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Fluorescence and EPR studies on the collapse of poly(*N*-isopropyl acrylamide)-*g*-poly(ethylene oxide) in water

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Abstract

Studies on the thermal behaviour of copolymers, poly(*N*-isopropyl acrylamide), PNIPA, grafted with different amounts of poly(ethylene oxide), PEO, were conducted in aqueous solutions by fluorescence and EPR spectroscopies. The grafting reaction had been conducted under various reaction conditions and the number of PEO grafts per a PNIPA chain varied from 6 to 10. A fluorescence probe, 4-(dicyano methylene)-2-methyl-6-(*p*-dimethyl amino styryl)-4H-pyran (4HP) was observed to localise in the hydrophobic parts of the polymers. The changes in the polarity and microviscosity in the surroundings of 4HP upon the collapse of PNIPA depended in general on the amount of PEO in the polymer, polymer concentration, and sample-heating rate. However, fluorescence data revealed differences between the polymers, owing to the varying distribution of the PEO grafts along the PNIPA main chain. The polymer grafted in water at an elevated temperature ($T \sim$ LCST) differed clearly from the others, those synthesised either in cold water or in an organic solvent. This particular polymer formed dense local hydrophobic domains inside the collapsed globule. Two ways of preparing the samples for EPR measurements resulted in a different localisation of a spin probe 5-doxyl stearic acid in the polymers. The probe resided either in the outer PEO shell close to the aqueous phase or in a less polar phase close to the PNIPA core of the polymer. The rate of the rotational diffusion of the radical increased discontinuously during the polymer collapse in the former case but in the latter, a typical linear activation of the thermal motion was observed. The results strongly suggest that the distribution of the PEO grafts along the PNIPA chains depends on the conformation of the functionalised PNIPA during the grafting, and this affects the behaviour of the graft copolymers. © 2001 Published by Elsevier Science Ltd.

Keywords: Thermally responsive polymers; Poly(*N*-isopropyl acrylamide); Fluorescence spectroscopy

1. Introduction

Solution properties and self-assembling of amphiphilic graft copolymers containing poly(ethylene oxide) as a hydrophilic part is an essential topic in polymer science not only from the theoretical but also from the practical point of view [1–8]. Such polymers have been used for stabilisation of dispersions and emulsions. Hydrophilic PEO can also be used to modify the surface properties of polymer particles [9]. The biocompatibility of PEO has motivated the syntheses of PEO containing amphiphilic polymers as vehicles in drug delivery systems [10–12].

Of responsive polymers which respond to chemical and physical stimuli like temperature, pH, ionic strength, or solvent composition, poly(*N*-isopropyl acrylamide), PNIPA, is one of the most studied. In an aqueous solution, PNIPA has a coiled structure at room temperature. Upon heating the polymer shrinks and at the lower critical solution temperature, LCST, collapses to a globular structure and precipitates. This paper describes the properties of linear graft copolymers for which PNIPA was chosen as the main chain because of its thermosensitivity. Poly(ethylene oxide) was used as a hydrophilic graft in order to solubilise the collapsed PNIPA chains at $T >$ LCST. PEO is well soluble in water in a wide temperature range, also above the LCST $\sim$ 32°C of PNIPA.

Qiu et al. investigated the formation of a core-shell structure of aggregates and of a single chain having a PNIPA core shielded by a PEO shell [8]. Recently, we have studied the influence of the number of PEO grafts on the shrinking and especially on the aggregation of PNIPA-*g*-PEOs [13,14]. In these studies, the number of PEO grafts with 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. To prepare

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polymers with fairly low numbers of PEO grafts (from 6 to 10) the grafting reactions between a functional copolymer, poly(*N*-isopropylacrylamide-*co*-glycidylmethacrylate), PNIPA-*co*-GMA, and amino terminated PEO were conducted either in water at different temperatures or in an organic solvent. It turned out that the copolymers grafted under various conditions behaved differently. One reason for the different properties of the polymers was concluded to be a slight difference in the number of PEO grafts. However, it may be assumed that the conformation of the functionalised PNIPA during the grafting affects also the localisation of the side chains. When PNIPA-*co*-GMA is grafted in water, reaction temperature ($T \sim$ LCST or $T <$ LCST) determines the hydrodynamic volume of the polymer and correspondingly also the accessibility of the functional groups in the PNIPA chain. Detailed knowledge of the properties of the graft copolymers with a slight difference in the amount of PEO grafts and in their distribution along the backbone is necessary for evaluating the effect that the conformation of the parent polymer during the grafting reaction may have on the behaviour of the product copolymers.

Fluorescence probes are widely used for monitoring specific properties of the medium in which they are incorporated. Especially, the formation of hydrophobic microdomains in water-soluble polymers has attracted attention [15–20].

Fluorescent donor–acceptor probes have been used to follow polymerisation processes [21–29]. One of these probes is 4-(dicyano methylene)-2-methyl-6-(*p*-dimethyl amino styryl)-4H-pyran, 4HP, which exhibits intramolecular charge transfer (ICT) property and stereoisomerism. 4HP contains both electron donor and acceptor moieties being linked by an aromatic chromofore. Because both twisting and charge separation are involved in the formation of intramolecular charge transfer states, the fluorescence emission of 4HP is sensitive to both the solvent polarity and the microviscosity of the medium. The sensitivity of 4HP to local changes in its environment makes it possible to study the formation, structure and stability of hydrophobic microdomains in a polymer system.

Nitroxide spin probes derived from fatty acids have traditionally been used in studies of organised structures like lipid bilayer membranes or synthetic liquid crystals [32]. In the present case it was of interest to compare amphiphilic spin probes with hydrophobic 4HP, and to see whether the capability of the graft copolymers to solubilise the probes 5-doxyl stearic acid, 5-DSA, and 16-doxyl stearic acid, 16-DSA, differs and changes upon the collapse of the PNIPA main chain.

As the probes used in this study are non-soluble or sparingly soluble in water but dissolve with the polymer, they are likely to be found in or close to the hydrophobic domains of the aqueous polymer system.

In this study, fluorescence and electron paramagnetic resonance techniques were used to study PNIPA-*co*-GMA and its derivatives, graft copolymers with a slightly varying number of PEO grafts. The purpose was to find out whether the properties of the polymers are solely determined by the number of the grafts or does the distribution of the grafts contribute to the thermal behaviour of the macromolecules.

2. Experimental

2.1. Materials

Table 1 summarises the compositions of the copolymers. The details of the synthesis of the polymers have been reported recently [13]. In this work, the studied polymers were a copolymer of *N*-isopropyl acrylamide and glycidyl methacrylate, PNIPA-*co*-GMA, and the PEO grafted derivatives PNIPA-*g*-PEO-6/15, PNIPA-*g*-PEO-7/29 and PNIPA-*g*-PEO-10. The grafting reactions of the PNIPA-*g*-PEO-6/15 and PNIPA-*g*-PEO-7/29 were conducted in water. The number codes indicate the number of grafts/grafting reaction temperature. The polymer with 10 PEO grafts per one chain was grafted in boiling dioxane and is denoted as PNIPA-*g*-PEO-10.

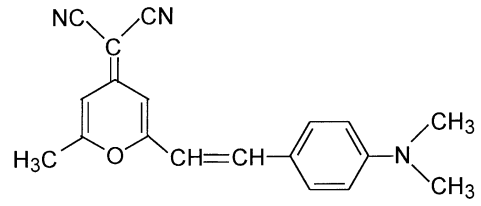
A fluorescent probe 4-(dicyano methylene)-2-methyl-6-(*p*-dimethyl amino styryl)-4H-pyran (4HP, Aldrich) (**1**), and spin probes 5-doxyl stearic acid (5-DSA, Sigma) and 16-doxyl stearic acid (16-DSA, Sigma) (**2**) were used as

Table 1
Summary of compositions of linear and graft polymers

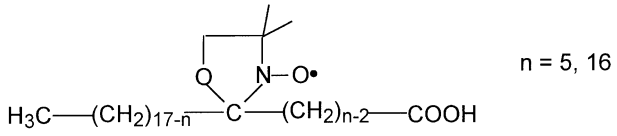
Sample	Comonomer (mol%) PEO (mol%)	Number of PEO grafts	Mass percent of PEO (wt%)	M_w (g/mol)
PNIPA-*co*-GMA	1.5[a]			1.78×10^5 [b]
PNIPA-*g*-PEO-6/15	0.38[c]	6	17	2.14×10^5 [d]
PNIPA-*g*-PEO-7/29	0.44[c]	7	19	2.20×10^5 [d]
PNIPA-*g*-PEO-10	0.6[c]	10	25	2.40×10^5 [d]

[a] Determined by ^{1}H NMR.
[b] Measured by static laser light scattering.
[c] Determined by ^{1}H NMR and ^{13}C NMR.
[d] Calculated by M_w (backbone polymer) + number of PEO grafts $\times M_w$(PEO).

received. The chemical structures of the probes are shown below.



(1)



(2)

2.2. Instrumentation and characterisation

Light scattering. Dynamic light scattering (DLS) measurements were conducted with a Brookhaven Instruments BI-200SM goniometer and a BI-9000AT digital correlator. The light source was Lexel 85 Argon laser (514.5 nm, power range 15–150 mW). Time correlation functions were analysed with a Laplace inversion program (CONTIN). The range of polymer concentrations was 0.01–1.0 g/l. The solutions were clarified by filtering through Millipore membranes (0.45 μm pore size). Experiments were carried out in a temperature range from 20 to 45°C, at the scattering angle of 90°. The samples were heated fast from ambient temperature by placing them into the goniometer where temperature was 45°C. The samples were equilibrated at this temperature at least for 2 h.

Fluorescence spectroscopy. Fluorescence spectra were recorded using a Spex Fluorolog 3 spectrofluorometer at right angles. The temperature of the water-jacketed cell holder was controlled by a programmable circulation bath. The temperature was measured with a thermocouple connected to the cell holder. Samples were excited at 469 nm. The slit widths were set at 2.0 nm for both the excitation and the emission. In a 1 cm cell, the samples were heated with a slow and fast rate. In the former the heating rate was 0.2°C/min. In the latter, the cell holder was preheated at 45°C after which the sample was placed into the cell holder, and the emission spectra were recorded with an interval of 60 s starting at $t = 0$ s.

A minute amount of the probe, 4HP, dissolves in water and exhibits two weak emission maxima at 558 and 626 nm. The maxima are only moderately resolved in the spectra of 4HP incorporated in polymer samples. As may be seen in Fig. 1, the emission intensity increases upon heating the polymer solution. Also, a blue shift takes place. The increase of intensity may be attributed to an increase in the microviscosity inside a collapsing polymer, whereas

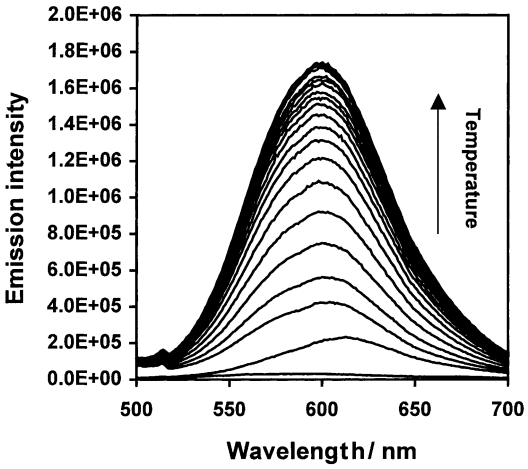


Fig. 1. Increase of fluorescence emission intensity of 4HP with increasing temperature during the fast heating of aqueous PNIPA-*g*-PEO-10.

the blue shift owes to the decreasing polarity of the surroundings of the probe.

Electron paramagnetic resonance. EPR spectra were recorded with a Varian E4 spectrometer. In the registration of spectra, microwave power 1 mW and modulation amplitude 1 G were used. In a cell with 1 mm o.d., the samples were heated with a rate of 0.4°C/min.

2.3. Sample preparation

Light scattering and fluorescence spectroscopy. Aqueous polymer samples for light scattering and fluorescence measurements were prepared by first dissolving the polymers and 4HP separately in chloroform. Known amounts of polymer and probe solutions were carefully mixed, and the solvent was evaporated with a stream of air. Then, known volumes of deionised water were added on the thin films of polymer containing the probe. The solutions were kept in a refrigerator for 24 h for the complete dissolution. This way of preparing the samples will be referred to as *an indirect dissolution method*. In the light scattering experiments the polymer concentration was kept constant (0.1 g/l) whereas the probe concentration varied from 3.33×10^{-8} to 3.33×10^{-6} M. In the fluorescence measurements the polymer concentration varied from 0.01 to 1.0 g/l and the probe concentration was kept constant, 3.33×10^{-6} M. All the solutions were clear and yellowish, and no sign of undissolved material was seen.

Electron paramagnetic resonance. Two series of samples prepared in a different way were studied by EPR. The first series was prepared in the indirect way described above, using 5-DSA and 16-DSA. The solutions were kept at room temperature for at least one week before measurements. The concentrations of the polymers and the spin probes were 0.1 g/l and 10^{-4} M, respectively. No sign of a broad singlet line typical for an insolubilised probe was detected but all the measured spectra were motionally narrowed ones with three sharp lines. In the second series, the spin probe 5-DSA 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 solubilised in the solution. The samples were diluted with the polymer solutions until no sign of a broad singlet line due to the insolubilised probe could be detected. 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 [14].

3. Results and discussion

3.1. Light scattering

The fluorescent probe, 4HP, is practically non-soluble in water but dissolves together with the polymer. Thus, the probe is expected to localise in the hydrophobic parts of the polymer and enhance the hydrophobic interactions between the macromolecular chains. The effect of the probe, and that of the method to prepare the samples, on the aggregation of the polymers was studied by DLS. All polymer solutions prepared in the indirect method (first dissolving the polymer in chloroform, and then dissolving the dried solid material in water) were observed to form aggregates in aqueous solutions at room temperature. This was the case whether the sample contained the probe 4HP or not. No aggregate formation was observed when the polymer solution was prepared by adding the polymer into water (a *direct* dissolution). The size distributions of the polymers in aqueous solutions with and without the added probe are shown in Fig. 2. These observations suggest that the aggregation is not solely due to the presence of the probe but the method of the sample preparation plays an important role in the intercatenary binding of the polymers. During the evaporation of chloroform, hydrogen bonds may form between the polar groups of the polymers. It is well known that in certain cases, extensive hydrogen bonding may render polymers insoluble.

The concentration of the probe was observed to have an influence on the size distributions of the polymer aggregates. The lower was the concentration of the probe, that narrower was the size distribution. Increasing the concentration of the probe beyond the upper limit used in this work led to more complex aggregate structures, observed as bimodal size distributions.

Fig. 3 shows the average size distribution of the collapsed aggregates at 45°C. The probe concentration was 3.33×10^{-6} M. The most significant difference between the polymers was detected in their aggregate sizes and, particularly, in the size distributions. The aggregates of PNIPA-*g*-PEO-7/29 and PNIPA-*g*-PEO-10 had very narrow size distributions. The distributions of the PNIPA-*g*-PEO-6/15 and PNIPA-*co*-GMA were broad but slightly narrower than those of the pure polymers with no added probe. An important observation was that the collapsed aggregates with no probe (not shown in the figure) showed the same order in their mean sizes as those containing the probe, PNIPA-*g*-PEO-7/29 < PNIPA-*g*-PEO-6/15 < PNIPA-*g*-PEO-10, regardless of the dissolution method. This order has been observed earlier in pure aqueous solutions with varying polymer concentrations [14]. The aggregation behaviour of the polymers is concluded to depend on the degree of grafting. The observed difference between the polymers with either six or seven grafts is striking, however, and suggests that the distribution of the grafts along the chain plays a role in determining the properties of the polymers.

4. Fluorescence studies

4.1. Fast heating of the samples

The aqueous samples were quickly heated from ambient

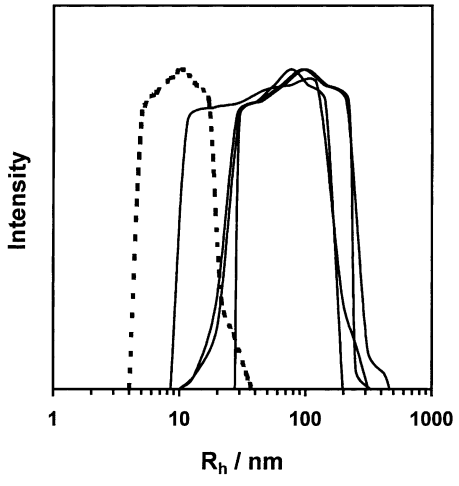


Fig. 2. Size distribution of the aggregates at 20°C in water. Polymer concentration 0.1 g/l and probe 4HP concentration 3.33×10^{-6} M. In the figure the size distribution of PNIPA-*g*-PEO-10 dissolved by the direct method without the probe is shown by a dotted line.

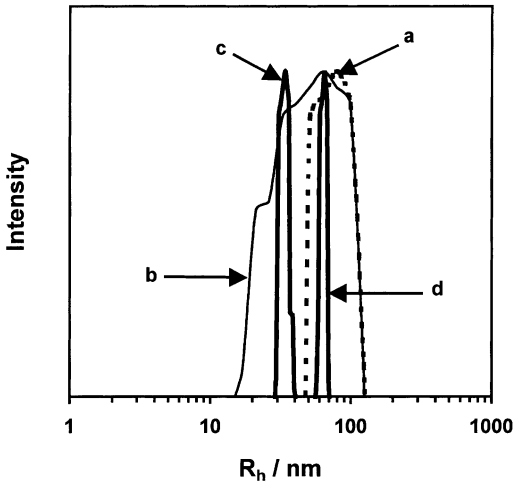


Fig. 3. Size distribution of the aggregates at 45°C in water. Polymer concentration 0.1 g/l and probe 4HP concentration 3.33×10^{-6} M. (a) PNIPA-*co*-GMA; (b) PNIPA-*g*-PEO-6/15; (c) PNIPA-*g*-PEO-7/29; (d) PNIPA-*g*-PEO-10.

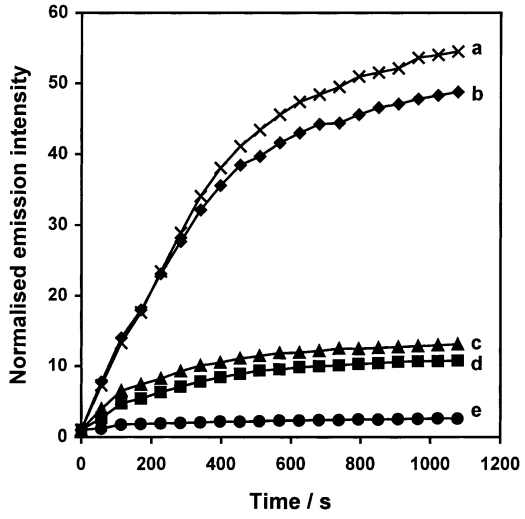


Fig. 4. Normalised maximum intensities of the emission spectra of 4HP in aqueous PNIPA-*g*-PEO-7/29 solutions with varying concentrations measured during the fast heating. Polymer concentration: (a) 1.0 g/l; (b) 0.5 g/l; (c) 0.1 g/l; (d) 0.05 g/l; (e) 0.01 g/l.

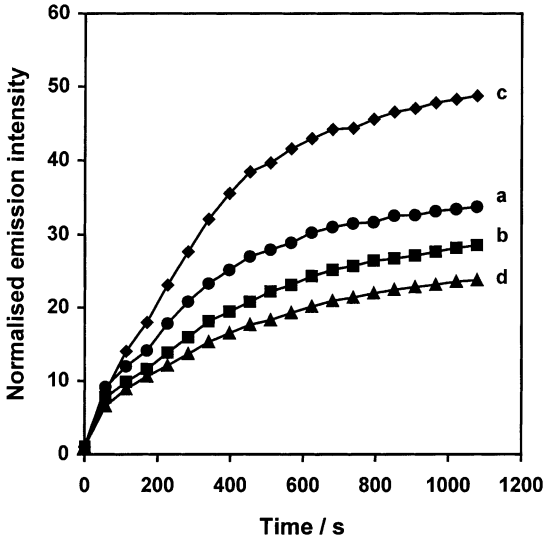


Fig. 5. Normalised maximum intensities of the emission spectra of 4HP during the fast heating of aqueous polymer solutions: (a) PNIPA-*co*-GMA; (b) PNIPA-*g*-PEO-6/15; (c) PNIPA-*g*-PEO-7/29; (d) PNIPA-*g*-PEO-10. Polymer concentration 0.5 g/l. Probe concentration 3.33×10^{-6} M.

temperature to 45°C. Normalised emission intensity maxima are shown as functions of time in Fig. 4. The maximum intensities of the spectra first increased fast, the increase slowing down with time. The intensities of the spectra increased faster with increasing polymer concentration showing that the changes in the microviscosity in the vicinity of the probe were biggest in the upper end of the studied concentration regime. The stabilisation of the collapsing polymer in the surroundings of the probe takes longer time when the polymer concentration is increased. This is because the diffusion of water out from the polymer determines the rate of collapse and an increase of the polymer concentration slows down the diffusion. Curves a and b in Fig. 4 corresponding to the solutions with the highest concentrations, differ considerably from the others. The changes in the surroundings of the probe get more intense above a certain polymer concentration. This is probably connected to the density of the aggregates, which has been shown to depend on the polymer concentration [14].

Of the studied polymers, the intensities of the emission bands were lowest in the case of PNIPA-*g*-PEO-10, see Fig. 5. PEO grafts tend to swell the polymer coil, and the collapsed aggregate of the PNIPA-PEO-10 is not as densely packed as that of the other polymers [13]. On the other hand, the increase of the emission intensity was highest in the case of PNIPA-*g*-PEO-7/29. If PEO chains in the PNIPA-*g*-PEO-7/29 are localised differently along the backbone during the grafting reaction as suggested in the introduction part, the polymer then may contain long strands of PNIPA segments not mixed with PEO. These strands should be capable of compressing more intensely than the polymers in which PNIPA and PEO are more randomly mixed. It may be concluded that the polymer grafted in an aqueous solution at a temperature close to the LCST has the highest ability to collapse. This conclusion is supported by the fact that of the grafted copolymers, only PNIPA-*g*-PEO-7/29 has maximum intensity higher than the parent polymer PNIPA-*co*-GMA.

The shift of the emission bands to shorter wavelengths upon heating was dependent on the number of PEO grafts in the polymer; PNIPA-*co*-GMA (3–5 nm) ~ PNIPA-*g*-PEO-6/15 (3–5 nm) < PNIPA-*g*-PEO-7/29 (10–13 nm) < PNIPA-*g*-PEO-10 (17 nm). PEO increases the solubility of PNIPA in water especially at low temperatures, therefore the changes in polarity due to the collapse of PNIPA become more distinct as the amount of PEO increases.

4.2. Slow heating of the samples

The samples were also heated slowly, with a heating rate 0.2°C/min. The intensities of the bands increased drastically at distinct transition temperatures of the polymers indicating an increase in the microviscosity of the probe surroundings, see Fig. 6. The transition temperatures observed in this experiment are close to those measured previously by light scattering [13]. The increase in the emission intensity is highest in the case of PNIPA-*co*-GMA, and the PEO grafts slow down the increase. Again, the increase is more moderate in the case of the polymers with six or 10 grafts than in the case of PNIPA-*g*-PEO-7/29.

The shift of the emission bands to shorter wavelengths during the compression of the polymers varied in the following series; PNIPA-*g*-PEO-6/15 (~4 nm) < PNIPA-*g*-PEO-10 (~8 nm) < PNIPA-*co*-GMA (~10 nm) < PNIPA-*g*-PEO-7/29 (~17 nm). The order is different from that observed in the fast heating experiments, i.e. the blue shift was not directly proportional to the number of the PEO

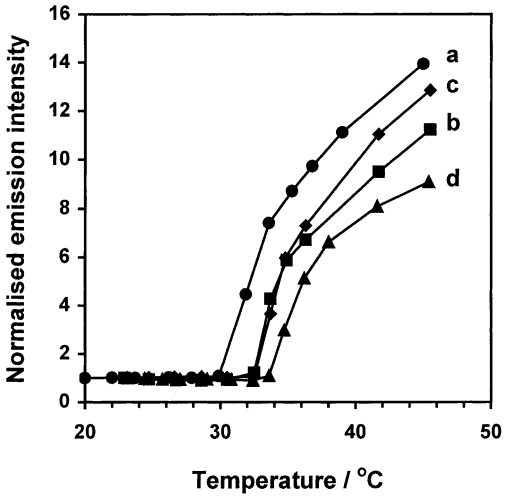


Fig. 6. Normalised maximum intensities of the emission spectra of 4HP during the slow heating of aqueous polymer solutions: (a) PNIPA-*co*-GMA; (b) PNIPA-*g*-PEO-6/15; (c) PNIPA-*g*-PEO-7/29; (d) PNIPA-*g*-PEO-10. Polymer concentration 0.1 g/l. Probe concentration 3.33×10^{-6} M.

grafts. An important observation is that of four polymers including also PNIPA-*co*-GMA, the polarity in the surroundings of the probe changes most during the compression of PNIPA-*g*-PEO-7/29. During the slow heating, the polymer aggregates have time to occupy stabile conformations. Thus, the structural differences between the polymers can be observed. PNIPA-*g*-PEO-7/29 is suggested to contain long PNIPA segments, and dense domains consisting only of PNIPA may build up inside the polymer.

5. EPR measurements

Doxyl stearates used as spin probes in this study are amphiphiles, the water solubilities of which increase with temperature. This type of sonds were expected to localise into more polar parts of the polymers than 4HP.

We have recently studied 5-DSA that was allowed to spontaneously dissolve into aqueous solutions of PNIPA-*g*-PEO-6/15 and PNIPA-*g*-PEO-7/29 (Section 2, *second series*) [14]. The rotational correlation time of the probe decreased abruptly with increasing temperature, showing that the rate of tumbling of the probe increased upon the collapse of the polymers. This was interpreted as the diffusion of the probe outwards from the collapsing polymer.

As the way of preparation of the sample solutions was observed to affect the aggregation of polymers, we compare here the previous results with those obtained using a similar dissolution method as with 4HP (Section 2, *first series*).

The way of preparing the samples was observed to determine the localisation of the probe. In the solutions where the nitroxide was allowed to dissolve spontaneously from the wall of the sample cell, the probe was found in much more polar surrounding than in the case where the polymer and the probe were first mixed together in chloroform (cf. the indirect dissolution method). In the former case the nitrogen hyperfine coupling constant was 16.4 G, in the latter it was 15.6 G. The former value is close to that typical for nitroxides in water, the latter approaches the coupling constant observed in a single crystal [30–32]. The probes 5-DSA and 16-DSA did not show any difference in their coupling constants.

Baglioni et al. have studied micelles of non-ionic detergents containing aliphatic and oligo(oxyethylene) segments [33]. They showed that a doxyl stearate is predominantly localised in the oligo(oxyethylene) part of the micelle. Correspondingly it may be concluded that in the present case the radicals are located either in the outer PEO shell of the polymers (a_N 16.4 G) or deeper inside, close to the PNIPA–PEO interface (a_N 15.6 G), depending on the way of preparing the sample.

The hyperfine coupling constants of the nitroxides did not change upon the collapse of the polymers. The nitroxides residing in a fairly non-polar surroundings showed only a typical thermal activation in the rate of their rotational motion. Nitroxides close to the aqueous phase, however, showed a discontinuous increase in the rate of rotational diffusion upon increasing temperature. The logarithmic rotational correlation times (τ_R) of the probes, estimated with the classical Kivelson formalism [34] are shown against inverse temperature in Fig. 7. It may be seen from the figure that the radical close to the water phase, the τ_R of which changes non-linearly detects differences between the

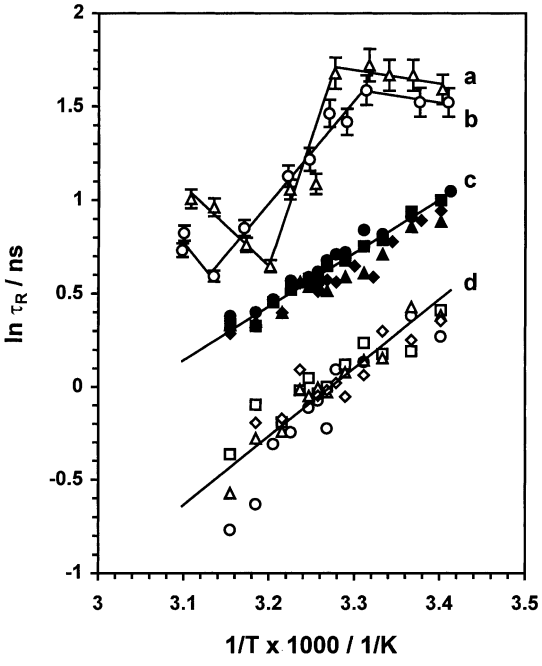


Fig. 7. Logarithmic rotational correlation times of 5-DSA and 16-DSA versus inverse temperature of aqueous polymer solutions. First series: PNIPA-*co*-GMA, PNIPA-*g*-PEO-6/15, PNIPA-*g*-PEO-7/29 and PNIPA-*g*-PEO-10 with (c) 5-DSA and (d) 16-DSA; polymer concentration 0.1 g/l. Second series: (a) PNIPA-*g*-PEO-6/15 with 5-DSA; (b) PNIPA-*g*-PEO-7/29 with 5-DSA; polymer concentration 1.0 g/l.

polymers. The probes closer to the hydrophobic core give identical results for every polymer.

It should be noted that in some cases the estimated rotational correlation times are of an order of nanoseconds. These values are too high for nitroxides, the spectra of which are clearly motionally narrowed ones. This indicates that the assumption of the isotropic rotation fails. Because the number of grafts should not affect the rotational motion of the probe it can be assumed that the data in a correct way reflects the difference between the polymers. Furthermore, the distinct difference between the samples a and b in Fig. 7 has been previously verified by viscosity measurements [14].

Comparison of the fluorescence and EPR results shows that upon the collapse of the NIPA-*g*-PEOs, the most dramatic changes are detectable in the hydrophobic core of the polymer and in the outermost part of the hydrophilic shell. Because only the fluorescent probe 4HP evidently concentrates in the core, it gives information on the densities of the hydrophobic clusters in various polymers.

6. Conclusions

The way of preparing the polymer solutions was observed to affect the degree of aggregation of the polymers. The aggregate sizes of the polymers with different 4HP concentrations were investigated by light scattering. At room temperature, any particular differences in the aggregate sizes between the polymers were not observed within the probe concentration range used. In spite of the aggregate formation at room temperature the use of fluorescent and paramagnetic sonds was considered useful because the sonds predominantly localise inside the polymer coils where they detect changes in their microenvironment.

At the transition temperatures (LCST) the intensities of the fluorescence emission spectra suddenly increased. 4HP was localised in the hydrophobic domains inside the polymers, and showed a blue shift of the emission maximum upon the collapse of the polymers, due to the decreasing polarity of the surroundings of the probe.

Fluorescence measurements conducted during the fast and slow heating of the polymer solutions showed that of the graft copolymers, PNIPA-*g*-PEO-7/29 has the strongest tendency to form firm collapsed sructures. Comparison of the results of the fast and slow heating experiments allows one to conclude that of the four studied polymers, PNIPA-*g*-PEO-7/29 formed aggregates with densest collapsed PNIPA microdomains. The only rational explanation for the observed difference between PNIPA-*g*-PEO-7/29 and the other graft copolymers is the different distribution of the PEO grafts along the PNIPA chain.

The localisation of the spin probes was different depending on the method of the sample preparation. The probes were found inside the polymers either close to the hydrophobic core or in the polar aqueous surroundings near the surface of the polymers. In the former case the rotational correlation times changed monotonically with temperature but in the latter case, a discontinuous increase in the rate of tumbling of the label was observed upon the collapse of the polymer. The label close to the aqueous phase detected differences in the hydrophilic shells of the polymers. The use of the amphiphilic spin probes confirmed the expected localisation of 4HP in the hydrophobic core of the collapsing polymers.

The results support the assumption of different distributions of the PEO grafts on the PNIPA main chain which owe to the different conditions under which the polymers have been grafted and, correspondingly, to the different conformations of the polymers during the grafting reaction.

References

[1] Wesslén B. Macromol Symp 1998;130:403.
[2] Kawaquchi S, Winnik MA, Ito K. Macromolecules 1996;29:4465.
[3] Chiu H, Chern C, Lee C, Chang H. Polymer 1998;39:1609.
[4] Talingting MR, Munk P, Webber SE, Tuzar Z. Macromolecules 1999;32:1593.
[5] Heitz C, Prud'homme RK, Kohn J. Macromolecules 1999;32:6658.
[6] Jankova K, Chen X, Kops J, Batsberg W. Macromolecules 1998;31:538.
[7] Xu B, Li L, Yekta A, Masoumi Z, Kanagalingam S, Winnik MA, Zhang K, Macdonald PM. Langmuir 1997;13:2447.
[8] Qiu X, Wu C. Macromolecules 1997;30:7921.
[9] Caldwell KD. Poly(ethylene glycol) chemistry and biological applications. Harris JM, Zalipsky S, editors. ACS, Symp Ser 1997;680:400.
[10] Harris JM, Zalipsky S. Poly(ethylene glycol) chemistry and biological applications. ACS, Symp Ser 1997:680.
[11] Uhrich KE, Cannizzaro SM, Langer RS, Shakesheff KM. Chem Rev 1999;99:3181.
[12] Bromberg LE, Ron ES. Adv Drug Delivery Rev 1998;31:197.
[13] Virtanen J, Baron C, Tenhu H. Macromolecules 2000;33:336.
[14] Virtanen J, Tenhu H. Macromolecules 2000;33:5970.
[15] Yamazaki A, Song JM, Winnik FM, Brash JL. Macromolecules 1998;31:109.
[16] Ringsdorf H, Simon J, Winnik FM. Macromolecules 1992;25:5353.
[17] Winnik FM, Davidson AR, Hamer GK, Kitano H. Macromolecules 1992;25:1876.
[18] Ringsdorf H, Venzmer J, Winnik FM. Macromolecules 1991;24:1678.
[19] Chen J, Jiang M, Zhang Y, Zhou H. Macromolecules 1999;32:4861.
[20] Noda T, Morishima Y. Macromolecules 1999;32:4631.
[21] Loutfy RO. Pure Appl Chem 1980;58:1239.
[22] Loutfy RO. Macromolecules 1981;14:270.
[23] Loutfy RO. J Polym Sci 1982;20:825.
[24] Dousa P, Konak C, Fidler V, Dusek K. Polym Bull 1989;22:585.
[25] Vatanparast R, Li S, Hakala K, Lemmetyinen H. Macromolecules 2000;33:438.
[26] Hakala K, Vatanparast R, Li S, Peinado C, Bosch P, Catalina F, Lemmetyinen H. Macromolecules 2000;33:5954.
[27] Wang ZJ, Song JC, Bao R, Neckers DC. J Polym Sci, Part B: Polym Phys 1996;34:325.
[28] Song JC, Neckers DC. J Polym Engng Sci 1996;36:3.
[29] Paczkowski J, Neckers DC. Macromolecules 1992;25:548.
[30] Winnik FM, Ottaviani MF, Bossman SH, Garcia-Garibay M, Turro NJ. Macromolecules 1992;25:6007.
[31] Winnik FM, Ottaviani MF, Bossman SH, Pan W, Garcia-Garibay M, Turro NJ. Macromolecules 1993;26:4577.
[32] Berliner LJ. Spin labeling theory and applications. New York: Academic Press, 1976.
[33] Baglioni P, Bongiovanni R, Rivara-Minten E, Kevan LJ. Phys Chem 1989;93:5574.
[34] Kivelson DJ. Chem Phys 1960;33:1094.