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Raman Spectroscopic Characterization of Association and Thermoreversible Gelation in Aqueous Systems of Poly(*N*-acetamidoacrylamide)

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ABSTRACT: Polymer self-association and thermoreversible gelation in aqueous solutions of poly(*N*-acetamidoacrylamide) (PAAA) have been investigated by Raman spectroscopy. We found that aqueous solutions of PAAA, even at low concentrations, revealed the presence of polymer−polymer coordination, suggesting formation of polymer clusters. The effect of denaturant (sodium thiocyanate) on the inter- and intramolecular interactions in the system has been also studied. It is shown that the SCN ions destroy the macroscopic hydrogen-bond network of water, as well as reduce the polymer's ability for self-association by occupying the NH groups. The changes observed in the Raman spectra upon the gel transition show that in the gel state the equilibrium of the system is shifted toward polymer−polymer coordination, whereas in the solution it is mostly polymer−water coordination. The influence of the different factors such as polymer concentration, level of denaturant addition, and type of solvent on the gel formation is discussed.

Introduction

In recent years, a great deal of effort has been devoted to understanding the associative behavior of many water-soluble polymers.[1−6] This type of polymer has found importance in various biomedical and pharmaceutical applications. To describe the structure and dynamical behavior of systems consisting of associating units (monomers or macromolecules) able under certain conditions to form the infinite cluster, i.e., the gel fraction, constitutes a classical problem of polymer physics.[7] The formation of clusters or associative networks may be a result of attractive forces such as hydrogen bonding and/or hydrophobic interaction between the network chains.

In a recent light scattering study,[8] the structural and dynamical properties of dilute aqueous solutions of the associating polymer poly(*N*-acetamidoacrylamide), PAAA, have been investigated with and without added denaturant. Since there are no specific sites on the polymer chain available for the formation of intermolecular hydrophobic associations (see Figure 1), we can consider this macromolecule as a hydrophilic associating homopolymer. In semidilute solutions of PAAA in water this system forms a thermoreversible gel:[8,9] at elevated temperatures, this system is a moderately viscous solution, which upon cooling can be transformed to a transparent gel. The light scattering measurements[8] revealed the evolution of aggregates, and these aggregates were disrupted upon addition of excess amounts of the hydrogen-bond-breaking agent sodium thiocynate. It was argued that the attractive forces responsible for the formation of clusters are due to hydrogen bonding between the amide groups on the polymer backbone.

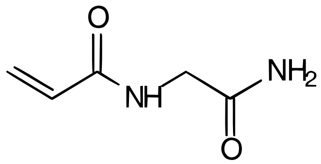

Figure 1. Illustration of the structure of the monomer *N*-acetamidoacrylamide.

In the present work, we have studied local structural changes during the sol−gel transition and during association processes of semidilute solutions by means of Raman spectroscopy. Generally, the vibrational spectroscopy provides unique information about the system on a molecular level and thus constitutes a complement to the dimensional scale probed in an elastic light scattering experiment. In addition, Raman spectroscopy is an effective tool for the study of hydrogen bonding in different type of materials,[10−12] in particular, for structural analysis of and characterization of the interactions in water-soluble polymers.[1] The effect of denaturant on association and gelation processes has been surveyed.

The general aim of this paper is to gain further insight into the role played by hydrogen bonds in the association and gelation processes of this type of polymer system. In addition, taking into account that the mechanism of hydrogen-bond formation in the system under consideration (through CO and NH groups) is close to that in biological objects, the observations made in the present study may be of interest for the problem of protein folding and denaturation.

Experimental Details

Sample Preparation. The PAAA was prepared according to the standard procedures. The details for the synthesis and polymerization of N-acetamido acrylamide have been described elsewhere,[8] and the structure of the monomer is shown in Figure 1. On the basis of the stoichiometry of the components and the polymerization conditions, the molecular weight of the

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Table 1. Characteristic Data of the PAAA Samples

sample no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
PAAA (wt %)	0.79	1.19	0.80	1.19	0.49	1.39	0.50	1.39	1.19	1.19	4.01	4.01	1.18	4.00
NaSCN (M)	0	0	2	2	0	0	2	2	0	2	0	2	5	2
solvent	H_2O	H_2O	H_2O	H_2O	H_2O	H_2O	H_2O	H_2O	D_2O	D_2O	H_2O	D_2O	H_2O	H_2O
gel temp[a] (°C)	38	52		37	−	55	−		60	42				

[a] $T_{gel} \pm 2$ °C; − indicates no gel formation, open cell indicates no measurement.

polymer was estimated to be approximately 500 000. The polymer formed during this process is known to exhibit a rather broad molecular weight distribution with a polydispersity index (M_w/M_n) usually[8] about 1.5.

Samples were prepared by weighing appropriate amounts of polymer (PAAA) and solvent (H_2O/D_2O/NaSCN solutions), followed by swelling of polymer (24 h) and repeated rapid heating to liquid solutions (~70 °C) under stirring until homogeneous solutions were obtained. The compound NaSCN (Merck) was used as a denaturant in these experiments. The characteristics of the PAAA samples used in this work are given in Table 1, together with the approximate gel temperatures. The gel point was determined as the temperature at which a sample in a test tube showed no flow when turned upside down,[13] and it is evident from Table 1 that gel temperature depends on polymer concentration, amount of denaturant, and type of solvent (H_2O or D_2O). We are aware of that the method used in the determination of the gel point is crude, but it is sufficient for the present purpose, namely to establish whether the measurements are performed in the sol or the gel state.

To carry out detailed band assignment, the sample of dry deuterated PAAA has been prepared by double dissolving of a protonated form of PAAA in heavy water (Aldrich, 99.9%) following with drying in a vacuum at about 80 °C.

Experimental Setup. Raman spectra were recorded with a Dilor-Labram confocal monochromator equipped with a 1800 lines/mm holographic grating and a Peltier cooled CCD detector. The 638 nm line of an He−Ne laser was used as an excitation source. Spectral resolution was set to 2 cm^{-1}. Each spectrum presented in this work is an average of at least 25 scans.

For all samples, Raman spectra were taken at two different temperatures corresponding to the gel (10 °C) and the liquid state (60 °C for PAAA concentrations 0.5−1.5 wt %; 75 °C for higher concentrations). Temperature control was provided by a THMS600 stage (Linkam Scientific Instruments Ltd.) operated with a TMS 93 controller; temperature accuracy was about 0.5 °C.

Results and Discussion

Raman Spectra. Polymer Band Assignments. Despite the simple structure of the PAAA monomer (see Figure 1), the correct assignment of the observed spectra is a complex procedure. First of all, it should be noted that, among the body of the literature devoted to the vibrational spectra of different kinds of amide and amine compounds (both experimental and theoretical investigations),[14−24] very few studies are concerned with the polymer forms of these materials.[14,15] In addition, theoretical calculations of potential energy distribution[16−20] show that even in a single amide-like molecule most of the spectral bands are overlapped or originate from a mixture of several vibrations. However, since the main goal of the present study is to characterize the gel-forming processes, which are believed to be due to the hydrogen bonding, our following investigation will be focused on the spectral evidences of the NH (ND) groups participating in hydrogen bonding.

Figure 2 compares Raman spectra of dry PAAA in hydrated and deuterated forms, and it is immediately seen that spectra are quite different, although the only difference between two compounds is the NH → ND substitution. Below we will analyze the whole spectral interval on the basis of known (reported) theoretical and experimental data on different amine/amide systems.[14−24]

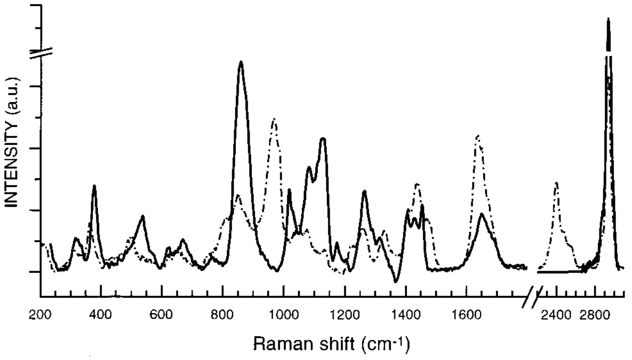

Figure 2. Raman spectra of solid PAAA in protonated (solid line) and deuterated (dashed line) forms.

The spectral region *below 800 cm^{-1}* corresponds mostly to the polymer skeletal vibrations[14,15] and is expected to be practically insensitive to the isotopic effect. Indeed, spectra of both forms of polymer look the same in this region, in agreement with reported predictions. The minor changes observed with deuteration reflect the complicated nature of the spectral bands in polymers, namely that each spectral band involves the contributions from several different vibrations. Thus, the small downward frequency shift of some lines in this region with isotopic substitution can be attributed to the participation of NH/ND (or even CN) vibrations in the skeletal modes.[16−18]

The frequency interval *800−1500 cm^{-1}* is the most interesting and informative with regard to determination of NH fingerprints. At the same time a band assignment in this region is most difficult, since it contains a number of basically mixed and/or overlapped vibrations.

It is seen from Figure 2 that the most striking change occurring with deuteration is the significant decrease of intensity of the band around 850 cm^{-1}, along with the appearance of a new strong band centered at 975 cm^{-1}. We also note essential damping of the series of the bands between 1000 and 1200 cm^{-1}, while in the interval 1380−1500 cm^{-1}, assigned to the different nonstretching CH_2 vibrations with possible participation of various types of CN and CC stretching,[17,18] the changes are quite small.

In accordance with numerous reports on spectral transformations with isotopic substitution in amide-like compounds,[16,18,19] several bands will be shifted from their positions and fall into the spectral region between 900 and 1100 cm^{-1}. It is important to stress that due to the mixing of different vibrational modes one may observe the shift toward both lower and higher frequencies. Thus, for the deuterated systems the region 900−1000 cm^{-1} was reported[18,19] as characteristic for ND_2 bending and rocking vibrations coupled with CN stretch and C═O bending. It is also seen from Figure 2 that even after deuteration in the spectral regions 800−900 and 1000−1150 cm^{-1}, there are some “remainders” which are evidences of the different CC and CN modes not affected by deuteration.[16−19]

On the basis of the above considerations, we can establish for each material the spectral regions where NH (ND) groups have an essential contribution. For the H-form of the polymer these are the bands around 800−900 cm^{-1} and the interval between 1000 and 1150 cm^{-1}, whereas for the D-form it is mostly the band centered at 975 cm^{-1}.

It should be noted that all the literature assignments proposed above were made (theoretically and experimentally) mainly for simple molecules of different structure, and these models are quite reliable for the description of CN, C═O, and NH vibrations. However, the frequency values for all C−C like modes will be significantly different for simple molecules and polymers. Therefore, to describe these modes correctly, one should take into account the assignments made for the corresponding polymers.[14,15] Thus, studying the polyacrylamide gels with varying monomer/comonomer ratio, Bansil and Gupta[15] have found that the region 800−900 cm^{-1} includes significant contribution of C−C vibrations of the side chains of polymer, while in the region 1000−1150 cm^{-1} it is mostly due to C−C vibrations of the polymer skeleton.

The amide I mode (1550−1750 cm^{-1}) is primarily the C═O stretch, but it can contain small variable amounts of other vibrations such as CN and CCN stretching and NH bending.[18] Due to the latter, the isotopic substitution is expected to lower the frequency of this mode. Indeed, in the D-form of the material the main peak of amide I band is slightly shifted toward lower frequencies (about 8 cm^{-1}), which is in a good agreement with previously reported data.[20,21]

The region *above 2200 cm^{-1}* (2700−3100 cm^{-1} for the protonated and 2200−3100 cm^{-1} for the deuterated form) is characteristic for different types of CH and NH (ND) stretching vibrations. The observed spectral changes in this envelope show a good correspondence to those reported in the literature for different amides.[14−19,22]

It should also be stressed that similar spectral behavior at isotopic substitution, i.e., the appearance of a new band at 950 cm^{-1}, spectral changes in the region 1000−1600 cm^{-1}, and stability of the band around 800 cm^{-1} (polymer skeletal vibrations), were also confirmed in IR absorption experiments (not shown).

The Role and Behavior of NaSCN. In general, the role of the denaturants in the hydrogen-bond reconfiguration, particularly in the case of protein denaturation in aqueous solutions, is still far from clear.[25] Two mechanisms were proposed: the direct binding of the molecules to the polymer (protein) or indirect action by a change of the hydrogen-bond structure of water.[26−28] It was also suggested that both mechanisms are operating.[25] However, it has been suggested recently[29] that the denaturant does not function as a structure breaker as was previously supposed in order to explain the denaturation mechanism.[28] On the basis of theoretical calculations, Wallquist et al.[29] proposed the “outside-in” mechanism of denaturation, where the denaturant molecules give rise to swelling of a protein that causes, in turn, the onset of water into the interior of a macromolecule, leading to the destabilization of the native state and denaturation. Although this approach has some experimental support,[30] the general conclusion about unchanging of the water structure with denaturant addition seriously contradicts numerous experimental findings.[25,28] Therefore, it is extremely important to understand the behavior of NaSCN and its action on the polymer chains.

The vibrational spectrum of thiocyanate ion has three fundamental frequencies:[31−33] ν_1 (C−N stretching) at 2065 cm^{-1}, ν_2 (bending) at 470 cm^{-1}, and ν_3 (C−S stretching) at 750 cm^{-1}. Both C−S and C−N stretchings are reported to be sensitive to the complex formation,[32,33] and therefore the knowledge about their spectral behavior in each certain system can bring information about the SCN ion configuration.

Figure 3 shows the temperature behavior of C−S stretching band for different samples. All the spectra of the gels in Figure 3 were normalized using the intensities of polymer bands in the range 1200−1500 cm^{-1} which were unchanged during the gel transition (see below), while the spectra of aqueous solution were normalized using the intensity of the $\nu_2(H_2O)$ band (around 1640 cm^{-1}) which is stable in the considered temperature range.[34]

It is important to stress that observed frequency of C−S stretching vibration (750 cm^{-1}) is higher than that of free SCN$^-$ ion (735 cm^{-1}),[31,32] suggesting that thiocyanate anions are involved in the interaction. Taking into account that most common ion complexes of dissolved thiocyanate salt (contact ion pairs and dimers) are usually unstable in water,[32] it is reasonable to suppose that SCN$^-$ ions are hydrogen bonded with water molecules.

It is seen from Figure 3 that in the case of aqueous solution (the uppermost plot) a temperature decrease from 60 to 10 °C causes only a minor frequency shift and a very small intensity decrease of the considered band. It is interesting to note that the same behavior was observed for a wide range of salt concentrations (0.5−8 M), implying that even at such concentrations the salt is completely dissolved. In the presence of PAAA, however, spectral transformation of the discussed band with temperature becomes more significant. While minor frequency shift is still observed for all samples, the intensity decrease with lowering temperature is more obvious, suggesting that there is interaction between PAAA chains and CS groups of the thiocyanate ion. Unfortunately, the large scattering of the experimental data from sample to sample does not allow us to carry out any quantitative evaluation of this behavior, but the difference in the state of SCN$^-$ anion in the presence of PAAA is obvious.

It should also be noted that no differences in the spectral profiles between aqueous solutions with and without polymer were observed for CN stretching band (~2060 cm^{-1}), supposing that SCN$^-$ anions are coordinated to the PAAA chains by CS groups. This last observation is consistent with previously reported data on the SCN$^-$ coordination in different solvents[32] and agrees with the general findings for S-bonded thiocyanate complexes.

It was also interesting to check the effect of NaSCN on hydrogen-bond structure of water. Figure 4 compares polarized Raman spectra of water and NaSCN aqueous solutions of different concentration in the frequency region of the OH stretching vibrations. The Raman spectrum of water (curve a) is well-known and has been (and still is) intensively investigated.[34−37] In the frequency region 2700−4000 cm^{-1} two intensive wide bands centered at about 3200 and 3400 cm^{-1}, respec-

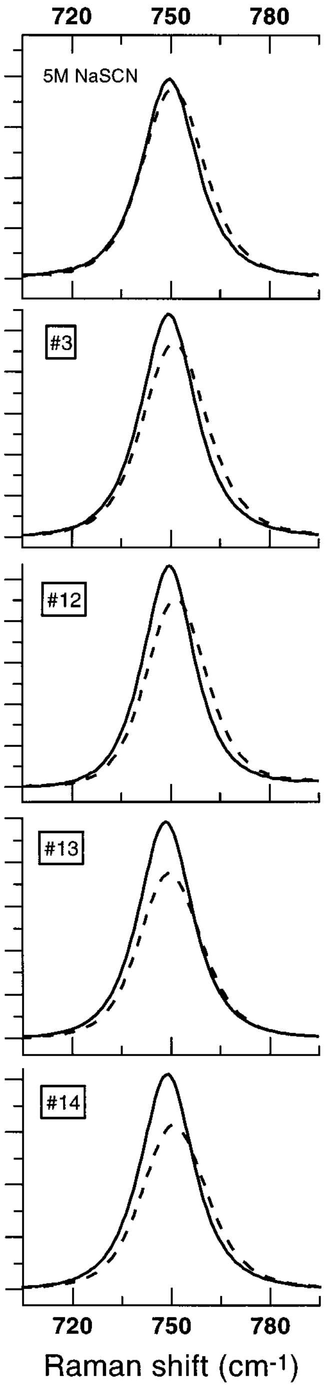

Figure 3. Raman spectra of different systems in the envelope of thiocyanate ion CS stretching vibrations: (- - -) 10 °C; (—) 60 or 75 °C, see text. Labels correspond to those given in Table 1.

tively, are present. The latter band, assigned to the symmetric stretching vibrations of hydrogen-bonded water molecules (ν_1(H_2O)),[34,35] has a high-frequency shoulder at 3600 cm^{-1} which is attributed to the asymmetric H_2O stretching vibrations (ν_3(H_2O)) and/or vibrations of free (non-hydrogen-bonded) OH oscillators.[35] The band at 3200 cm^{-1} contains the contributions from H_2O bending vibration overtone (2 × 1600 cm^{-1})[35] and the vibrations of an extent continuum of water molecules connected by hydrogen bonds (so-called "collective mode" of water).[36,37] The last concept is now well developed, and the change of the intensity of this band was used to characterize the degree of "collectivization" of water molecules in different systems such as polymer gels[36] and polymer solutions.[37] In particular, it was shown that the intensity of the collective band is proportional to the size of compartmentalized water clusters[36] and that the analysis of the OH stretching vibration in Raman spectra may be applied to define the relative hydrophilicities of water-soluble polymers.[37]

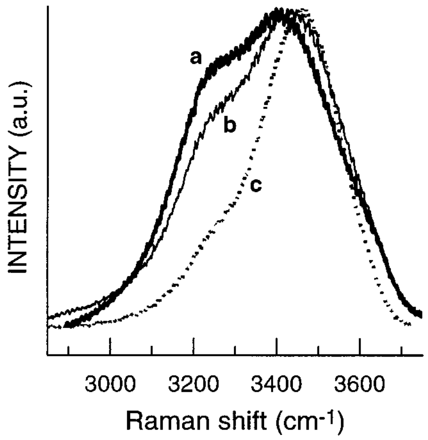

Figure 4. Polarized Raman spectra of water and aqueous NaSCN solutions in the frequency region of OH stretching vibrations: a, water; b, 2 M solution; c, 8 M solution. $T = 60$ °C.

If we consider the Raman spectrum of thiocyanate aqueous solution (curves b and c), it is immediately seen that the intensity of the collective mode is essentially lower than in water. This trend may be attributed to the disruption of the continuous macroscopic hydrogen-bond network by SCN^- ions, which completely conforms with the known facts about water behavior in different solutions.[38] Another important feature that can be noted from Figure 4 is that the peak of H_2O symmetric stretching vibrations (around 3400 cm^{-1}) demonstrates progressive shifts toward higher frequencies with increasing salt concentration (up to 40 cm^{-1} for 8 M concentration). This is a clear evidence of the total weakening of the intermolecular hydrogen bonds between water molecules, in support of the previously made conclusion. In addition, the Raman spectrum of pure water has a quite pronounced shoulder in the region around 3600 cm^{-1}, which is usually assigned to the vibrations of non-hydrogen-bonded (or very weakly bonded) OH oscillators. At the same time, spectra of thiocyanate solutions do not show such a shoulder. This feature may be explained by hydrogen bonding of free OH groups to SCN^-.

Polymer Dissolution and Effect of Polymer Concentration. Figure 5 presents Raman spectra of solid and dissolved PAAA, and it is seen that no crucial spectral transformations happen upon dissolving the polymer. For the protonated form we only note an intensity decrease in the spectral regions characteristic of NH vibrations (800−900 and 1000−1150 cm^{-1}). In the case of deuterated material there is an intensity redistribution between the skeletal modes at 750−900 cm^{-1} and visible alteration of the C═O stretching band at 1600−1700 cm^{-1}. The center of gravity of the latter band shifts toward lower frequencies upon polymer dissolving, and this observation is the same as that reported previously for polyacrylamide.[14] This is interpreted as a weakening of the polymer−polymer interaction upon dissolving.[14] A more detailed discussion of the spectral behavior of the amide I band is given below.

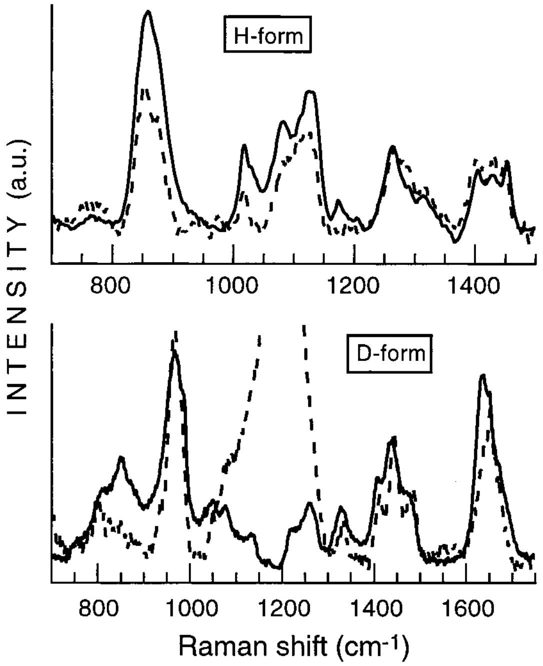

Figure 5. Comparison of the Raman spectra of solid (solid line) and dissolved (dashed line) PAAA. For PAAA solution: $T = 60$ °C; polymer concentration of 1.2 wt %.

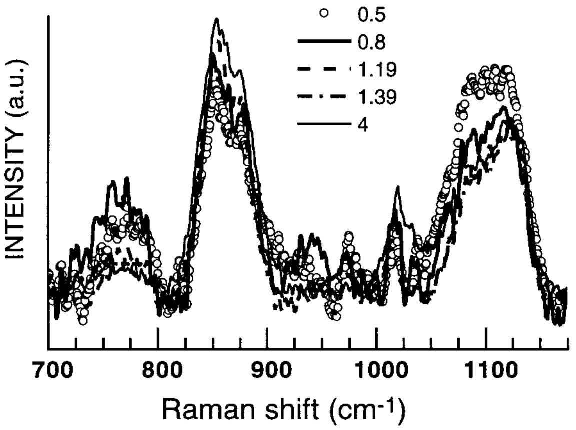

Figure 6. Raman spectra of aqueous PAAA solutions in the liquid state. Legend shows the polymer concentration in weight percent.

Comparing PAAA solutions of different concentrations (Figure 6), it is seen that spectral changes are quite small, although several general tendencies may be noted as the polymer concentration increases: (a) decrease of skeletal vibrations band around 780 cm^{-1}; (b) increase of the intensity of the band around 870 cm^{-1}; (c) decrease of the band in the region 1050−1150 cm^{-1}. Moreover, the observed changes are not linear with concentration, so that the effect is more pronounced when going from very low to intermediate concentrations (0.5−1 wt %), whereas with further increase of PAAA content (1−4 wt %) all mentioned spectral changes are significantly weaker. It should be noted, however, that the system with 0.5 wt % polymer concentration does not form a gel, whereas all other samples (PAAA concentrations higher than 0.8 wt %) have a gel transition (see Table 1). From a general point of view, the predominant condition for gel formation is that the concentration of polymer in the solution must be sufficient to create the transient network or to establish the connectivity necessary for the evolution of the gel network. As the polymer concentration increases, the entanglement couplings become progressively more important, and a connected gellike network may be created[39] in the sol phase. In this case, the transition from the sol to the gel phase should be less drastic. In light of these aspects, the nonlinear spectral transformations with polymer concentration may be due to the difference in general state of the system, i.e., separated polymer clusters at low concentration versus interpenetrating or entangled polymer network at higher concentrations.

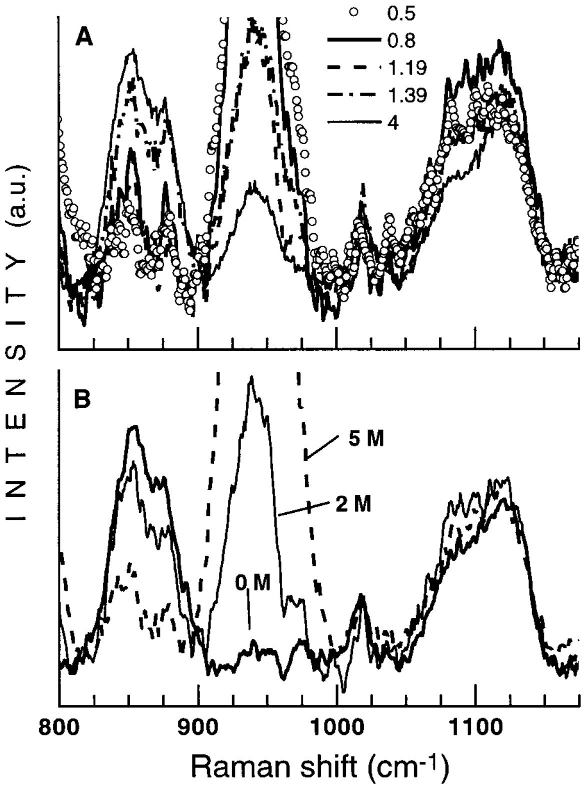

Figure 7. Raman spectra of denatured PAAA solutions in the liquid state: (A) varying the polymer content (see the inset legend) at a fixed NaSCN concentration (2 M); (B) varying NaSCN concentration at a fixed PAAA concentration (about 1.2 wt %). Note: the intensive band around 950 cm^{-1} is due to overtone of SCN bending vibration ($2\nu_2$).[33] Since this band does not show any specific behavior, it is not discussed in this work.

In contrast, turning to [2 M NaSCN−water] PAAA solutions (Figure 7A), a different behavior is demonstrated. As the polymer concentration increases at fixed NaSCN concentration, we note a small decrease of the relative intensity of the band around 1050−1150 cm^{-1} (similar to that in aqueous solutions, Figure 6), but the increase of the band at 870 cm^{-1} is much more pronounced in the present case than without denaturant. Furthermore, the relative intensity of the latter band exhibits a gradual concentration dependence. This finding supports the conjecture that the presence of SCN$^-$ ions essentially alters the coordination of the polymer chains.

The last statement should be explained in more detail. Since the probed volume in the Raman experiments is macroscopic on the molecular scale, the resulting spectrum will describe the state of the extended continuum of the polymer network, taking into account all the characteristic features of this ensemble such as the mutual coordination of macromolecules, etc. If we

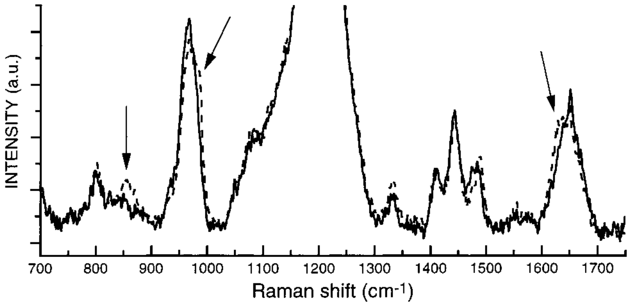

Figure 8. Raman spectra of sample 9 (1.2 wt % PAAA in D_2O) in the sol and gel states.

compare the general spectral profiles observed for the different systems under consideration (namely, the relative intensities of the different groups of spectral bands), it is seen that in the case of aqueous solutions (Figure 6) it is close to that observed in solid polymer (Figure 5). This observation can be understood taking into account that dissolved PAAA forms clusters,[8] which, in conformity with the solid material, are characterized by dominant polymer−polymer interaction. In contrast, the general spectral profile of denaturated systems (Figure 7A) essentially differs from that of solid material, suggesting thereby that the state of the polymer chains is now significantly changed. In this case, the network connectivity is probably not established. However, upon raising the polymer concentration, the Raman spectrum becomes very similar to that of pure aqueous solution (compare spectra for the systems of 4 wt % concentration in Figures 6 and 7A). On the other hand, this system forms a gel at lower temperatures (see Table 1) and should be characterized by a connected polymer network with strong polymer−polymer interactions.

Summarizing all experimental observations discussed above, we may conclude that aqueous solutions, even at fairly low concentrations, have a structure similar to that of the polymer in the gel phase, while the networks in denaturant-containing systems are less “connected” and are transformed to the gel-like structure only in systems of higher polymer concentrations.

In Figure 7A we observe the effect when the PAAA concentration changes at fixed NaSCN content (2 M). It is instructive to know that the same effect appears when the polymer concentration is kept constant, and the level of NaSCN addition is varied instead (Figure 7B). This means that in reality it may be better to consider the ratio between PAAA and NaSCN concentrations rather than their absolute concentrations.

Changes in Polymer Coordination with Temperature. Spectral changes with gelation are best seen in the case of D_2O solutions. Figure 8 presents the spectra of the sample 9 (~1.2 wt % PAAA in D_2O) in sol (60 °C) and gel (10 °C) state. There are three spectral changes occurring with gelation (marked with arrows): (i) increase of the band around 850 cm^{-1} (primarily including side chains vibrations) which can be interpreted as a formation of a rigid polymer network; (ii) intensity redistribution in the spectral regions characteristic for ND bending and rocking vibrations (around 960 cm^{-1}); (iii) intensity redistribution in the amide I spectral region (C=O stretch; around 1650 cm^{-1}).

The last two changes should be discussed in more detail since both ND and CO groups participate in the hydrogen-bond formation. Figure 9 depicts the Raman spectra of different systems in the spectral envelop of ND vibrations. All spectra were normalized using the intensity of the spectral region 1300−1600 cm^{-1} which is not affected by gelation (see Figure 8). It is immediately seen that in the transition from sol to gel the profile of the considered band is significantly changed (see the plot marked as no. 9). An inspection of the spectra for the sol and the gel states reveals an intensity redistribution between the two spectral components of the band. Moreover, the character of this redistribution is similar to that observed when the polymer is dissolved (cf. the uppermost plot). In the presence of NaSCN (2 M) however, this effect is significantly smaller (no. 10), suggesting that some of the ND groups are strongly interacting with SCN anions and do not change their state with temperature. Upon further increase of the PAAA concentration at fixed NaSCN content, the effect becomes more pronounced (compare nos.10 and 12), but it is still smaller than in pure aqueous solution. All these facts support the hypothesis that the SCN ions obstruct the gelation process by binding to the ND groups.

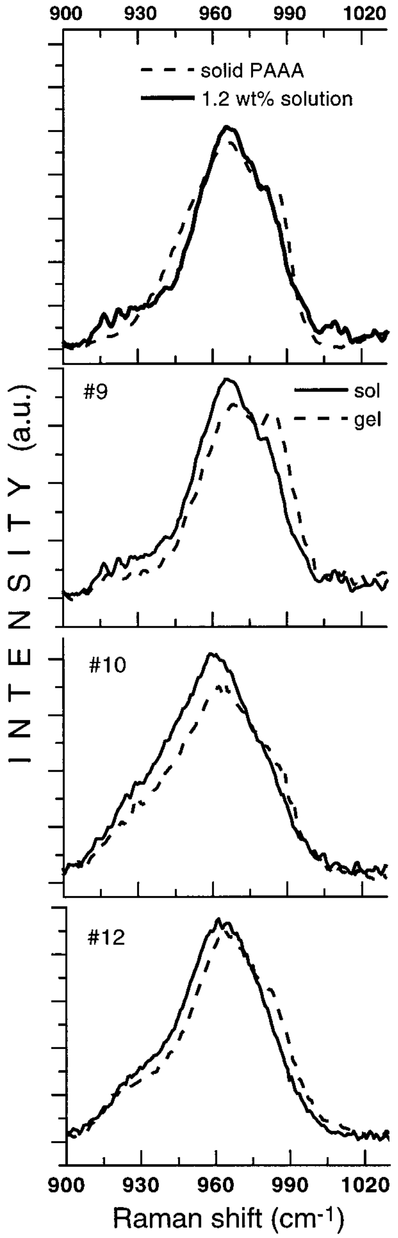

Figure 9. Raman spectra of the different systems indicated in the frequency region of ND vibrations.

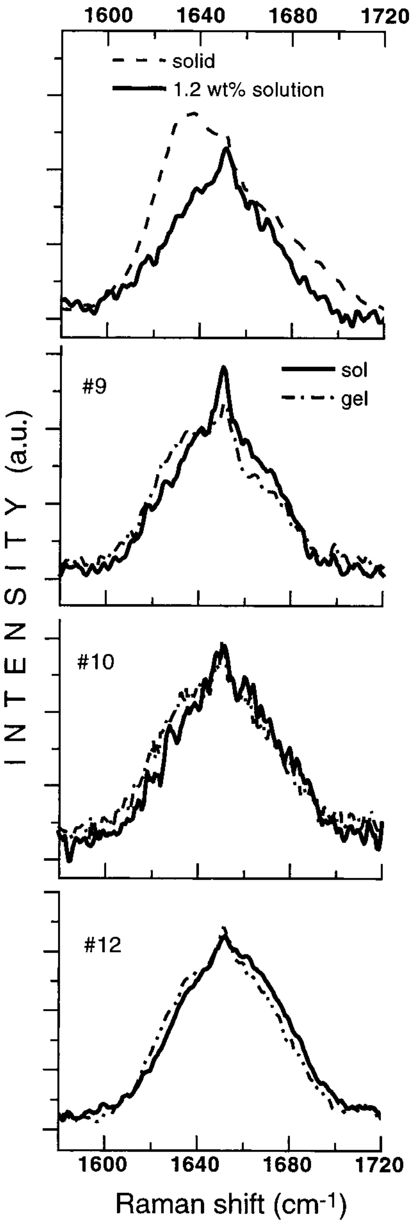

Figure 10. Raman spectra of the different systems indicated in the frequency region of amide I.

Figure 10 shows Raman spectra of the same systems as in Figure 9 but in the amide I frequency envelope. It is clearly seen that the considered band also displays a multicomponent structure. In accordance with results of ab initio calculations, the low-frequency part is mostly due to C=O stretch, while the high-frequency component includes essential contributions from the CN stretching vibration.[18,19] The sharp peak in the center of the band was tentatively ascribed as an evidence of the hydrogen bonding.[14]

The change of this band with temperature is also clear. If we compare the results (Figure 10) for the sol and the gel phase of the sample dissolved in D_2O (no. 9), an intensity decrease of the high-frequency part of the envelope, accompanied by an increase of the low-frequency part, is observed when going from the sol to the gel phase. This difference between the sol and the gel phase may be interpreted as a weakening of the CN stretching vibrations due to stronger bonding of H (D) atoms of NH (ND) groups and as increasing bonding of the O atoms when the gel is formed. It is also seen that the presence of NaSCN blocks this process (compare nos. 9 and 10, 12). It is important to stress that, similar to the case with ND bands considered above, observed changes of the amide I band during the sol–gel transition have the same character as those detected for dissolved polymer (upper plot).

Evidence of Hydrogen Bonding in the System. Since the gelling process in the present samples is believed to be due to the hydrogen bonding, it is important to detect all possible spectral features that can be attributed to the presence (or absence) of H–bonds. Below we give some general aspects on the possible ways of hydrogen bonding in the systems under consideration. To create a hydrogen bond, one must have both H-bond donor and acceptor. For the studied solutions and gels the situation is characterized by

H-bond donors: NH_{PAAA}, OH_{water}

H-bond acceptors: CO_{PAAA}, OH_{water}, (–SCN)

Note that the same consideration can be made for the deuterated systems.

Thus, in the pure aqueous PAAA solution there are four possible types of hydrogen bonding, while in the presence of NaSCN there are six types. Below we will try to establish the correlation between all these interactions and the results of our spectroscopic investigation.

OH_w–OH_w. This type of bonding is obvious and well observable. Indeed, all spectral profiles in the OH stretching region corresponded to the liquid water, even if it was affected by NaSCN (see Figure 4).

OH_w–SCN. This interaction is also well observed and was assigned to destroying of the hydrogen-bond network of water by SCN^- (Figure 4).

CO_{PAAA}–NH_{PAAA} and **CO_{PAAA}–OH_w.** We have observed that there are significant changes in the state of C=O groups with dissolving (see the uppermost plot of Figure 10). If we assume that in the dry polymer all the CO groups are bonded with NH groups only, then it is expected that the observed new band profiles result from the change in the bonding type, which suggests, in turn, that CO groups are bonded now with water molecules. In view of this, we can conclude that our spectra resolve two different states of the CO groups; i.e., in other words, we observe two different types of bonding.

NH_{PAAA}–CO_{PAAA} and **NH_{PAAA}–OH_w.** Similarly to the previous case, the spectral profile of the NH band changes significantly when the polymer is dissolved (the uppermost plot of Figure 9). Again, assuming that in the solid state the polymer is characterized by NH–CO type bonding only, the change of the band shape as the polymer is dissolved can be attributed to the appearance of a new type of interaction.

NH_{PAAA}–SCN. This interaction is quite well seen from Figure 7. We observed that addition of NaSCN significantly changed the intensity of the band around 870 cm^{-1}, which includes an essential contribution of the NH groups. Similarly, in deuterated systems the presence of NaSCN affects the behavior of the band around 945–1000 cm^{-1} which is due to ND vibrations (Figure 9). In addition, the temperature behavior of the SCN^- ions in the presence of PAAA differs significantly from that observed for the NaSCN aqueous solutions without polymer (Figure 3).

Thus, in our spectroscopic investigation we resolve all possible types of the hydrogen bonding in the systems. It is very important to stress that spectral changes occurring between the sol and the gel phase are similar to those observed when going from “solid polymer ⇒ polymer solution”.

Remarks on the Gel Formation. It appears that the interplay between the different types of interactions discussed above is very delicate, and therefore the gelation process is expected to be sensitive to conditions such as polymer (and denaturant) concentration, temperature, type of denaturant, and solvent (H_2O or D_2O).

It has been found[40] that the change in intermolecular interaction energy associated with the hydrogen-bond exchange process

$$\text{amide(NH)}\cdots\text{water} + \text{water}\cdots\text{(OC)amide} \Leftrightarrow \text{amide(NH)}\cdots\text{(OC)amide} + \text{water}\cdots\text{water}$$

is small, with a magnitude of less than 5 kJ/mol. This means that NH···OC hydrogen-bond formation in an aqueous environment should be approximately isoenergetic.[40]

Obviously, to create the macroscopic polymer network, it is necessary to have sufficient concentrations (or space density) of NH and C=O groups. If the concentration and distribution of these groups are not sufficient for the network formation, the solution will contain clusters that are formed through intramolecular bonding. In this context, it is important to remember that we must be in the semidilute concentration regime, so that the connectivity of the network can be established. The results from the present study indicate that the denaturant occupies part of the NH groups, thereby reducing the total amount of available sites needed for the gel formation. On the other hand, it is clear that the presence of NaSCN itself does not guarantee prevention of the gel network formation. As the concentration of the polymer increases (at fixed denaturant content), the temperature-induced gel phase appears again. This indicates that the most important factor is the ratio between polymer and denaturant concentrations. In other words, there must be enough sites for hydrogen bonding in order to establish the network connectivity.

Another interesting question is why at the same conditions the gel temperatures are different for gels formed in H_2O than in D_2O (compare samples 2 and 9 and samples 4 and 10 in Table 1). It has been shown[41] that the general strength of the hydrogen bonds is higher in heavy water than in water. In other words, at the same conditions (bond length and bond angle), the D···O bond is stronger than the H···O bond. When applied to the present systems, this suggests that in heavy water the polymer−polymer interaction becomes stronger due to NH → ND substitution. Thus, the conditions concerning polymer−polymer coordination and concentration of denaturant are shifted in such a way that the gel temperatures are higher in heavy water than in water.

General Conclusions

I. Raman spectroscopy appears to be an effective tool for the characterization of association and gelation processes in systems of water-soluble polymers. Despite low polymer concentrations, in this investigation we have detected manifestations of all possible types of bonding. Moreover, comparing the degree of the spectral changes as a function of polymer concentration, it seems that the Raman technique is able to distinguish between the states of the solution with and without the formation of a three-dimensional network.

II. The present study shows an obvious aggregation of PAAA in aqueous solutions (concentration effect). Our observations suggest that even at concentrations lower than necessary to establish the connected polymer network, the PAAA polymer in aqueous solution shows the presence of polymer−polymer coordination that is consistent with the previously developed approach about clusters formation and growth. In contrast, the networks formed in denaturant-containing systems are more “disconnected”, and these systems reveal pronounced polymer association only at higher concentrations.

III. When going from the sol to the gel phase, there is evidence of a change in the state of both NH and CO groups of the polymer, and the character of these alterations is similar to that observed during the dissolving of the solid polymer. This indicates that the gel state equilibrium of the system is shifted toward polymer−polymer coordination, while in the solution the equilibrium coordination is of polymer−water type.

IV. The presence of denaturant affects critically the general state of the system. Two different processes are clearly observed with NaSCN addition:

1. Disruption of the macroscopic hydrogen-bond structure of water. The process is due to attraction of a great number of H-bonds by SCN ions.
2. Direct binding of the SCN ions to the NH (ND) groups of PAAA. This process significantly reduces the number of free agents for polymer−polymer association (CO−HN bonding) and thereby prevents gel formation.

Although our study cannot give a direct answer concerning the question of which of the two above-mentioned effects is the most critical for polymer denaturation, taking into account that at higher polymer concentrations the gelation process appears even in the denaturated solvent, we may conclude that direct binding of the denaturant to the polymer chains plays a more important role in the denaturation process.

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