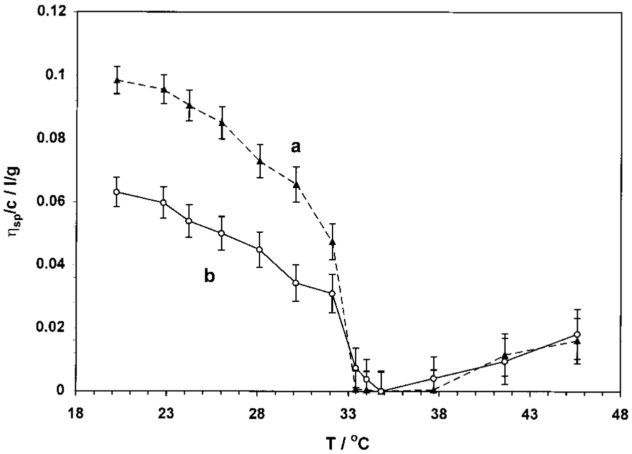

Figure 4. Reduced viscosities versus temperature of aqueous polymer solutions of (a) PNIPA-*g*-PEO-6/15 and (b) PNIPA-*g*-PEO-7/29. Polymer concentration = 1.0 g/L.

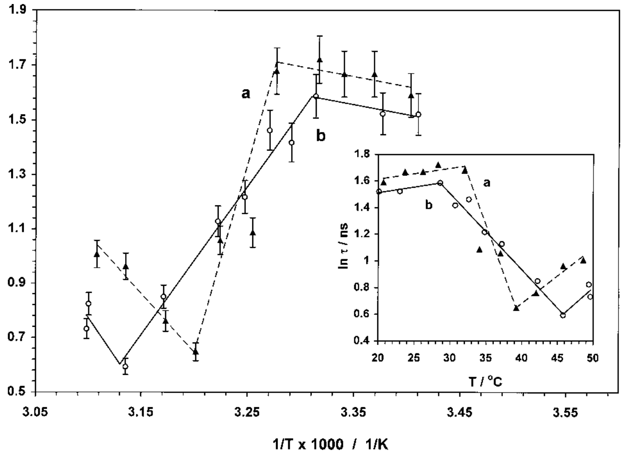

Figure 5. Logarithmic rotational correlation times of 5-doxylstearic acid versus inverse temperature of aqueous polymer solutions of (a) PNIPA-*g*-PEO-6/15 and (b) PNIPA-*g*-PEO-7/29. Polymer concentration = 1.0 g/L.

out from the polymer with more PEO grafts. This indicates that the hydrophobic core in which the hydrophobic tail of the probe may be embedded is bigger in PNIPA-*g*-PEO-6/15 than in PNIPA-*g*-PEO-7/29.

Microcalorimetry. Influences of different factors such as polymer concentration and the addition of salt on the thermal behavior of aqueous PNIPA as well as kinetics of the collapse have been studied calorimetrically by Schild et al.[30] and Tiktopulo et al.[31] The breakage of hydrogen bonds formed by ordered water molecules around PNIPA taking place upon heating is an endothermic process. In the case of PNIPA-*g*-PEO copolymers, differences not only in the LCST but also in the enthalpy change could be expected. The solubilizing effect of an additional PEO chain is observed in the microcalorimetric measurements. The peak temperatures of the collapse were 33.5 and 34.3 °C for PNIPA-*g*-PEO-6/15 and PNIPA-*g*-PEO-7/29, respectively. The difference in LCSTs is of the same order of magnitude as was observed by light scattering. The PNIPA-*g*-PEO-6/15 showed an enthalpy change (2.5 kJ/mol) twice larger than PNIPA-*g*-PEO-7/29 (1.3 kJ/mol). The difference in ΔH is high, taking into account the small difference in the chemical structure of the polymers. This supports the conclusion from the EPR measurements that the hydrophobic core is bigger in PNIPA-*g*-PEO-6/15 than in PNIPA-*g*-PEO-7/29.

Conclusions

PNIPA copolymers have been grafted with PEO under various conditions. The grafting was conducted in water at $T \ll$ LCST as well as at $T \sim$ LCST. A reference polymer was prepared in an organic solvent. The number of grafts was slightly different in every polymer, this indicating that the conformation of the polymer affects the accessibility of the functional side groups in PNIPA.

The number of PEO grafts affects the LCST and the viscosity of the solutions. According to the viscosity measurements the polymers collapse and form rigid spheres at the LCST. By light scattering, formation of polymer aggregates was observed at the LCST. The size of the aggregates is dependent on the polymer concentration; an increase of concentration increases the size of the aggregates. The aggregates are hard spheres, the densities of which depend on concentration and the number of PEO side chains.

An important observation is that the sizes of the aggregates at $T >$ LCST are not directly proportional to the number of the PEO grafts but evidently reflect the localization of the PEO chains on the surface of the collapsed chains. This finding implies that the polymers may show a tendency of readopting the conformation in which they were synthesized.

EPR measurements on a fatty acid spin probe solubilized in the aqueous polymers revealed that a small change in the number of PEO chains, and probably also in their distribution along the PNIPA chain, affects the binding of the probe to the polymer. The number of PEO chains changes the size of the hydrophobic core in the collapsing polymer. This conclusion is of special importance for the investigation of the interactions of low molar mass substances with this type of polymers and thus also for their potential applications.

To summarize, not all the differences in the behavior of the polymers are understood by the differences in the number of the PEO grafts. To further clarify the affects of the number and distribution of the grafts, investigations on the kinetics of the grafting reaction under various conditions as well as on the properties of the product polymers are in progress.

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