Poly(*N*-isopropyl acrylamide) derivatives with pendent sulfonic acid groups and nitroxide radicals

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Copolymers of *N*-isopropyl acrylamide, 2-acrylamido-2-methylpropane sulfonic acid and an acrylic ester of a nitroxide radical have been synthesized by radical polymerization. The products are polymers where the nitroxide side group is reversibly protonated by the sulfonic acid groups. The lower critical solution temperature (*LCST*) and the amount and size of the aggregates formed in aqueous solutions above the *LCST* are dependent not only on the amount of sulfonic acid groups in the polymer, but also on the pH and ionic strength of the solvent. Electron paramagnetic resonance spectra of the labelled polymers are sensitive to the rate and amplitude of the segmental motion of the polymer chain, and also to the rate of proton exchange between the sulfonic acid and the nitroxide.

(Keywords: poly(*N*-isopropyl acrylamide); sulfonic acid; spin labelling)

INTRODUCTION

Poly(*N*-isopropyl acrylamide) (PNIPAAM) is a water-soluble polymer which is being studied intensively in several laboratories. The considerable interest focused on this polymer is mainly due to its lower critical solution temperature (*LCST*) in water (32°C). Heating an aqueous solution of PNIPAAM over the *LCST* leads to phase separation. Correspondingly, a chemically crosslinked PNIPAAM swells in cold water but shrinks during heating, the gel collapse being thermoreversible. The *LCST* of PNIPAAM has been shown to be sensitive even to small amounts of additives, either copolymerized into the polymer chain or dissolved in the water phase[1].

PNIPAAM is a promising starting material for various types of responsive gels, i.e. polymer gels which respond to changes in the chemical or physical state of their surroundings. In general, a discontinuous and reversible volume change of a polymer gel may be produced by changing the solvent composition, temperature, pH or ionic strength of the system[2].

By copolymerizing *N*-isopropyl acrylamide (NIPAAM) with ionic comonomers, it should be possible to prepare polymers and gels which respond to changes in temperature as well as to changes in pH and ionic strength. The purpose of this study has been to modify the properties of PNIPAAM by introducing sulfonic acid groups into the polymer chain. 2-Acrylamido-2-methylpropane sulfonic acid (AMPS) has been used as a comonomer. Also, nitroxide radicals have been introduced into the polymer to allow the investigation of the dynamic properties of the polymer with electron paramagnetic resonance (e.p.r.) spectroscopy.

Nitroxide radicals are extremely suitable reporter groups in aqueous polymer systems. E.p.r. spectra obtainable from spin-labelled polymers are sensitive to the rate and amplitude of the reorientational motion of the label. The nitrogen hyperfine coupling constant is dependent on solvent polarity, and thus e.p.r. spectra of the nitroxide give information of the immediate surroundings of the label. Recently, Winnik *et al.*[3,4] have used the spin-labelling technique to study the phase separation and preferential solvation of PNIPAAM in water–methanol and water–tetrahydrofuran mixtures. The labelled polymers were synthesized by reacting a copolymer of *N*-isopropyl acrylamide and *N*-acryloyloxysuccinimide with an aminofunctionalized nitroxide radical. The same method has been earlier utilized to produce fluorescence-labelled PNIPAAM[5–7].

Nitroxides are known to act as inhibitors in radical polymerization[8]. If a polymer is synthesized by the radical mechanism, its spin-labelled analogues are usually prepared either by functionalizing a preformed polymer with the label or by using a comonomer containing a precursor of the nitroxide (the corresponding secondary amine) which may be turned to nitroxide by oxidation after the polymerization.

This paper describes the synthesis and properties of spin-labelled AMPS–NIPAAM copolymers. It has been observed that the acrylic ester of a nitroxide radical 2,2,6,6-tetramethyl-4-hydroxy-piperidine-1-oxyl (TEMPOL) may be copolymerized by a radical mechanism in a mixture containing AMPS and NIPAAM. The

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polymerizability is suggested to be due to the protonation of the nitroxide by sulfonic acid groups present in the reaction mixture. The products are interesting polymers acting like pH indicators: the intensity of the e.p.r. spectrum is dependent on the protonation–deprotonation equilibrium of the nitroxide group. Also, if the amount of AMPS in the polymer is low enough, the copolymer shows the phase separation typical of PNIPAAM.

EXPERIMENTAL

Synthesis of polymers

Spin-labelled copolymers were prepared which contained either 5 or 40 mol% of AMPS. These are referred to as copolymers 5:95 and 40:60, respectively. The amount of the label was kept constant, 3 mol%. These compositions were selected in an attempt to prepare two distinctly different copolymers, the properties of which are mainly determined either by NIPAAM or by the extremely hydrophilic AMPS. With increasing amount of AMPS the copolymers become sticky and somewhat difficult to isolate and purify.

Spin-labelled PNIPAAM containing approximately 40 mol% AMPS (AMPS–NIPAAM 40:60) was synthesized by a radical copolymerization. A solution of NIPAAM (3.4 g, 0.030 mol), AMPS (4.2 g, 0.020 mol) and the label, acrylic ester of TEMPOL (0.34 g, 1.5 mmol) in ethyl alcohol (51 ml) was heated to 70°C under nitrogen. Azobisisobutyronitrile (161 mg) in ethyl alcohol was added and the solution was stirred for 24 h at 70°C. After the reaction, the polymer was precipitated into diethyl ether several times. Upon filtering and drying, 6.2 g of polymer was obtained (yield 77%). The copolymer containing 5 mol% AMPS (AMPS–NIPAAM 5:95) was prepared in an analogous manner.

The chemical structure of the polymers was checked by elemental analysis, i.r., n.m.r. and e.p.r.. The main spectral characteristics of the copolymers are as follows. The chemical shifts in D_2O by ^1H n.m.r. (200 MHz): 1.1 ($-CH_3$ of NIPAAM), 1.45 ($-CH_3$ of AMPS), 1.5–2.2 ($-CH_2CH-$), 3.0–3.6 ($-CH-$), 3.7–4.0 ($-CH_2-$). I.r. (KBr): 3300, 2970, 1648, 1550, 1222, 1039, 625 cm^{-1}. The composition of the polymers was concluded to be the same as that in the feed mixtures.

The molar masses of the polymers relative to poly(ethylene oxide) standards were measured by g.p.c. using 0.1 M aqueous NaCl solution as an eluent. According to g.p.c., the copolymer 40:60 has $M_w = 17 \times 10^3$ and $M_n = 10 \times 10^3$. M_w obtained from light scattering is 20×10^3. The light scattering measurements were conducted using 0.1 M NaCl as a solvent. In this solvent the polymers show no polyelectrolyte effects; the measured Zimm plots are like those usually observed with uncharged polymers. However, the M_w measured using light scattering should be taken as an apparent value owing to the possible preferential absorption of the salt to the polymer, as well as to the copolymeric nature of the polymers. For the labelled copolymer 5:95, $M_w = 9 \times 10^3$ and $M_n = 6 \times 10^3$ by g.p.c. For reference, polymers with no label were also prepared; the molar masses are of the same order of magnitude.

Characterization of aqueous solutions

Polymers were studied in water and 0.1 M aqueous NaCl solutions, as well as in aqueous buffer solutions. Buffers were Titrisol pH 11 from Merck (boric acid/potassium chloride–sodium hydroxide buffer), and a potassium dihydrogen phosphate–sodium hydroxide buffer of pH 6.

E.p.r. spectra were recorded with a Varian E4 spectrometer. Light scattering measurements were conducted using a Brookhaven BI-2030AT instrument with 35 mW He-Ne laser.

RESULTS AND DISCUSSION

The paramagnetic centre of a nitroxide is easily destroyed by acids owing to the formation of hydroxyl amine. However, it has been shown earlier that at least some nitroxides may be protonated by strong acids. Malatesta and Ingold[9] have recorded a spectrum of protonated 2,2,6,6-tetramethylpiperidine 1-oxyl in concentrated sulfuric acid. According to these authors, the e.p.r. spectrum disappears entirely if the sample contains 54% sulfuric acid by weight, owing to the fast protonation and deprotonation of the radicals.

In the present case it was observed that the intensity of the e.p.r. spectrum of the aqueous solution of acrylic ester of TEMPOL decreases when a considerable amount of AMPS is added. With an increasing amount of AMPS, the intensity reaches a minimum but the spectrum does not totally disappear by further addition of the acidic monomer. As can be seen from *Figure 1*, the original spectrum is almost recovered when an AMPS-containing nitroxide solution is turned basic by adding sodium hydroxide. The observed difference between the original spectrum (*Figure 1*, spectrum a) and that recorded after the addition of AMPS and sodium hydroxide (spectrum e) is due to the dilution of the sample. It is noteworthy that a very large excess of AMPS is needed to reach the minimum in the intensity of the e.p.r. spectrum.

The spectra shown in *Figure 1* were recorded 15 min after each addition of either AMPS or sodium hydroxide. They show that the sulfonic acid groups of AMPS

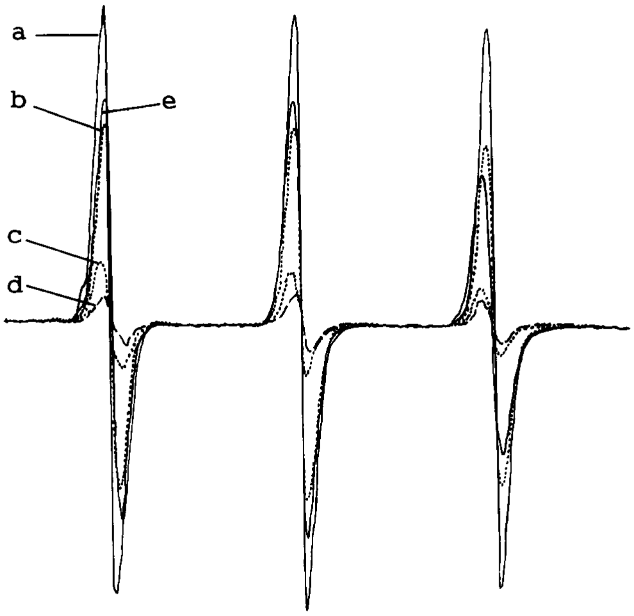

Figure 1 E.p.r. spectrum of (a) 10^{-4} M aqueous solution of the acrylic ester of TEMPOL recorded at room temperature. Concentration of added 2-acrylamido-2-methylpropane sulfonic acid: (b) 1 M; (c) 3 M; (d) 4 M. (e) Spectrum after addition of NaOH (7 mol l^{-1})

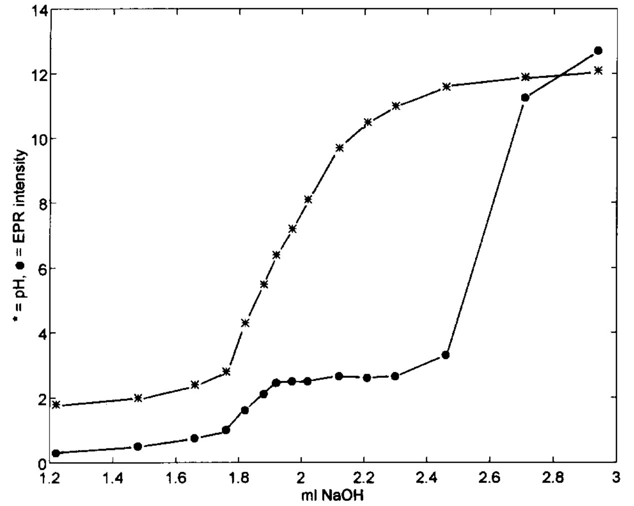

Figure 2 Titration curves of an aqueous solution (50 mg ml^{-1}, sample volume 20 ml) of the labelled AMPS–NIPAAM 40:60 copolymer. ●, Intensity of the e.p.r. spectrum (in arbitrary units); *, pH of the solution. 1 M NaOH solution was used

reversibly protonate the nitroxide. The conclusion is further supported by the results from experiments conducted using the labelled polymers.

Polymerization of AMPS increases the local concentration of the acidic groups in the polymer solution. Even in dilute solutions, nitroxide radicals attached to the polymer chain are protonated by the sulfonic acid, in spite of the fact that the overall concentration of the latter is several orders of magnitude lower than in the monomer solutions described above. This is seen surprisingly clearly by following the intensity of the e.p.r. spectra from the solutions of the labelled copolymers. When the polymers are dissolved in pure distilled water, only very weak spectra can be detected. However, by adding sodium hydroxide, the intensity of the spectra may be greatly increased. In *Figure 2*, the intensity of the central line ($m = 0$) of the e.p.r. spectrum is shown as a function of added 1 M sodium hydroxide solution for an aqueous sample of the copolymer 40:60. The pH titration curve is also included in *Figure 2*.

After the titration shown in *Figure 2*, the sample solution was acidified with hydrochloric acid, and the titration was repeated. The intensity of the e.p.r. spectra was observed to be constant over the whole pH range, evidently because the sulfonic acid was now in its sodium salt form and was thus incapable of protonating the nitroxide.

The labelled copolymers were studied by dynamic light scattering. The diffusion coefficients of the polymers were measured in various solvents, where the polymer concentration was kept constant at 20 mg ml^{-1}. *LCST* was detected as an abrupt decrease of the diffusion coefficient of the polymer as well as a considerable increase of the scattered light intensity. The average diffusion coefficient as a function of temperature is shown in *Figure 3*.

The copolymer 40:60 does not show a clear phase separation in water. Its diffusion coefficient increases with temperature up to 43°C, above which the diffusion rate seems to have a nearly constant value. This may be an indication of a conformational transition or aggregation; the hydrodynamic radius of the polymer starts to increase at temperatures above 43°C. The copolymer 5:95 shows the *LCST* at 36°C in pure water. Its critical temperature is lowest (32°C) in the basic buffer solution; in the acidic buffer as well as in the aqueous sodium chloride solution the *LCST* is 34°C. The differences are small, especially when the solutions containing low molar mass electrolytes are compared with each other. However, some conclusions may be drawn. All the low molar mass electrolytes used in the measurements shield the electric charges of the sulfonic groups and decrease the *LCST* of the polymer closer to the value reported for pure PNIPAAM in water (32°C). Evidently due to the shielding effect, in the electrolyte solutions below the *LCST* the diffusion coefficient of the polymer increases with temperature, whereas just the opposite behaviour is observed in pure water.

The finding that the *LCST* for the copolymer 5:95 is lower at pH 11 than it is at pH 6 or in pure water was not expected. If the dissociation of the sulfonic acid groups in AMPS were the only factor affecting the critical temperature of phase separation, then the *LCST* would be expected to be lowest in the acidic buffer solution. In the acidic buffer, sulfonium ions are to some extent protonated, whereas in water or in the basic buffer they are totally dissociated. This shows that the protonation of the amide nitrogen also has to be taken into account; this process is hindered only in the basic buffer solution.

To gain a further understanding about the extent of aggregation, the phase separation process was followed as a function of time by measuring the intensity of light scattered from polymer solutions at 36°C. A sample tube containing the polymer solution (AMPS–NIPAAM 5:95, 5 mg ml^{-1}) equilibrated at room temperature, was immersed in the goniometer where the temperature was kept constant at 36°C. Measurement of the scattered light intensity at the scattering angle 90° was started immediately. The methanol solution was omitted from this measurement, because it did not show any sign of phase separation (*Figure 3*).

The intensity of scattered light is shown in *Figure 4* as a function of time for four samples. Differences in the time delay before the beginning of the aggregation process are due to the observed differences in the critical temperature, and are in accordance with the data shown in *Figure 3*. The relative increase in the scattered light

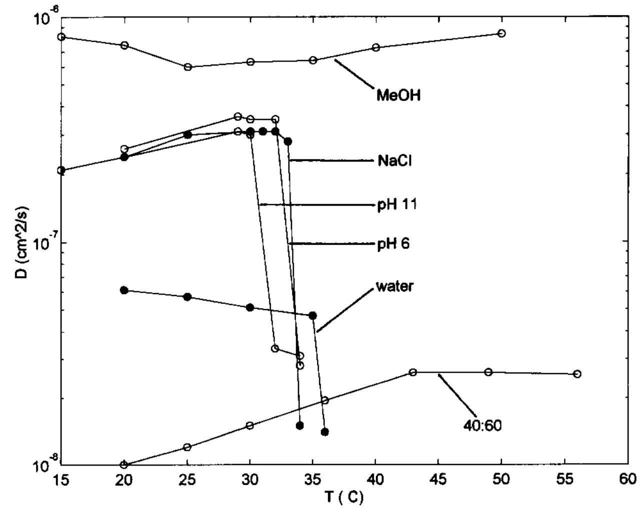

Figure 3 Average diffusion coefficient of AMPS–NIPAAM copolymers as a function of temperature in various solvents. The lowest curve represents the copolymer 40:60 in water. The other curves represent the copolymer 5:95 in water, 0.1 M aqueous sodium chloride, buffer pH 6, buffer pH 11, and in methanol

intensity differs greatly depending on the solvent. The number, and possibly also the size, of the aggregates is largest in pure water; the scattered light intensity increases by a factor of 200 during the phase separation. As a comparison, the intensity of light scattered from the polymer dissolved in the basic buffer solution increases only two-fold. The difference between the basic solution and all the other samples is remarkable in this case.

The scattered light intensity from aqueous solutions of the copolymer 40:60 also increases at elevated temperatures. The increase is only moderate, and a high polymer concentration is needed to detect it. Intensity from an aqueous sample (40 mg ml^{-1}) increases by a factor of five to eight during a 30 min measurement at either 50 or 60°C.

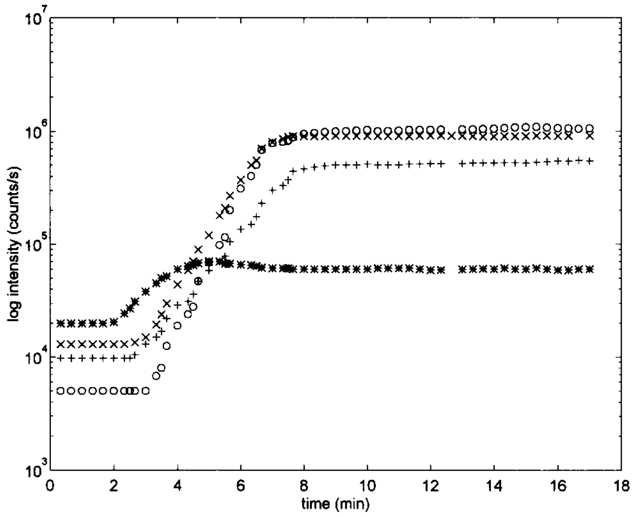

Figure 4 Intensity of scattered light (counts per second, in a logarithmic scale) from four solutions of AMPS–NIPAAM 5:95 as a function of time at 36°C. Polymer concentration 5 mg ml^{-1}. Solvents: water (○); 0.1 M aqueous NaCl (×); buffer pH 6 (+); buffer pH 11 (*)

To conclude, it has been shown that the polymer containing 5 mol% AMPS has *LCST* 4°C higher than that reported for homopolymer PNIPAAM in water. The critical temperature and the amount and size of the aggregates formed are dependent on the ionic strength and pH of the aqueous solvent. Increasing the amount of sulfonic acid groups in the polymer increases the critical temperature considerably. In the case of copolymer 40:60, signs of an aggregation process were seen at temperatures above 43°C.

The copolymer 5:95, which undergoes a phase separation very similar to that of homopolymer PNIPAAM, was further studied by e.p.r. Polymers dissolved in the buffer solutions were measured as a function of temperature, starting at 2°C. Two polymer concentrations were studied, 3 and 20 mg ml^{-1}. Some representative e.p.r. spectra are shown in *Figure 5*.

The most important feature of the e.p.r. spectra is the absence of any clear indication of aggregation above *LCST*. As reported by Winnik *et al.*[3,4], spin-labelled aggregated PNIPAAM in water produces a composite spectrum; a slow motional component appears at temperatures above 31°C. Spectra of labelled PNIPAAM were analysed as combinations of three superimposed spectra, each having a different correlation time and a tilt angle of the label.

The spectra obtained from the copolymer 5:95 are motionally narrowed, where the line width decreases with increasing temperature. Some broadening of the spectra is observed only in the more concentrated sample at the lowest temperatures. The broadening is not due to aggregation but is a phenomenon typical of labelled

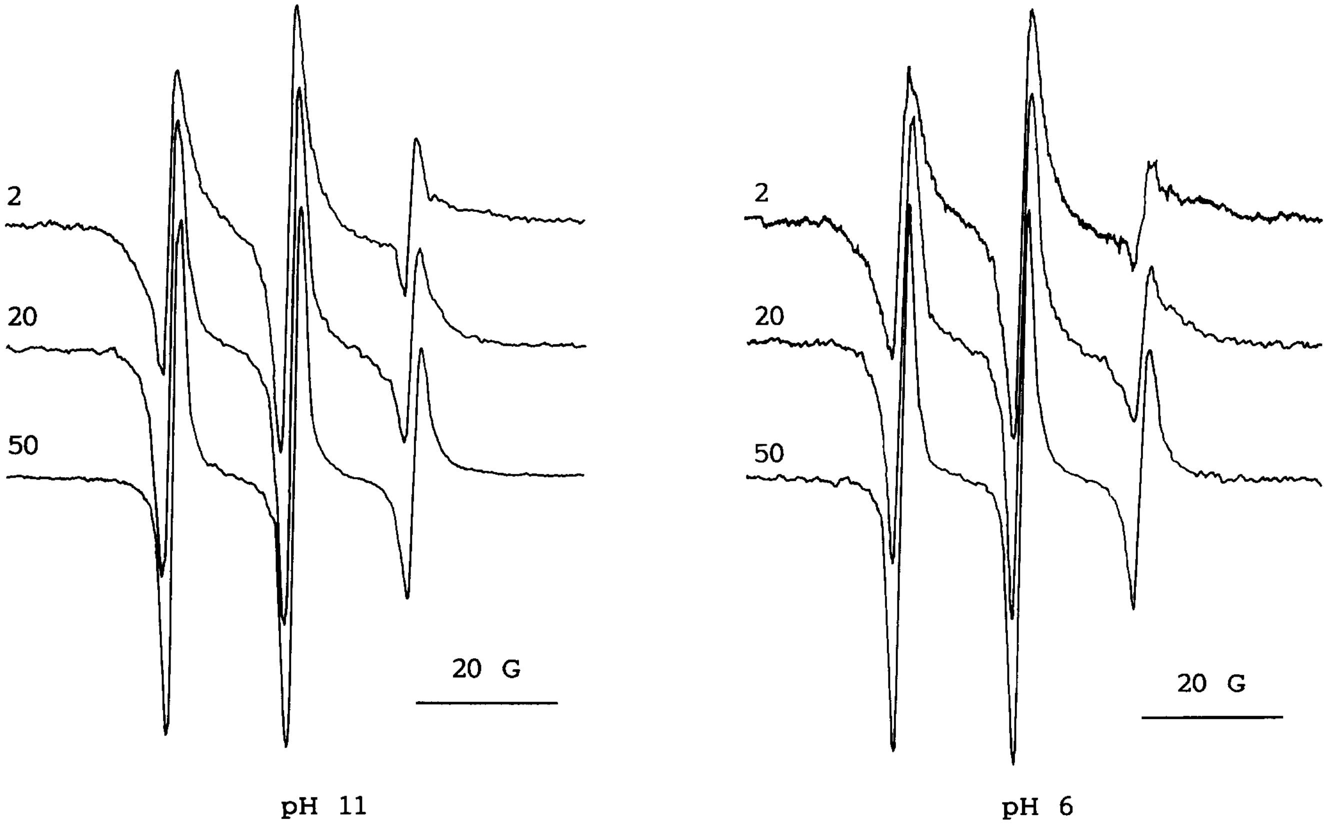

Figure 5 E.p.r. spectra of the copolymer 5:95 (20 mg ml^{-1}) in buffer solutions at temperatures 2, 20 and 50°C

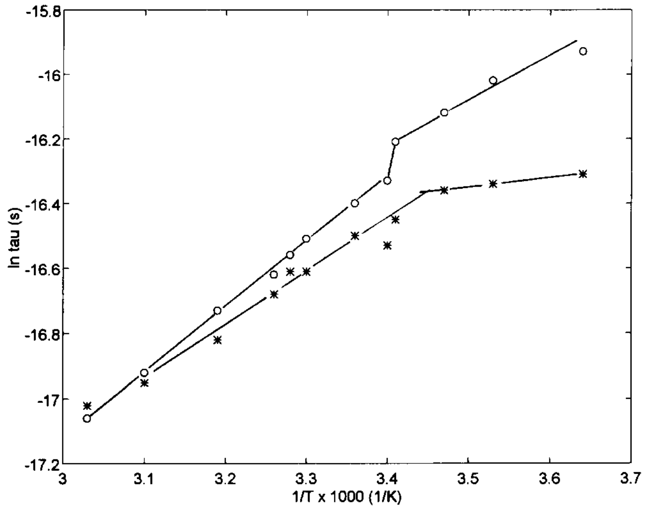

Figure 6 Logarithm of τ_R against inverse temperature for the copolymer 5:95 (20 mg ml^{-1}) in pH 6 (○) and pH 11 (*)

polymers; the origin of this phenomenon is in the fast reorientational motion of the label with respect to the slow diffusion of the macromolecule. In the e.p.r. spectra of labelled polymers, two wide outer peaks usually appear with increasing viscosity of the solution[10].

The spectra shown in *Figure 5* raise a question about the effect of molar mass on aggregation. One could suggest that the motionally narrowed spectra are due to the low molar mass; this point is to be studied further. However, it is also reasonable to think that the observed characteristics of the spectra are due to the presence of the sulfonic acid groups, which strongly interact with the nitroxides. The acid groups are capable of keeping the labels out of the hydrophobic clusters formed during the aggregation. This suggestion is supported by the observed constancy of the nitrogen hyperfine coupling constant over the whole measured temperature range.

It is worth noting that the molar masses of the labelled polymers and those containing no nitroxides, prepared in an analogous manner, are of the same order of magnitude. Thus the low molar mass is not due to the presence or absence of nitroxide radicals in the polymerization reaction mixture. The dependence of the molar mass on the amount of initiator, as well as on the molar ratio of the comonomers, will be the subject of further studies.

Dilute polymer solutions with different pH values are somewhat difficult to compare because of the low intensity of the spectra of the acidic samples. A simple way was chosen to analyse the spectra: rotation of the label was assumed to be isotropic and the rotational correlation times (τ_R) were calculated from the intensities of the three lines using the conventional Kivelson formalism[11]. As a result, Arrhenius type plots are obtained by representing logarithmic τ_R *versus* inverse absolute temperature. The activation energy of the rotational motion of the label, obtained from these plots, may be used to decide whether or not the label can detect the changes in the polymer conformation. Results for the concentrated samples (20 mg ml^{-1}) are shown in *Figure 6*. The concentrated samples give reproducible spectra from which the correlation times may well be calculated. The dilute solution gives qualitatively the same results although the correlation times naturally have lower values.

From *Figure 6* it can be seen that the calculated points do not fall on a single straight line, but the acidic and basic solutions behave in a different way. In both cases the data are best fitted by two straight lines, i.e. one representing the low temperature regime (below *LCST*) and the other, temperatures above *LCST*. The fact that the straight lines intersect around room temperature (not *LCST*) may be due to several reasons. It may just indicate that the motion of the label is too simply described by only one (isotropic) correlation time, but more importantly, it probably shows that the reorientation of the label is affected by the rate of proton exchange between the sulfonic acid group and the nitroxide. Dependence of the proton exchange on temperature is in this case seen also as a steady increase of the intensity of the e.p.r. spectra with increasing temperature.

The activation energies of the label rotation in the low temperature region are 10 and 2.5 kJ mol^{-1} at pH 6 and pH 11, respectively. The corresponding values at higher temperatures are 16.5 and 12.5 kJ mol^{-1}. The difference between the two samples is pronounced at low temperatures; the rotation of the label is more restricted in the acid solution where the nitroxide is effectively protonated.

CONCLUSIONS

It has been shown that 2-acrylamido-2-methylpropane sulfonic acid can reversibly protonate a nitroxide radical and thus allow a radical polymerization in the presence of nitroxides, which usually act as inhibitors.

Spin-labelled poly(*N*-isopropyl acrylamide) derivatives containing various amounts of 2-acrylamido-2-methylpropane sulfonic acid as a comonomer, are polymers which are sensitive to the temperature, pH and ionic strength of the surrounding solvent. They undergo a change in conformation at a certain temperature, dependent on the composition of the polymer as well as on the solvent. If the amount of AMPS is low enough, a phase separation similar to that of homopolymer PNIPAAM can be detected at *LCST*. E.p.r. spectra of these polymers are sensitive not only to the segmental motion of the polymer but also to the rate of proton exchange between the sulfonic acid and the nitroxide.

The spin labelling method has been shown to be useful in the case of linear polymers, and will be utilized in future in the characterization of the gels prepared from derivatives of PNIPAAM.

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