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Charge density effects on the fluorescence behavior of dansyl labels to strong polyelectrolytes

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Abstract

Fluorescent spectra of dansyl-labeled copolymers containing 2-acrylamido-2-methylpropanesulphonic acid (AMPS) and *N*,*N*-dimethylacrylamide (DMAA) with 0.2 mol% chromophore were observed in aqueous solution. Two emission bands appeared at 336 and 533 nm for protonated and unprotonated species of the label, respectively. These wavelengths were independent of the mole fraction F_{AMPS} of AMPS ranging from 0.186 to 0.738 but the relative intensity I_{336}/I_{533} increased proportionally to F_{AMPS}. The locally excited emission (LE), b* band of the dansyl label was found at 405 nm when the chromophore concentration was decreased below 1.45×10^{-5} mol/l. With increasing charge density in the solid copolymer the b* band was strengthened and the a* band for the twisted intramolecular charge-transfer state was weakened and even disappeared owing to the existence of ion-pair attractions. It was found that for the copolymer in dimethyl sulfoxide (DMSO)/tetrahydrofuran (THF) mixtures the change in charge density did not alter the emission wavelength λ_{em} of the a* band. The charge density dependence for the threshold of DMSO/THF composition, at which the relative emission intensity R_{F} abruptly decreased, was similar to that inducing the volume phase transition in the corresponding polyelectrolyte gels.

Keywords: Polyelectrolyte; Dansyl; Fluorescence

1. Introduction

Electrostatic interaction is the strongest primary interaction in macromolecular systems comparing with the other primary interactions of van der Waals force and hydrogen bonding. Polyelectrolyte, carrying ionizable groups on the backbone or side chain is classically considered as repulsive dominant in polar medium with an extended rod conformation or a wormlike chain with large electrostatic resistance length [1]. Consequently, polyelectrolyte is inferred to be molecularly dispersed in dilute aqueous solution as confirmed by the rapid increase in the reduced viscosity with dilution [2]. However, some abnormal phenomena have been reported since 1988. Hara et al. [3] found polyelectrolyte behavior on halatotelechelic ionomers in polar solvent by measuring the reduced viscosity. This is regarded as an evidence of intramolecular repulsion for polymer chain even with only two charges at the chain ends. If we look at this fact from another viewpoint, it appears possible that the local polyelectrolyte concentration would not be simply reduced by dilution. In other words, the polyelectrolyte chains mutually aggregate even in polar solvent rather than molecularly disperse.

This consideration has been supported by the recent intensive studies of dynamic light scattering, small-angle X-ray scattering and small-angle neutron scattering on polyelectrolyte in dilute polar solutions. Two diffusion modes were found at the magnitude of about 10^{-6} and 10^{-8} cm^2 s^{-1} [4–8]. The former is clearly corresponding to the chain translation, but it is difficult to attribute the latter to a specified substance in the framework of the classic polyelectrolyte theory. The ambiguous situation is also the case for the appearance of up-turn and peak on the curve of scattering intensity against scattering vector [8–10]. All of the above abnormal phenomena can be reasonably explained by the existence of chain aggregation in the polyelectrolyte solution with a larger size and a slower mobility than a single molecule, which cannot be destroyed by dilution. The attractive force inducing the aggregation is based upon the dipole–dipole interaction between the ion pairs in polyelectrolyte–polar solvent system.

The charges on polyelectrolyte chains cannot be evenly dispersed in solution as they are restricted by the chain backbone, the distance between the charges is about 0.25 nm for a completely dissociated poly(acrylic acid) chain in water. This value is equivalent to the ion distance

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Table 1
Composition of dansyl-labeled polyelectrolytes ADDan (containing 0.2 mol% of dansyl label monomer DANSAEP)

Sample	AMPS/DMAA (mole ratio)	Yield (wt%)	Elemental analysis (wt%)		F_{AMPS} (mol%)[a]
			S	N	
ADDan5	5/95	71.52	1.91	12.24	6.48
ADDan15	15/85	80.63	4.46	11.75	18.59
ADDan30	30/70	77.60	7.58	10.55	31.14
ADDan45	45/55	85.21	9.71	9.55	44.00
ADDan60	60/40	85.76	11.97	8.36	63.32
ADDan75	75/25	90.25	13.31	7.83	73.81

[a] Estimated from the N content.

in aqueous NaCl solution, more concentrated than 10 mol/l if it is accessible. In order to minimize such a high free energy of electrostatic repulsion, the counterion should be condensed on the polyion as described in the terms of counterion condensation [11,12]. The charged site on the polyelectrolyte with condensed counterion forms a loose ion pair that acts as a dipole in polar solvent, leading to the contraction between each other on the same chain or different chains. Khokhlov et al. proposed a theoretical simulation to show the electrostatic aggregation for polyelectrolyte gels in its poor solvent [13,14].

Recently Liu et al. found that highly charged gels of chemically crosslinked 2-(acrylamido)-2-methylpropanesulfonic acid (AMPS) and *N*,*N*-dimethylacrylamide (DMAA) underwent the volume phase transition in the mixture of dimethyl sulfoxide (DMSO) and tetrahydrofuran (THF) by increasing the volume fraction of THF [15]. A swelling hysteresis was observed in this kind of gel when the shrinking–swelling process was repeated, indicating the existence of ion pair aggregations which was not destroyed thoroughly by swelling [16]. This seems to be the cause of the chain aggregation mentioned above.

Fluorescence technique is a suitable tool to investigate the interaction in polyelectrolyte systems, due to its effective distance and sensitivity to the change in local environment [17]. Available methods include emission spectra, excimer formation, fluorescent quench, no-radiation-energy-transfer, time-resolved spectrum, etc. Dansyl chromophore labeled to polyelectrolyte chains is popularly used for this purpose [18]. The protonated state of the dimethylamino group in the dansyl can be easily altered by changing the proton concentration in the solution. Strauss and Vesnaver [19,20] investigated the pH-value dependence of the absorption and emission spectra of the dansyl chromophore labeled to alkyl vinyl ether and maleic anhydride copolymers and discussed the equilibrium between the acidic and basic species of dansyl labels.

We are planning to reveal the chain aggregation induced by the ion pair attraction for our sulfonate polyelectrolyte in polar solvent using the fluorescent technique. Since the sulfonate group is a strong electrolyte, the change in charge density of the sample will change the protonated state of the dansyl group showing different emission spectra. The corresponding experimental results are given in the present paper.

2. Experimental

Syntheses of dansyl label, *N*-[2-[[[5-(*N*,*N*-dimethylamino)-1-naphthalenyl]-sulfonyl]amino]ethyl]-2-propenamide (DANSAEP) copolymerizable in aqueous solution and preparation of dansyl-labeled strong polyelectrolytes containing 2-acrylamido-2-methylpropanesulphonic acid (AMPS) monomer unit have been described previously [21,22]. Here, we give a brief introduction only. The dansyl-labeled polyelectrolyte was made from radical copolymerization of AMPS (Fluka), DMAA (Kohjin, Japan) and DANSAEP in water at 70°C for 16 h, using 0.2 mol% of ammonium persulfate as the initiator. The total monomer concentration was 0.46 mol/l with 0.2 mol% DANSAEP. The product was dialyzed for 2 weeks and freeze-dried followed by vacuum-drying at 60°C to obtain a series of dansylated samples with different mole fractions, F_{AMPS}, of AMPS refereed to as ADDan series. The F_{AMPS} value of the copolymer was evaluated from the N content determined by elemental analysis with a Heraeus CHN–O apparatus. The dansyl label content was detected by UV absorption in a buffer of $pH = 6.98$ and the results were close to the fed ratio. The composition of the ADDan copolymer samples is listed in Table 1.

The fluorescence measurement of dansyl-labeled ADDan samples in water solution and in DMSO/THF mixtures was carried out on a Hitachi F-4500 fluorescence spectrophotometer at 25°C. The stock solution of ADDan in the DMSO/THF mixtures was prepared by dissolving ADDan in pure DMSO and then adding THF to the desired solvent composition. The stock solution was diluted with the mixed solvent of the same composition.

3. Results and discussion

3.1. Emission spectra of acidic and basic species of dansyl label

The dansyl chromophore in ground state exists as both

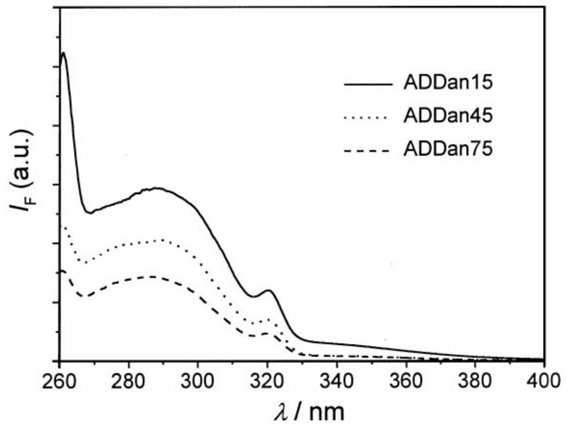

Fig. 1. Excitation spectra of indicated ADDan copolymers in 0.60 wt% aqueous solutions at emission wavelength λ_{em} of 533 nm.

protonated and unprotonated species with respect to its dimethylamino group, depending on the pH value of the solution. These species exhibit their characterizing absorption spectra as well as emission spectra. The excitation spectra recorded at emission wavelength λ_{em} of 533 nm from aqueous solutions of three dansyl-labeled ADDan copolymers with different charge densities are shown in Fig. 1. The spectra obviously consist of two bands with maxima at 286 and 320 nm corresponding to the above two species of the label. Because the sulfonate group on the copolymer side chain is a strong electrolyte, protonated dansyl species is dominant in water solution, resulting in a strong exciting band at 286 nm.

Fig. 2 depicts the corrected emission spectra, i.e. subtracting the water emission from the total emission, of the dansyl-labeled copolymer ADDan75 excited at four wavelengths. The spectra are characterized by two bands, at 336 and 533 nm for the protonated and unprotonated species of the dansyl residue, respectively. The emission wavelength is independent of the exciting wavelength.

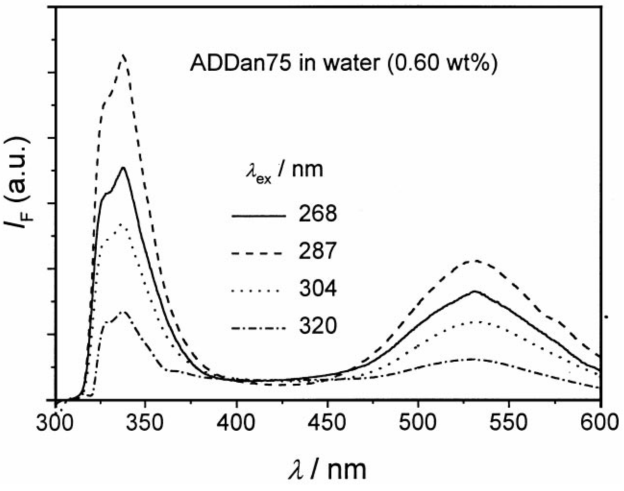

Fig. 2. Emission spectra of ADDan75 in 0.60 wt% aqueous solution excited at indicated wavelengths λ_{ex}; the water emission is subtracted.

In order to investigate the charge density effect on the emitting behavior of the dansyl label, we plotted in Fig. 3 the relative emission intensity at 336 nm to that at 533 nm, I_{336}/I_{533}, as a function of the AMPS mole fraction F_{AMPS} in our labeled copolymers. It is evident that the I_{336}/I_{533} value increases linearly with an increase in the F_{AMPS} of the copolymer, indicating an increase in the relative concentration of the protonated species to that of the unprotonated species for dimethylamino groups in the dansyl label. The corresponding change in pH value estimated from the F_{AMPS} value, with polymer concentration, is from 2.03 to 1.61. Increase in the charge density for our strong polyelectrolyte at constant concentration means increasing the proton concentration in the solution, leading to more protonated dansyl labels. This is consistent with that reported by Strauss and Vesnaver [19,20], who found two emission bands at 336 nm and at about 550 nm for dansyl-labeled copolymer of maleic anhydride and alkyl vinyl ether in the acidic and basic forms, respectively. With increasing pH value of the aqueous solution, the emission at 336 nm was weakened and a redshift was observed for the basic species emission at about 550 nm. Their I_{336}/I_{550} is always lower than unity owing to the high pH value required for dissociating the carboxylate groups.

3.2. Locally excited and twisted intramolecular charge-transfer emission of the dansyl label

For the unprotonated species of the dansyl chromophore, there are two excited electronic states related to the emission spectrum according to the angle between the plans of dimethylamino group and naphthalene ring [23,24]. One is the locally excited state (LE) for the emission at high energy (b^* band) in which both groups are almost coplanar. The other is the twisted intramolecular charge-transfer (TICT) state for the emission at low energy (a^* band) called as emission maximum in which the dimethylamino plan is

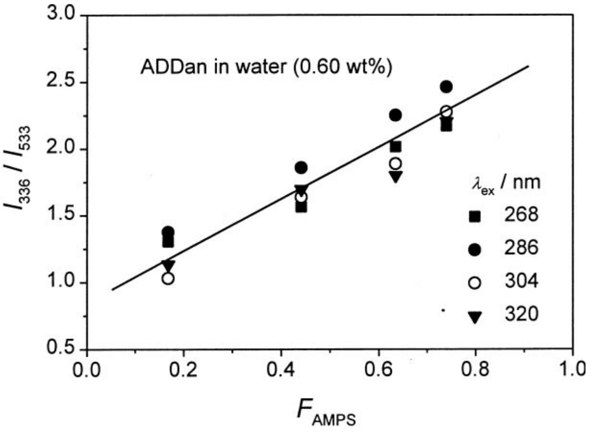

Fig. 3. Relative intensity I_{336}/I_{533} of emission at 336 nm to that at 533 nm as a function of AMPS mole fraction F_{AMPS} excited at indicated four wavelengths.

nearly perpendicular to the naphthalene plan. The TICT emission is sensitive to the local polarity surrounding the chromophore and the segment mobility [23,25].

The wavelength of TICT emission (a* band) λ_{em} observed from these copolymers in water solution appears within 532–533 nm over the concentration ranging from 0.015 to 10.0 wt%. However, no b* band emission was observed from the solution of moderate concentration as shown in Fig. 2. To find the trace of b* band emission, much more dilute polymer solutions were used in our experiments. A broad emission peak of b* band at 405 nm can be recognized in Fig. 4 as long as the solution concentration is reduced below 0.122 wt% (equivalent to 1.45×10^{-5} mol/l of the chromophore), its intensity increases slightly with the concentration except for the solution of 0.122 wt%. At the same time the emission of the a* band is obviously weakened with dilution. There may be two factors of dilution affecting the emission intensity: decrease in the chromophore concentration and increase in the concentration of unprotonated dansyl label due to the rise in pH. The I_{336}/I_{533} value estimated for these dilute solutions scatters from 2.0 to 2.2, close to the value for ADDan60 solution of 0.60 wt% in Fig. 3, suggesting that the relative concentration of unprotonated species of dansyl labels is not increased by dilution. In fact, no change can be expected in the relative concentration of proton to dansyl label for a given ADDan sample in aqueous solution by dilution. Therefore, the increase in emission intensity of the a* and b* bands with solution concentration is simply attributed to the increase in the chromophore concentration. However, the concentration dependence of the b* band is so weak that it cannot even be recognized from the curves in Fig. 2 with a strong a* band emission at the moderate polymer concentration. This is due to the fast torsional motion of the dimethylamino group around amino-naphthalene bond in dilute solutions, which makes the TICT state dominant.

If the above argument is true, we should observe the LE emission at solid state where the torsional motion will be frozen so that the formation of the TICT state becomes very slow. Fig. 5 shows the fluorescence spectra of three solid ADDan samples excited at 317 nm to avoid the emission from the acidic species. The b* band at 405 nm is clearly seen while the a* band emission at ca. 510 nm becomes a shoulder peak. Furthermore, the higher the charge density in the copolymer, the stronger the b* band emission. Because the free energy of ionization becomes very high due to the low medium polarity contributed by the polymer backbone, the sulfonate group on our polyelectrolytes will form the ion pair in solid state. The dipole attraction between these ion pairs restricts the segment mobility and TICT state formation, leading to a higher b* band emission. In this case, the time required for the dimethylamino group rotating to perpendicular to the naphthalene plane is longer than the lifetime of excited LE state.

Interestingly, if these samples are excited at 333 nm only the b* band can be observed as the insert in Fig. 5, where the trend of charge density dependence of the b* band intensity

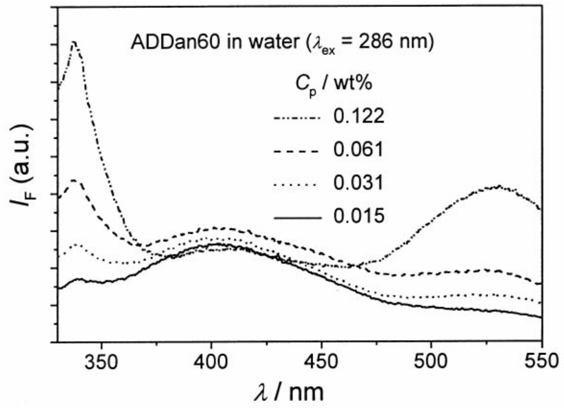

Fig. 4. Emission spectra of ADDan60 in dilute aqueous solutions of indicated concentrations, $\lambda_{ex} = 286$ nm.

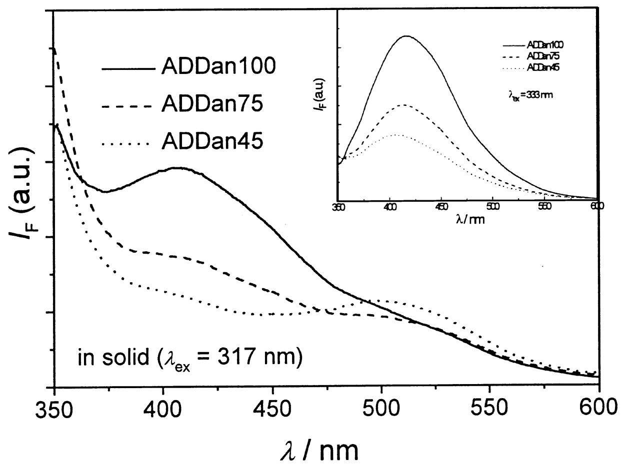

Fig. 5. Emission spectra of the indicated ADDan samples in solid state excited at 317 nm. The insert is the emission spectra of the same samples in solid state but excited at 333 nm.

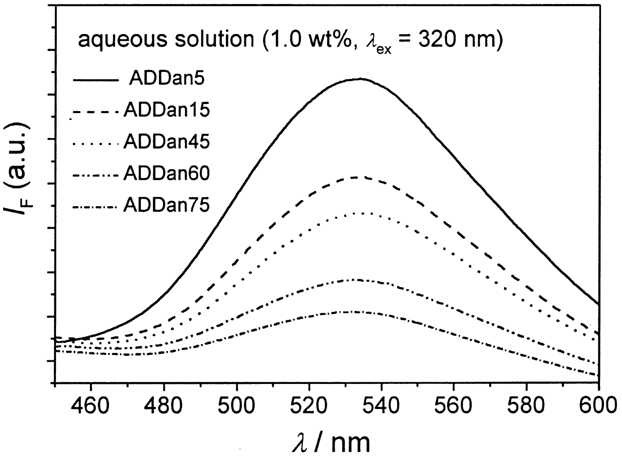

Fig. 6. Emission spectra of the indicated ADDan samples in 1.0 wt% aqueous solutions excited at 320 nm.

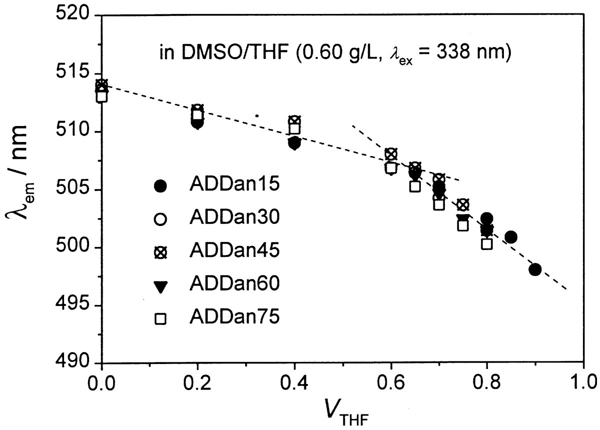

Fig. 7. Emission maximum wavelength λ_{em} of the indicated ADDan samples against the volume fraction V_{THF} of THF in DMSO/THF mixtures at polymer concentration of 0.6 g/l excited at 338 nm.

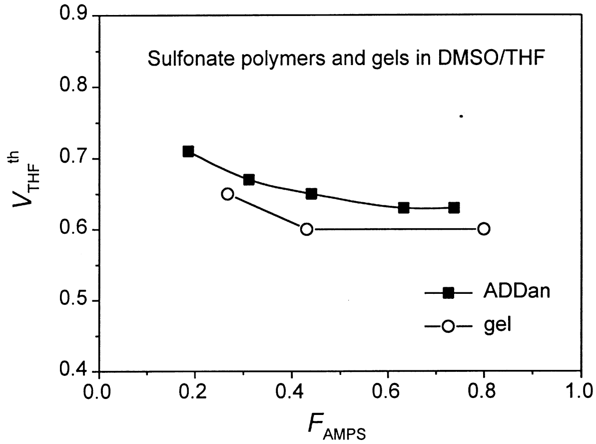

Fig. 9. The charge density dependence of the threshold composition V_{THF}^{th} of DMSO/THF mixtures, at which R_F abruptly drops (■) and the volume phase transition occurs (○) in the corresponding polyelectrolyte gels.

becomes even more obvious. At present, the origin for the disappearance of the TICT emission remains unknown.

It is important to understand the charge density dependence of the a* band emission before using it as a measure of microenvironment. The emission spectra of the ADDan copolymers with different AMPS mole fractions are illustrated in Fig. 6. The emission maximum λ_{em} is a constant of 533 nm for all of the samples while the fluorescent intensity decreases with the increase in the F_{AMPS} of labeled copolymers. This fact seems to suggest that the local polarity surrounding the dansyl chromophore does not change with the charge density of copolymer, but the formation of TICT state is hindered by the increase in the charge density. Another possibility for the decrease in emission intensity with increasing F_{AMPS} may be the decrease in concentration of the unprotonated species of dansyl labels induced by decreasing pH.

3.3. Emission spectra in the DMSO/THF mixture

We have reported on the fluorescence of dansyl labels that the polyelectrolyte is able to maintain its solvation layer stable when the composition of bulk solvent is gradually changed below a certain threshold value, at which a sudden drop occurs in the emission wavelength and intensity [22]. Does this threshold depend on the charge density of the polyelectrolyte? Does this threshold composition correspond to that inducing the volume phase transition in the corresponding polyelectrolyte gels? We investigated the emission spectra of these dansyl-labeled polyelectrolytes in DMSO/THF mixtures of different composition.

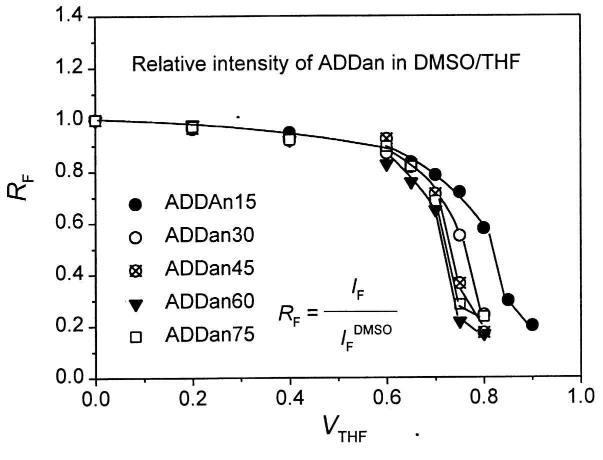

Fig. 8. Relative intensity R_F of emission maximum in DMSO/THF to that in DMSO as a function of V_{THF} for indicated ADDan samples of 0.6 g/l.

The emission maximum wavelength λ_{em} and relative intensity R_F of the a* band emission in DMSO/THF to that in DMSO are plotted against the volume fraction V_{THF} of THF in the mixture in Figs. 7 and 8, respectively. The λ_{em} value for each ADDan polyelectrolyte decreases as V_{THF} increases and the data can be approximately fitted by two straight lines as the dashed lines in Fig. 7. Their intersecting point gives a threshold V_{THF}^{th} value as 0.65, below which the solvation layer surrounding the dansyl chromophore does not vary as much as the bulk solvent, and above which the original solvation layer is destroyed, as illustrated by the steeper decrease in λ_{em}. The most surprising finding is that the λ_{em} data for the five samples are almost overlapped, suggesting the independence of local polarity on the charge density of whole molecular chains, which is the same behavior as in aqueous solutions mentioned above. Although the polarity of the solvation layer is an integrated effect from the bulk solvent, polymer backbone, residues on the polymer chain, and the additives in the solution, this result emphasizes that the composition of the polyelectrolyte chain does not alter the averaged local polarity around the dansyl chromophore as much as expected.

The relative intensity R_F is a measure for the segment mobility of labeled chain molecules. Fig. 8 manifests a more evident drop in R_F for each copolymer with given charge density when V_{THF} is beyond a threshold value V_{THF}^{th} in DMSO/THF mixtures. The V_{THF}^{th} determined from the intersecting point of the tangent lines to the two parts of the curve is plotted as a function of AMPS mole fraction F_{AMPS} of the copolymer in Fig. 9 (filled square). The solvent composition at which the volume phase transition occurs in the corresponding polyelectrolyte gels [15] is also plotted in the same figure (unfilled circle) for comparison. One can find two features from the figure, that both the thresholds $V_{\mathrm{THF}}^{\mathrm{th}}$ exhibit similar charge density dependence and decrease with increasing charge density. This fact confirms that the volume phase transition in the polyelectrolyte gels is caused by the sudden drop of polarity in the solvation layer of the polymer chains, which is a local property less affected by integral property of the molecular chain.

Acknowledgements

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