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Spin-Labeled Polyelectrolyte Gels Based on Poly(*N*-isopropylacrylamide). Effects of the Network Structure and the Gel Collapse on the EPR Spectra

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ABSTRACT: The swelling and collapse of aqueous copolymer gels of *N*-isopropylacrylamide and 2-acrylamido-2-methylpropanesulfonic acid have been studied by EPR spectroscopy using spin-labeled polymers. The swelling of the gels is strongly affected by added electrolytes and pH of the aqueous solvent. The EPR spectra from aqueous solutions of the linear polymer are motionally narrowed three-line spectra at temperatures below and above the critical temperature. Spectra from the gels are composites, i.e. they contain also a broad slow motional component, and their shapes are dependent on temperature as well as on the cross-link density. The intensities of the EPR spectra are suggested to be affected by the protonation of the radical label. Changes of the spectral intensity with temperature may be used to detect conformational changes of the polymer.

Introduction

Responsive polymer gels are systems which undergo an abrupt collapse during a gradual change in the state of their surroundings. The gel collapse, a sudden decrease of the volume of the gel, may be induced by a change in temperature or in the solvent composition, or even by the application of an electric field.[1,2]

Poly(*N*-isopropylacrylamide) (PNIPA), has been studied extensively as a starting material for thermally responsive gels which might be used e.g. in drug delivery systems.[3] It has been applied also in various other systems, like thermosensitive coatings for polymer particles[4] and in graft copolymers.[5] The widespread use of PNIPA is at least partly due to its very convenient lower critical solution temperature (LCST), which is 32 °C in water. When *N*-isopropylacrylamide (NIPA) is polymerized with multifunctional monomers in water, the architecture of the products may be varied by the reaction temperature. Polymerization below the critical temperature produces clear homogeneous gels, whereas the gels synthesized above the LCST are turbid owing to the heterogeneity of the polymer network.

When NIPA is copolymerized with monomers containing dissociating groups, charged polymers are obtained which are sensitive not only to temperature but also to the pH and ionic strength of the aqueous solvent. In our recent study, linear copolymers of NIPA and 2-acrylamido-2-methylpropanesulfonic acid (AMPS) were investigated by light scattering and electron paramagnetic resonance (EPR) spectroscopy.[6] It was shown that small amounts of sulfonic acid in the polymer chain change the LCST of the aqueous solution; the degree of aggregation above the critical temperature changes as well.

In the EPR technique, stable nitroxide radicals are utilized as labels to detect changes in the segmental dynamics of the polymers. The EPR spectra of nitroxides are sensitive to the rate and amplitude of the motion of radicals; because these radicals are easily soluble in water, they are ideal label groups in aqueous systems. In addition, information on the polarity of the surroundings of the label may be obtained from the spectra. In aggregating aqueous polymer systems, this information may be used to follow the changes of the microenvironment of the label.[7]

Winnik and Ottaviani[8,9] have studied spin-labeled linear PNIPA in water and mixed solvents. The EPR spectra measured from aqueous polymer solutions showed clearly the change in polymer conformation at the LCST. Below the critical temperature, motionally narrowed three-line spectra were obtained, whereas the spectra measured above 32 °C were combinations of motionally narrowed and broad slow motional EPR spectra.

The behavior of a spin-labeled copolymer of NIPA and AMPS differs from that of the homopolymer PNIPA. The conformational change and aggregation of the polymer do not produce any dramatic changes in the shapes of the three-line spectra. This has been interpreted as an indication of an intimate interaction between the nitroxide and sulfonic acid side groups of the polymer. The water-soluble side groups are evidently kept in an aqueous environment, on the surface of the polymer coil during the collapse of the individual chains.[6]

The intensity of the EPR spectrum of an aqueous solution of the labeled AMPS−NIPA copolymer is dependent on pH of the solvent. The pH dependence of the spectral intensity varies with varying amount of sulfonic acids in the polymer, which implies that the intensity is determined by the interaction of the radical with the acid groups. Our experimental evidence supports a hypothesis that the sulfonic acids may reversibly protonate the nitroxides. If this really is the case, then the rate of protonation and deprotonation of the label radical should affect the intensity of the spectrum.[6]

It has been shown earlier that at least some nitroxides may be protonated by strong acids. Malatesta and Ingold have recorded a spectrum of protonated 2,2,6,6-tetramethylpiperidinyl-1-oxy in concentrated sulfuric acid. According to these authors, the EPR spectrum disappears entirely if the sample contains 54% sulfuric

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acid by weight, owing to the fast protonation and deprotonation of the radicals.[10]

This paper describes the differences in the EPR spectra of linear and cross-linked labeled AMPS−NIPA copolymers. The amount of AMPS has been kept low (5 mol % of the monomer feed) and thus the changes of the spectral intensities with varying pH of the solvent are less remarkable than with polymers containing higher amounts of AMPS. Instead, the focus is on the temperature dependence of the shape and intensity of the EPR spectra.

The purpose of the paper is to show how temperature and solvent composition affect the swelling and collapse of the AMPS−NIPA copolymer gels and, especially, how the gel collapse changes the EPR spectra of the spin-labeled gels.

Experimental Section

Syntheses of the Polymers. Gels for the swelling tests were prepared at two different temperatures to obtain both heterogeneous and homogeneous samples.

Heterogeneous Gels. A mixture of monomers NIPA and AMPS, with a molar ratio 95:5, was dissolved in water to obtain a 15 wt % solution. Two samples were prepared, with 2 and 5 mol % of the cross-linking monomer *N*,*N*′-methylene bisacrylamide, respectively. After deaerating the solutions with nitrogen gas, ammonium persulfate (1 mol % of the feed) was added and the solutions were injected into cylindrical molds with a diameter of 16 mm. Polymerization was carried out at 60 °C for 20 h. After the reaction, the gels were cut into 3 mm slices.

Homogeneous Gels. The monomers NIPA and AMPS, the molar ratio of which was 98:2, were dissolved in water. *N*,*N*′-Methylenebisacrylamide (1 mol %) and 1.3 mol % of an accelerator, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED), were added. The solution was bubbled with nitrogen, the initiator (1 mol % ammonium persulfate) was added, and the solution was injected between two glass plates separated by 3.8 mm spacers. The polymerization was conducted at room temperature for 18 h. The resulting gel was cut into disks with a diameter of 14 mm, washed thoroughly in water, and dried.

Labeled Linear Polymers. Copolymers with nitroxide radicals as side groups were synthesized using two methods in order to find out the possible effects of the microstructure on the polymer behavior. In the first, a one-step method, NIPA and AMPS (mole ratio 95:5) were dissolved in ethanol together with 3 mol % of an acrylic ester of TEMPOL (2,2,6,6-tetramethyl-4-hydroxy-piperidinyl-1-oxy). AIBN was used as an initiator. The procedure is the same as that used in our previous experiments except the amount of initiator was only half of that used earlier.[6] In a second synthesis, a two-step method was used where NIPA and AMPS were copolymerized with 4 mol % of *N*-acryloxysuccinimide. After the polymerization the product was allowed to react with amino-TEMPO (2,2,6,6-tetramethyl-4-aminopiperidinyl-1-oxy) to obtain the labeled polymer. The synthesis was conducted as described by Winnik and Ottaviani.[8,9] Both syntheses gave polymers with $M_w = 30\,000$.

Labeled Cross-Linked Polymers. The one-step method described above was used in the syntheses of the gels. NIPA and AMPS were used in the molar ratio 95:5. Gels with the cross-link densities 2 and 5%, respectively, were prepared with *N*,*N*′-methylenebisacrylamide as a cross-linker. The polymers were labeled with the acrylic ester of TEMPOL (3 mol % of the feed). Potassium persulfate was used as an initiator, and the polymerization was conducted in water at 60 °C for 18 h.

Swelling Measurements. The heterogeneous gels were allowed to swell in water, aqueous 0.1 M NaCl solution, and buffer solutions with pH 6 and 11. The 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. The degree of swelling at various temperatures was investigated using a video camera coupled with an image analyzing system which allowed the diameter of the gel cylinders to be measured.

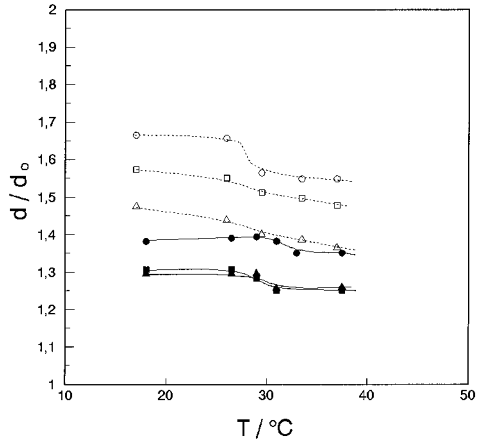

Figure 1. Swelling of the heterogeneous gels in various solvents. Open symbols refer to the gel with 2 mol % cross-linker, filled symbols to that with 5 mol %. The solvents are buffer with pH 6 (circles), buffer with pH 11 (triangles), and 0.1 M aqueous NaCl solution (squares). d_0 is the initial diameter of the gel cylinder and d is the diameter at a certain temperature.

The homogeneous gels were swelled in the same solvents as the heterogeneous ones. Because of better mechanical strength of the homogeneous samples, it was possible to measure the swelling ratio by weighing the samples equilibrated at a certain temperature. The swelling ratios were calculated as the weight of absorbed water per weight of dried polymer disk, $(w_{\text{wet gel}} - w_{\text{dry gel}})/w_{\text{dry gel}}$).

EPR spectra were recorded with a Varian E4 spectrometer. An attempt was made to simulate the spectra using the program by Schneider and Freed.[11] For that, the spectra were first digitized; this also facilitated the integration of the spectra.

Results and Discussion

The turbid heterogeneous AMPS−NIPA gels which were obtained by polymerization above the critical temperature were very soft and showed a high capability of swelling. Gel samples were swollen in buffered and saline aqueous solutions. The degree of swelling was observed to be dependent not only on the degree of cross-linking but also on the nature of the solvent. Accurate measurement of the swelling ratios by weighing the samples turned out to be difficult owing to the softness and poor mechanical strength of the gels. An attempt was made to construct the swelling/shrinking curves by measuring the diameter of gel cylinders at various temperatures. Results are shown in Figure 1 in terms of d/d_0, where d is the diameter of the gel at a given temperature and d_0 is the initial diameter of the gel. The gels with varying cross-link densities swelled more in the acidic than in the basic buffer; the swelling was most pronounced in water. However, in water the surfaces of the gels became too diffuse to be measured accurately, and this data was omitted. The values shown in the figure are of the same order of magnitude as what has been reported for copolymer gels of NIPA and acrylic acid,[12] but much higher than the values reported for PNIPA gels.[13] Only the initial part of the curves describing the gel collapse is shown in Figure 1, because the gels started to break into pieces during the collapse.

To get a better understanding of the solvent effects, homogeneous gel samples were prepared which slightly differed from the heterogeneous ones in their chemical composition. Samples with much better mechanical

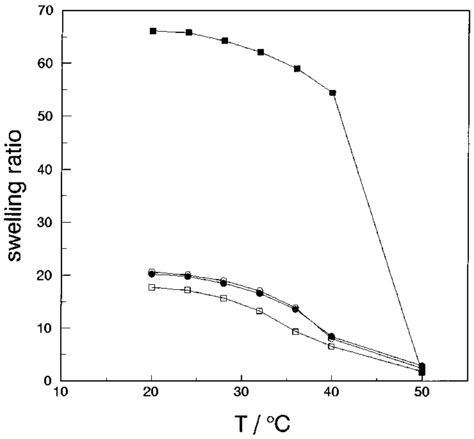

Figure 2. Swelling and collapse of the homogeneous gels. The swelling ratio is expressed by the weight of absorbed water per weight of dry polymer. Solvents are water (■), 0.1 M NaCl (○), buffer with pH 6 (●), and buffer with pH 11 (□).

strength were obtained by lowering the molar ratio of AMPS to 2 mol % and by conducting the polymerization at room temperature after adding an accelerator (TEMED) to the reaction mixture containing the monomers and an initiator. The swelling and shrinking of these samples could easily be followed by weighing the samples at various temperatures. The swelling ratio, expressed as the mass of absorbed water divided by the mass of the dry polymer, could then be plotted against temperature. The swelling ratios for a homogeneous gel with the content of the cross-linker equal to 1 mol % are shown in Figure 2. In water the swelling ratio is high and the transition to the collapsed state is discontinuous whereas in solutions with added electrolytes the gel swells considerably less. As in the case of heterogeneous samples, the gel swells more in the acidic than in the basic buffer.

The swelling and shrinking data of the gels are of importance when studying the EPR spectra of the spin-labeled gels. In the EPR measurements, heterogeneous gels with AMPS content of 5 mol % were used. Because the EPR technique gives information on the local dynamics of the polymer chains, the low mechanical strength of the samples does not complicate the spectroscopic measurements.

The EPR spectra of the linear and cross-linked samples change in a different way with temperature. Spectra from the solutions of the linear polymer retain their motionally narrowed shape through the whole temperature range studied, up to 75 °C. Two batches of the labeled linear polymer were synthesized (see Experimental Section). The polymers were observed to behave identically with increasing temperature; i.e. the EPR spectra of aqueous samples were identical. The polymerization method may affect the microstructure of the polymers; this experiment was conducted to ensure that the overall chemical composition of the copolymers is the most important factor determining the shapes of the spectra.

Spin-labeled gels with the higher cross-link density (5 mol % cross-linker) produce composite spectra throughout the measured temperature interval from 20 to 75 °C. Gels with the lower content of the cross-linking monomer (2 mol %) change from narrow three-line spectra to broadened composite ones at temperatures close to that reported as the LCST of the linear polymer. Even in the latter case, the spectra measured at room temperature are not typical motionally narrowed EPR spectra of nitroxides but show certain signs of polymer aggregation. Some representative spectra of the gels are shown in Figure 3.

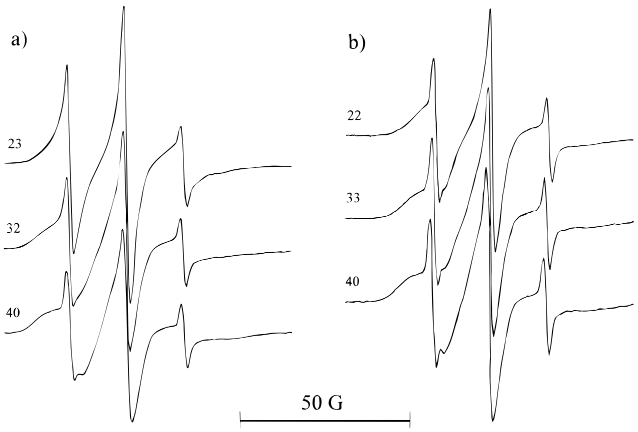

Figure 3. EPR spectra of spin-labeled heterogeneous gels at temperatures shown for each spectrum (in °C): (a) gels with 2 mol % cross-linker; (b) gels with 5 mol % cross-linker. The gels were swelled in the buffer with pH 11.

During the measurements, care was taken to use always identical amounts of the cross-linked polymer. A constant, excess amount of solvent was used to allow the gel to swell into its equilibrium. Using the same instrumental settings during each measurement, spectra were recorded which enabled one to compare not only the relative intensities of the individual lines but also the total integrated intensities of the spectra.

The changes in the shapes of the spectra show certain similarities with the observed macroscopic changes of the gels. When the temperature of the sample is increased, the intensities of the narrow lines increase but reach a maximum around the LCST. If the cross-link density is moderate, the slow motional spectral component appears upon passing the critical temperature. After this, during further heating the outer wide peaks increase in intensity whereas the intensity of the sharp lines changes in a more complex way. To describe the relative changes in the line intensities, parameters characterizing the slow motional (I_s) and motionally narrowed (I_f) components of the low-field line were used; the parameters are defined in Figure 4.

The peak intensities were normalized using I_f at 20 °C as a reference; in other words, for every sample I_f at 20 °C was given a value 1. Relative intensities of the lines are shown in Figures 5−7 for the gels with varying cross-link densities in various solvents.

The common feature in Figures 5−7 is that the intensity of the motionally narrowed peak passes through a maximum at around 30 °C. With increasing temperature, the slow motional component in the spectra increases in intensity owing to the slow collapse of the gels. The addition of salt does not affect considerably the shape of the spectra; more important factors seem to be pH of the solvent as well as the cross-link density.

It is evident that the composite structure of the EPR spectra is caused by the network structure. In a cross-linked polymer it is not possible for all of the polar side groups to be in an aqueous environment. The denser is the network structure, the larger is the number of nitroxide labels trapped in a viscous environment with a high concentration of network chains. So far, our attempts to simulate the spectra using the widely used program by Freed[11] have not been successful. Simulations were carried out by combining a slow motional and

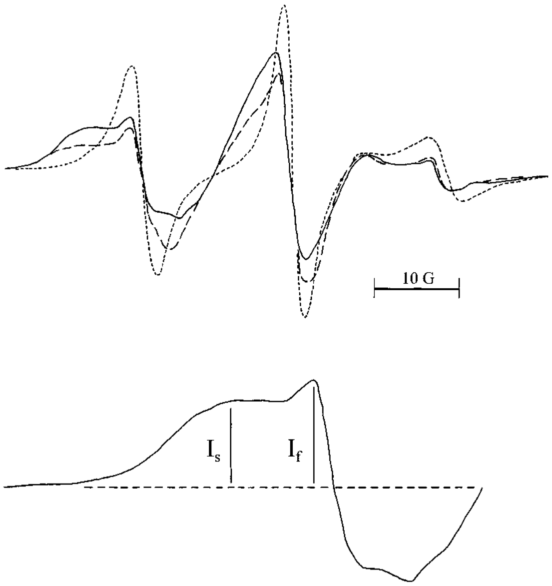

Figure 4. EPR spectra of a gel containing 2 mol % of the cross-linking monomer swelled in water. Spectra measured at 20 (dotted line), 50 (dashed line), and 70 °C (solid line). Below the spectra, the low-field line is enlarged in order to define the parameters I_s and I_f, which describe the slow and fast motional components of the spectra, respectively.

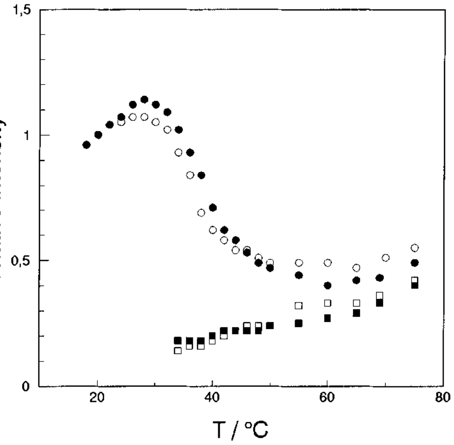

Figure 5. Relative intensities of the slow and fast motional spectral components against temperature. The points are obtained from I_s and I_f data by giving a value 1 for I_f at 20 °C. Sample is the gel with 2 mol % cross-linker swelled in water (filled symbols) and 0.1 M NaCl (open symbols). I_f is represented by circles, I_s by squares.

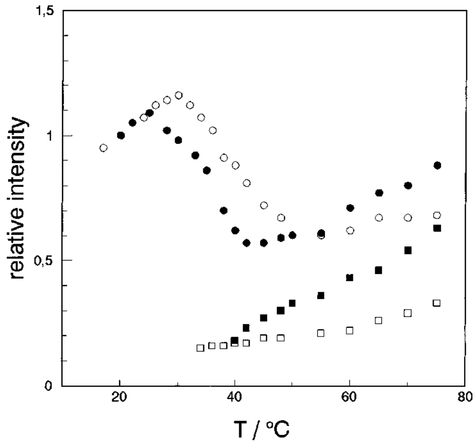

Figure 6. Comparison of the relative intensities (see text in Figure 5) from the gel with 2 mol % cross-linker swelled in the acidic buffer (filled symbols) and in the basic buffer (open symbols).

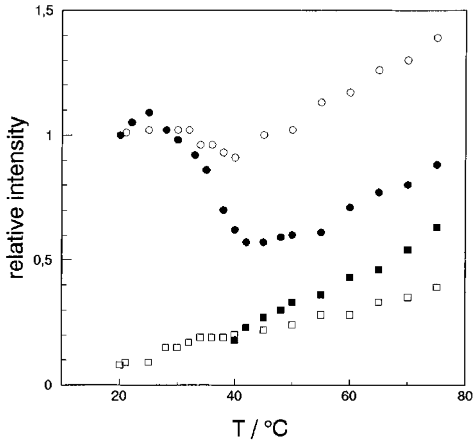

Figure 7. Relative intensities from two gels swelled in the acidic buffer. Filled symbols refer to the sample with 2 mol % cross-linker, open symbols to the denser network with 5 mol % cross-linker. Circles represent the normalized I_f, squares the normalized I_s data.

a motionally narrowed spectrum. It turned out, however, that spectra measured above the critical temperature are composites of much more than only two spectral components; several slow motional spectra should be used to construct a certain experimental spectrum. This severely complicates the simulation and indicates that in a collapsed gel the label radicals reside in various different environments where the restriction of the motion of the radicals varies. This fact can actually be seen by inspecting for example the low-field line of the broad spectrum in Figure 4. The slow motional component of the line is exceptionally broad and flat because it is a sum of several maxima.

One indication of the location of the label is the nitrogen hyperfine coupling constant of the radical, a_N. As may be seen in Figure 4, a_N decreases during the gel collapse. This has not been observed in solutions of linear copolymers. In cross-linked samples the change in the coupling constant is clearly observable in aqueous, saline, and basic solvents; in the acidic buffer the differences, if any, are below the accuracy of the measurement. Decrease of a_N in the gel samples shows that during the transition even the radicals with high mobility are drawn in an environment with a polarity lower than that of their aqueous surroundings below the LCST.

The protonation of a nitroxide attached to an AMPS–NIPA copolymer may be understood in terms of a high local concentration of sulfonic acid groups around the label. The observed temperature dependence of the intensity of the EPR spectra must then be due either to a change in the rate of the proton exchange or to the conformational change of the polymer, which could affect the distance between the side groups. Because the data in Figures 5–7 indicate that the conformational freedom of the polymer has an effect on the changes in the line intensities, this has been studied more closely by using the integral intensities of the spectra.

At constant temperature the intensity of an EPR spectrum is determined by the spin concentration in the sample and, as stated above, in the present case also by the rate of proton exchange between the nitroxide and sulfonic acid groups. When the intensity is measured as a function of increasing temperature, it is affected also by several other factors including the physical state of the sample. This means that care must

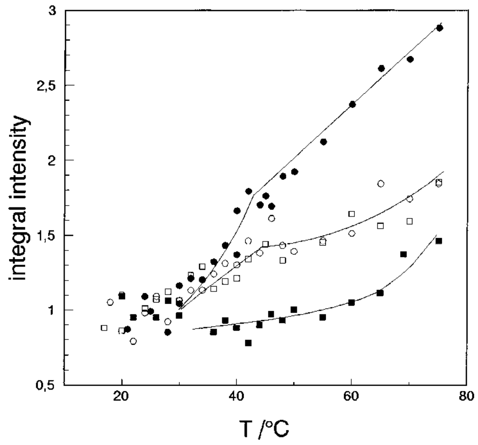

Figure 8. Integral intensities of the EPR spectra from the gels with 2 mol % cross-linker. The polymer was swollen in water (■), basic buffer (□), 0.1 M NaCl (○), and acidic buffer (●).

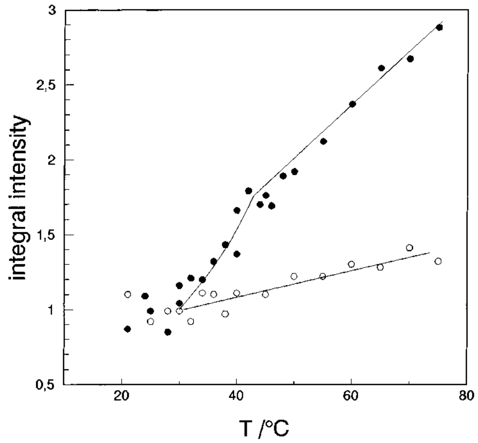

Figure 9. Integral intensities of two gels with varying cross-link densities. Both polymers were swollen in the acidic buffer. Filled circles represent the gel with 2 mol % cross-linker (reproduced from Figure 8), open circles the denser gel with 5 mol % cross-linker.

be taken not to overinterpret the data, which may contain a certain variance. The integrated intensities of the spectra are shown against temperature in Figures 8 and 9. Also these data have been normalized. Because the differences in the integrals were small and random below 30 °C, in each sample series the integral intensity at a given temperature was divided by the arithmetic average of the values obtained below 30 °C. In this way, the points in Figures 8 and 9 scatter around a value 1 at low temperatures. The lines in the figures are drawn somewhat freely to guide the eye; it is evident, however, that the intensity is not a linear function of temperature in any of the samples.

Figure 8 shows that all the added electrolytes change the temperature dependence of the spectral intensity. In water, where the gels swell more than in the other solvents and where the gel collapse is most striking, the intensity of the EPR spectra changes only moderately with temperature when compared to the other samples with the same cross-link density. The increase of the intensity of the spectra is thus not only due to the compression of the gel which might lead to an increase of the effective spin concentration in the spectrometer cavity. Because the curves representing the samples in salt and buffer solutions are not continuous but merely show a discontinuity around 40 °C, it is obvious that at least in these solvents the polymer conformation affects the intensities of the spectra.

That the increase of the intensity is strongest in the acidic solvent is probably because the acidic environment favors the protonation of the label. Gel samples with varying cross-link densities were studied and compared by measuring the spectra from the gels swollen in the acidic buffer. The integrated intensities of these samples are shown against temperature in Figure 9. The distinction between the two samples is clear, indicating that the topological differences in the polymer structure have a major impact on the protonation−deprotonation equilibrium of the label. In the sample with 5 mol % of the cross-linking monomer, the changes in the chain conformation with temperature are limited to such an extent that the deprotonation of the label is no more effective. It turns out that in the specific case of the spin-labeled AMPS−NIPA copolymers the EPR spectra may be used to monitor the conformational changes of the polymers in a way which has not, to our knowledge, been utilized so far. The proton exchange between the nitroxide and the acid groups close to the label is a process dependent not only on temperature or the solvent but also on the polymer conformation.

Conclusions

It has been shown that the introduction of 2-acrylamido-2-methylpropanesulfonic acid into a PNIPA network increases the temperature of the gel collapse when compared to the gels of pure PNIPA. Further, the noticeable effect of added electrolytes and pH on the swelling and collapse of the gels has been demonstrated.

The EPR spectra of the spin-labeled cross-linked copolymers are sensitive to the degree of cross-linking. Spectra from linear polymers are motionally narrowed below and above the critical temperature; the broadening of the spectra owes to the network structure and to the compression of the gel above the LCST.

Temperature-dependent changes in the gel samples have been studied by following the intensity of the components of the low-field line in the EPR spectra of the nitroxides. In gels with 2 mol % of the cross-linking monomer the intensities of the lines start to change right after the temperature has been raised above the LCST. In the gels with a higher degree of cross-linking, the changes are much less pronounced. Also the total integrated intensities of the spectra were followed with increasing temperature. The increase of the intensity is dependendent on solvent as well as on the cross-link density. Discontinuities in the curves representing the integral intensities against temperature occur usually above 40 °C.

The methods used in the present investigation show clearly how the conformational freedom of the polymers is reduced with the network formation. The intensity of the EPR spectrum has been observed in this case to reflect the polymer conformation.

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