Polymer Journal, Vol. 25, No. 2, pp 123—130 (1993)

Change in Micro-Environments in Poly(acrylamide) Gel with Pyrenyl Probe Due to Its Volume Phase Transition Induced by pH Change

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(Received April 3, 1992)

ABSTRACT: Changes in the micro-environments and interactions among the side groups during pH-induced volume phase transition of poly(acrylamide) gels having pyrenyl group as a fluorescent probe in acetone–water (9 : 11) mixed solvent were studied with fluorescence spectra and lifetime measurements. The results are as follows: (a) The volume phase transition is caused by hydrophobic interaction of polymer main chains and charge repulsive force of carboxylate anion at polymer side chains inside the gel, indicated by change in fluorescence intensity ratio I_1/I_3 of the pyrenyl probe and the shifts in fluorescence wavelength. (b) In the collapsed state, the gel consists of two phases: one is the portion of polymer main chain aggregate, and the other is the portion of polar side group assembly, which results from a competitive interaction between the hydrophobic interaction of polymer main chains and the charge repulsive force of carboxylate anion in the coexisting two phase regime.

KEY WORDS Poly(acrylamide) Gel / Volume Phase Transition / Fluorescence / Pyrenyl Group / Hydrogen Bond / Hydrophobic Interaction /

Some organic polymer hydrogels have widespread applications in medical, pharmaceutical, industrial, and related fields.[1] These hydrogels made of, *e.g.*, poly(acrylamide) (PAAm) or poly(isopropylacrylamide) (PNIPA) show volume phase transition with a reversible volume change of as large as several hundred times induced by various conditions.[1-6] Some macroscopic properties of gels, such as mechanical[7] and thermal[8] behavior have been widely investigated during the volume phase transition. The processes of swelling and shrinking of the PAAm or PNIPA gel were measured with light scattering[9,10] to monitor the density fluctuation of gel networks near the volume phase transition. It has been noted recently that detailed chemical structure, conformational change, interactions among the polymer chains and between the polymer chain and solvent molecules, and micro-environments inside the gels play very inportant roles in determining the volume phase transition. Though the light scattering study reveals the microscopic structure and dynamics of gel network for the volume phase transition,[10,11] NMR can also demonstrate the changes in the micro-environments and motions of the side groups and backbone polymer chains of PNIPA during the volume phase transition.[12] Fluorescence technique can give information

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not only on the microscopic structure and dynamics of a polymer network but also about micro-environment around polymer segments and interactions between various groups in polymer systems.[13] In our previous works,[14] changes in the interactions of various groups, micro-environments, and dynamic fluctuation of the networks near the volume phase transition point induced by pH[14a] or solvent composition[14b] change were studied for PAAm gels having fluorescent dansyl probe at side chains by measuring their fluorescence spectra, anisotropy, and lifetimes. The results revealed that there exist two transition points, the macroscopic transition point occurring at pH 5.0 and the microscopic transition point occurring at pH 3.8. The changes in hydrophobic interactions inside the gels and the mobilities of various groups in the gels were demonstrated at different transition points for the pH-induced system.

The pyrenyl group is frequently used as a hydrophobic fluorescent probe to study the hydrophobic interactions and conformational changes in micellar PNIPA[15] and polyelectrolytes.[16] The spectral shape of pyrene fluorescence can serve as a measure of solvent polarities around the pyrene, because relative intensity of the vibronic fine structure, I_1/I_3, directly depends on the solvent dipole moment.[17] In the present work, the PAAm gel showing a volume phase transition at pH 5.0 is studied by labeling its side chains with pyrenyl groups. The absorption spectra, fluorescence excitation spectra, fluorescence spectra were measured at various pH values in acetone–water (9 : 11) mixed solvent at 20°C in order to study hydrophobic interactions among various groups and polarities of micro-environments inside the PAAm gels in the swollen and collapsed states. The results are compared with the results of dansyl probe in the same system.

EXPERIMENTAL

Preparation of 1-(pyrenyl)-acrylamide (**I**)

All reagents and solvents in this work were obtained from commercial suppliers and purified before use. **I** was prepared and used as a fluorescent monomer for introducing the pyrenyl group to the side chain of PAAm gel and linear PAAm. Acryloyl chloride in THF was added dropwise to a solution of 1-aminopyrene and triethylamine in THF for 6 h at 0°C. The solution was stirred at room temperature overnight. The combined filtrates were evaporated, and the residue was chromatographed on silica with dichloromethane as eluent to provide yellowish solid of **I**.

Preparation of PAAm Gel and Linear PAAm

The chemical structures and molar ratios of monomers per 100 monomeric units of the PAAm gel and linear PAAm are shown in Figure 1. The PAAm gels and linear PAAm having pyrenyl probe were prepared by the copolymerization of 5.00 g of acrylamide as a main chain monomer for the gel (5.09 g of that for linear PAAm), 0.200 g of *N,N'*-methylenebisacrylamide as crosslinker for PAAm gel, 0.425 g of sodium methacrylate as an ionic agent for both, 7.0×10^{-4} g of 1-(pyrenyl)acrylamide as a fluorescent probe monomer for PAAm gel (1.5×10^{-3} g of that for linear PAAm), 40 μl of tetramethylethylenediamine (TEMED) as an accelerator, and 40 mg of ammonium persulfate as an initiator. All ingredients, with the exception of TEMED and ammonium persulfate, were dissolved in the DMF–water mixed solvent (2 : 3 volume ratio). The above solution was flushed with nitrogen. After TEMED and ammonium persulfate were added, the solution was diluted to a 100 ml with the DMF–water mixed solvent. (a): For PAAm gel, under stirring, the solution was divided into test tubes (1.5 cm in diameter); the test tubes were again flushed with nitrogen. At room temperature the copolymerization proceeded overnight. After gelation, the gels were

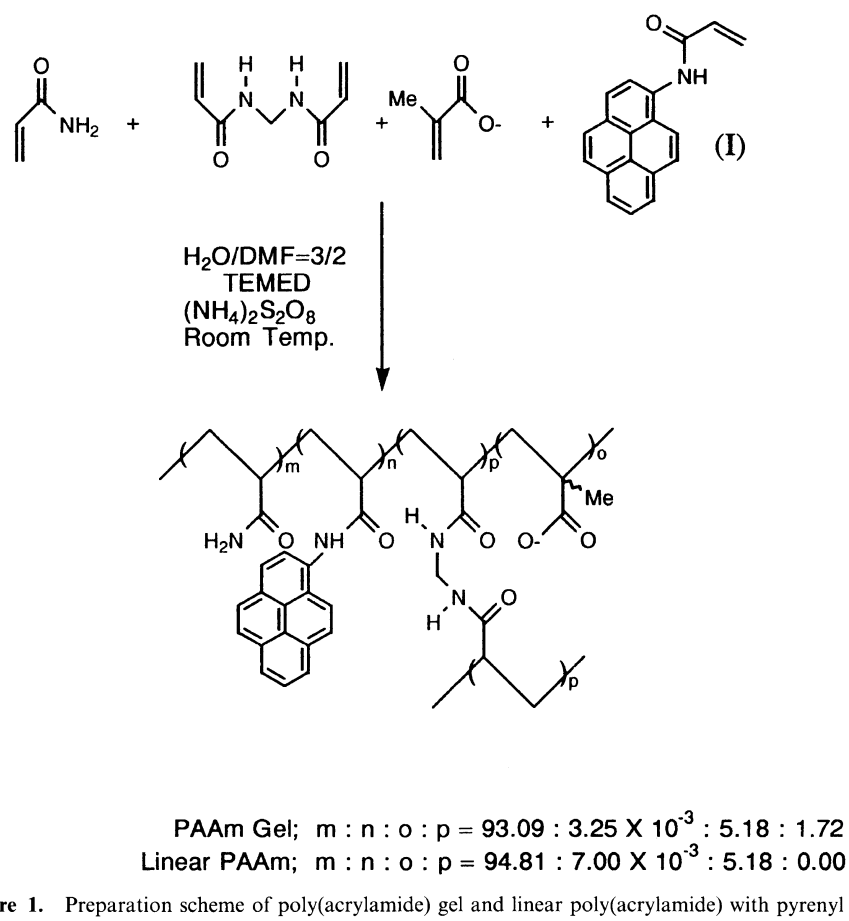

Figure 1. Preparation scheme of poly(acrylamide) gel and linear poly(acrylamide) with pyrenyl probe.

washed with pure water to remove residual chemicals. The gels were cut into 1.5 cm long samples, and immersed in the acetone–water mixed solvent (9:11 volume ratio) with different pH values, and then swollen or collapsed at 20°C. In order to make the same Na^+ concentration or the same ionic strength for different swelling systems, all of the acetone–water mixed solvents were adjusted to pH 12 at first with the same equivalent NaOH, and then were adjusted to different pH values with hydrochloric acid. After reaching the swelling equilibrium, the volume size and photophysical properties of the PPAm gels were measured. (b) For linear PAAm, the reaction was carried out in a taper flask overnight at room temperature. After copolymerization, the polymer was precipitated into methanol. Subsequent purification involved dissolving the polymer in DMF, reprecipitating into methanol, and drying under vacuum.

Measurements of Photophysical Properties

UV absorption spectra of all the samples were measured with a Jasco-660 UV/VIS spectrophotometer. The fluorescence excitation and emission spectra were measured with a Hitachi 850 fluorescence spectrophotometer. The excitation wavelength was set at 345 nm and the slit width was chosen to be 5 nm. A Horiba MAES-1100 photon-counting apparatus with a hydrogen flash lamp was used for fluorescence lifetime measurement of this system at pH 6.5. The FWHM of the lamp

pulse was 1.5—2.5 ns.

The PAAm gels which were swollen in the acetone–water mixed solvent having a certain pH value were cut into suitable size and put into a quartz cell. The original acetone–water mixed solvent having the same pH value was put into this quartz cell to steep the gel. The linear PAAm was dissolved into the acetone–water mixed solvents (9 : 11) having certain pH values. The mixed solvents were adjusted to have the same Na^+ concentration with the cases of the gels. The concentration of the linear polymer was 5.0×10^{-3} g ml^{-1}. All spectra of the gel and the linear polymer were obtained in the quartz cell under air condition.

RESULTS AND DISCUSSION

Microscopic Characterizations of PAAm Gel and Linear PAAm

By the above mentioned preparation method, the fluorescent pyrenyl probe and ionic groups are introduced into the side chains of PAAm system. All monomers in these systems are copolymerized into the gel which exhibits a volume phase transition at pH 5.0 or into the linear polymer. The relationship of the degree of equilibrium swelling of the gel, $V/V_0 = (D/D_0)^3$, to the pH value is shown in Figure 2. The V and V_0 are the volumes, and D and D_0 are the diameters of the gels after equilibrium swelling and just after preparation of the gels,

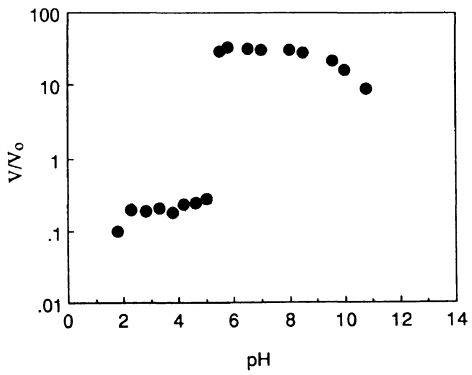

Figure 2. Degree of equilibrium swelling of PAAm gel having pyrenyl probe as a function of pH value.

respectively. Irrespective of the presence or absence of the pyrenyl probe labeled to the side chain of the gel, the gels shrink at pH ranging below 5.0. At pH 5.0 the gel shows a volume phase transition, and remains swollen up to pH 9.6. After swelling the gels are transparent in pH 5.0 to 9.6. The gels are pale yellow solids below pH 5.0, but this weak coloration did not disturb fluorescence measurements. For pH over 10.8, the swelling system becomes yellow. Since acrylamide groups are decomposed to acrylic groups and side chains connecting pyrenyl probes to PAAm main chain are also destroyed, the absorbance of the pyrenyl groups in the acetone–water mixed solvent withoug gel can be found with the UV absorption. For the linear polymer, the decomposition of PAAm side chains could be also observed above pH 10.4.

Photophysical Properties of Pyrenyl Probe in the Gel and the Linear Polymer

Both PAAm gels and linear PAAm exhibit the same characteristic absorption of the pyrenyl group after swelling in the acetone–water mixed solvent. The maxima absorption is at 341.5 nm. To avoid absorption of acetone in the mixed solvent, which occurs in the region shorter than 325 nm, an excitation wavelength for both the gels and linear polymer was set at 345 nm at 20°C. To eliminate the possibility of forming excimer of the pyrenyl probe in the system, very dilute concentrations were adopted for the fluorescent probe in copolymerization conditions (3.25×10^{-3} mol% for the gel and 7.0×10^{-3} mol% for linear polymer) (see Figure 1). No excimer emission was found from the fluorescence spectra in these systems. Typical excitation spectra and fluorescence spectra of pyrenyl probe labeled to the network of the gels are shown in Figure 3 for various pH. Identical excitation spectra were obtained for those monitored at 375 nm and 397 nm for collapsed state or at 387 nm and 407 nm for the swollen state. The excitation spectra correspond to the UV absorption spectra. The

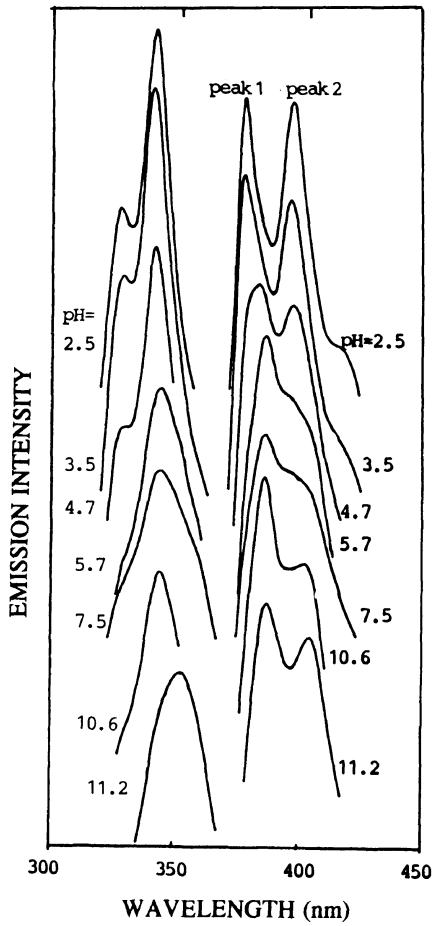

Figure 3. Typical fluorescence excitation spectra (left) and emission spectra (right) of pyrenyl probe labeled to the network of the PAAm gels after equilibrium swelling in acetone–water (9 : 11) mixed solvent at pH 2.5, 3.5, 4.7, 5.7, 7.5, 10.6, 11.2, respectively.

fluorescence spectra manifest the characteristics of micro-environments around the pyrenyl group. The shapes of the fluorescence spectra and positions of the peaks are affected by the pH condition. In the region below pH 5.0 where PAAm gels collapse, the fluorescence spectra clearly display two peaks, while in the region over pH 5.0 below pH 9.6 for swollen PAAm gels, the fluorescence displays only one peak and a shoulder for the longer wavelength side. The intensity ratios of I_1/I_3, where I_1 is the fluorescence intensity at 375 nm for the collapsed

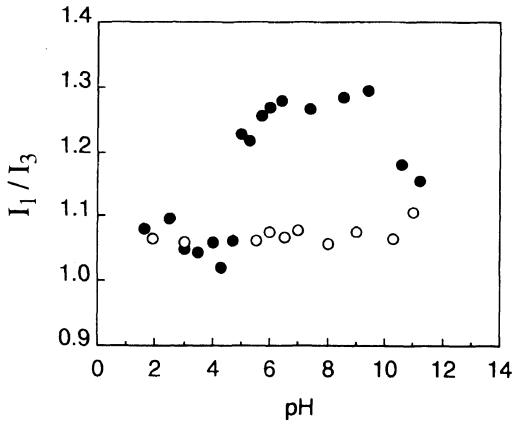

Figure 4. The pH dependence of fluorescence intensity ratio of the peak 1 to peak 3, I_1/I_3, for the pyrenyl probe attached to the PAAm gel (●) and linear PAAm (○).

state and at 387 nm for the swollen state, and I_3 is the fluorescence intensity at 397 nm for the collapsed state and at 407 nm for the swollen state, can be obtained by analyzing the fluorescence spectra into two peaks with gaussian shapes and are plotted in Figure 4. The shifts of these fluorescence wavelengths (λ_f) are shown in Figure 5 against the pH. An increase in I_1/I_3 takes place sharply at pH 5.0 for the PAAm gel with the increase in the pH value. The value of I_1/I_3 is remained unchanged virtually in the pH region from 5.0 to 10.0, and decreases over pH 10.0. The λ_f shifts gradually to longer wavelength from pH 4.7 to pH 5.7 and remains unchanged over pH 5.5 for the gels. However, no change in I_1/I_3 and no shift in λ_f occur in all pH regions for the linear polymer. The increase in the intensity ratio of vibronic fine structures, I_1/I_3, of pyrenyl fluorescence reflects the change in micro-environment of pyrenyl group in the PAAm gels to a less hydrophobic atmosphere.[17]

The change in polarity inside the gels was estimated by shifts in the fluorescence wavelength of dansyl probe in the same gel system,[14a] and also by the values of I_1/I_3 in the present work in Figure 4. An empirical scale of solvent polarity, E_T, based on the solvatochromic band of the pyridinium-*N*-phenol-

betaines was proposed by Dimroth,[18] and coorrelated to the fluorescence spectra of dansyl group[19] and pyrene.[17b] The value of E_T of the gels below pH 3.8 is 30 kcal mol^{-1} from the $\lambda_f = 460$ nm for dansyl probe,[14a] which corresponds to the dry polymer regime.[20] Over pH 3.8, E_T for the gels becomes 52 kcal mol^{-1}, while for all pH region the E_T for the linear polymer is 52 kcal mol^{-1} based on λ_f of the dansyl probe. From the value of I_1/I_3 for pyrenyl probe, the E_T inside the gel is estimated to be 48 kcal mol^{-1} below pH 5.0 and 52 kcal mol^{-1} over pH 5.0, while the E_T for the linear polymer is 48 kcal mol^{-1} in all pH ranges. The difference of E_T estimated by pyrenyl probe for the linear PAAm from E_T by dansyl probe for the linear polymer would be due to the error in estimating E_T for pyrenyl probe from the literature relationship between I_1/I_3 and E_T,[17b] since the amide group bonded directly to pyrene ring in the present polymers affects the excited electronic state of the amide-substituted pyrenyl group as will be shown below from the transient fluorescence decay measurements. The shorter spacer length of pyrenyl-labeled PAAm than that of dansyl-labeled PAAm may also lead to stronger interaction between chromophore and the polymer chain in the former case. This stronger interaction for the pyrenyl-labeled system would also result in smaller difference in E_T jump between collapsed and swollen states of the pyrenyl-labeled PAAm gels compared to that for dansyl-labelled gel system. The shifts of λ_f of the pyrenyl probe in Figure 5 also reflect changes in polarity[21] inside the gel.

A typical fluorescence decay profile with a residual is shown in Figure 6 for the pyrenyl probe attached to the side chain of the PAAm gel at pH 6.5. Good double exponential fittings were obtained for the gel with $I(t) = 0.926\exp(-t/9.6) + 0.074\exp(-t/33.9)$, and for the linear polymer at pH 6.5 with $I(t) = 0.926\exp(-t/9.3) + 0.074\exp(-t/32.7)$. The long lifetime (33 ns) corresponds to usual emission of pyrenyl group,[15a] while the short

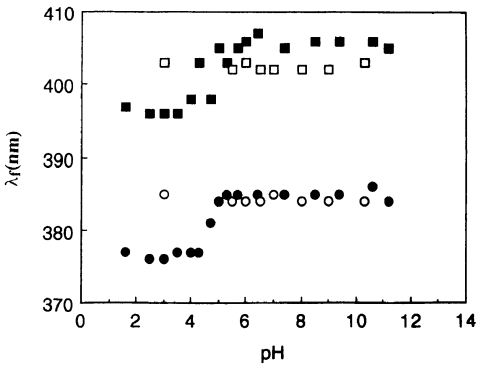

Figure 5. pH dependence of the fluorescence peak wavelengths of the probe attached to PAAm gel (■, ●) and linear PAAm (□, ○) for peak 1 (●, ○) and peak 3 (■, □) in Figure 3.

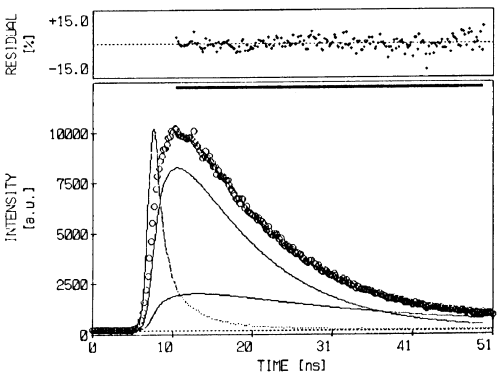

Figure 6. Fluorescence decay and a residual for curve fitting with a double exponential function for the pyrenyl prove attached to PAAm gel at pH = 6.5.

lifetime (9.5 ns) may be due to the existence of a new path of deactivation of excited amide-substituted pyrenyl group through intramolecular partial charge transfer between pyrenyl group and amide group caused by hydrophilic micro-environment around the amide-substituted pyrenyl group.

By comparing the experimental results for the PAAm gels with pyrenyl probe to those of dansyl probe, a distinct physical picture of the volume phase transition can be understood as follows: There exist hydrophobic interactions of polymer main chains and charge repulsive force among carboxylate anion group

($-COO^-$) inside the polymer gel. The hydrophobic interaction includes hydrogen bonds among carboxylic acid (–COOH) and amide group ($-CONH_2$).[22] The change of carboxylic acid group (–COOH) to carboxylate anion group ($-COO^-$) is carried out with increase in pH. In all pH ranges, the hydrophobic interaction and hydrogen bonds between polymer side chains give the system a tendency towards the collapsed state, while repulsive force among $-COO^-$ make the gel have a tendency towards the swollen state. In the pH region below 3.8, since the fraction of the carboxylic acid group (–COOH) is much more than that of carboxylate anion group ($-COO^-$), the hydrophobic interaction of the polymer main chains and hydrogen bonds among –COOH and $-CONH_2$ are the dominating interactions in the gel system to make the gels collapse macroscopically. In this state, the pyrenyl and dansyl probes are located completely in a hydrophobic micro-environment inside the gel which corresponds to the dry polymer regime.[20] A decrease in the proton concentration causes the transformation from –COOH to $-COO^-$ in the region of pH 3.8 to 5.0. This results in the fluorescence shift of dansyl and pyrenyl probes to the red side due to increase in micro-polarity inside the gel. But since the repulsive force among $-COO^-$ cannot overcome the hydrophobic interaction and hydrogen bonds, the gel remains in a collapsed state.

Below pH 5.0, an equilibrium between two coexisting phases is established,[12a] which are the aggregate of polymer main chains and polar side group assembly resulting from a competitive interaction between the hydrophobic interaction of polymer main chains and the charge repulsive force of $-COO^-$. Over pH 5.0, the charge repulsive force among $-COO^-$ becomes a dominant interaction in the system. In this situation, many solvent molecules are accessible to the gel and the gel is swollen to only one phase. The micro-environment inside the gel becomes hydrophilic which is indicated by the pyrenyl fluorescent probe.

Acknowledgments. The authors are grateful to Dr. T. Yamashita (University of Tokyo, RCAST) for his help in preparing the samples and Mr. F. Tsunomori (Tokyo University of Agriculture and Technology) for his help in fluorescence lifetime measurements. The authors also thank Prof. M. A. Winnik (University of Toronto) and Dr. F. M. Winnik (Xerox Research Center of Canada) for their suggestive discussion.

REFERENCES

1. A. Yamauchi and Y. Hirokawa, "Kinosei Gel," Shinsozai, One point series 24, (in Japanese), Kyoritsu Press, Tokyo, 1990.
2. T. Tanaka, *Phys. Rev. Lett.*, **40**, 820 (1978).
3. T. Tanaka, D. Fillmore, S. T. Sun, I. Swislow, and A. Shah, *Phys. Rev. Lett.*, **45**, 1636 (1980).
4. Y. Hirokawa and T. Tanaka, *J. Chem. Phys.*, **81**, 6379 (1984).
5. J. Ricka and T. Tanaka, *Macromolecules*, **18**, 83 (1985).
6. E. Kokufuta and T. Tanaka, *Macromolecules*, **24**, 1605 (1991).
7. (a) M. Ilavsky, *Macromolecules*, **15**, 782 (1982); (b) M. Ilavsky, K. Bouchal, and J. Hrouz, *Polym. Bull.*, **24**, 619 (1990).
8. K. Otaka, H. Inomata, M. Konno, and S. Saito, *Macromolecules*, **23**, 283 (1990).
9. A. Peters and S. J. Candau, *Macromolecules*, **21**, 2278 (1988).
10. (a) T. Tanaka and D. Fillmore, *J. Chem. Phys.*, **70**, 1214 (1979); (b) T. Tanaka, E. Saito, Y. Hirokawa, S. Hirotus, and J. Peetermena, *Phys. Rev. Lett.*, **55**, 2455 (1985).
11. H. H. Hooper, J. P. Baker, H. W. Blanch, and J. M. Prausnitz, *Macromolecules*, **23**, 1096 (1990).
12. (a) T. Tokuhiro, T. Amiya, A. Mamada, and T. Tanaka, *Macromolecules*, **24**, 2936 (1991); (b) H. Tanaka, K. Fukumori, and T. Nishi, *J. Chem. Phys.*, **89**, 3363 (1988).
13. See, for example, M. A. Winnik, Ed., "Photophysical and Photochemical Tools in Polymer Science," D. Reidel, Dordrecht, Holland, 1986.
14. (a) Y. Hu, K. Horie, and H. Ushiki, *Macromolecules*, **25**, 6040 (1992); (b) Y. Hu, K. Horie, H. Ushiki, F. Tsunomuri, and T. Yamashita, *Macromolecules*, **25**, 7324 (1992).
15. (a) H. Ringsdorf, J. Venzmer, and F. M. Winnik, *Macromolecules*, **24**, 1678 (1991); (b) F. M. Winnik,

Macromolecules, **23**, 233 (1991).
16. (a) K. Matusi, T. Nakazawa, and H. Morisaki, *J. Phys. Chem.*, **95**, 976 (1991); (b) M. Wilhelm, C. L. Zhao, Y. Wang, R. Xu, M. A. Winnik, J. L. Mura, G. Riess, and M. D. Croucher, *Macromolecules*, **24**, 1033 (1991); (c) D. Y. Chu and J. K. Thomas, *Macromolecules*, **17**, 2142 (1984).
17. (a) K. Kalyanasundaram and J. K. Thomas, *J. Am. Chem. Soc.*, **99**, 2039 (1977); (b) D. C. Dong and M. A. Winnik, *Can. J. Chem.*, **62**, 2560 (1984).
18. K. Dimroth, C. Reiehert, T. Siermann, and F. Bohlmann, *Ann. Chem.*, **661**, 1 (1963).
19. Y. H. Li, L. M. Chan, L. Tyer, T. Moody, C. M. Himel, and D. M. Hercules, *J. Am. Chem. Soc.*, **97**, 3118 (1975).
20. K. J. Shea, G. J. Stoddard, D. M. Shavelle, F. Wakui, and R. M. Choate, *Macromolecules*, **23**, 4497 (1990).
21. M. A. Winnik, H. Xu, and J. M. G. Martinho, *Polymer*, **32**, 84 (1991).
22. K. C. Tam and C. Tiu, *Polym. Commun.*, **31**, 102 (1990).