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Fluorescence study on multiple phase behavior of dimethylacrylamide–methacrylic acid copolymer gel

Masahiko Annaka*, Hiroaki Noda, Ryuuhei Motokawa, Takayuki Nakahira

Department of Materials Technology, Chiba University, Chiba 263-8522, Japan

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Abstract

Multiple phase behavior was found in copolymer gels consisting of dimethylacrylamide and methacrylic acid. They are characterized by distinct degrees of swelling; the gel can take one of a set of swelling values, but none of the intermediate values. Three different phases, denoted as phase051 (as-prepared), phase376 (swollen at high pH), phase440 (swollen after treatment by pH 12) were clearly resolved, where the three digits denote their linear swelling ratios in percentage with respect to the size at preparation. Each phase was stable and did not change its swelling ratio with pH or temperature as long as the values of pH or temperature were within limited ranges. Transitions among different phases were discrete with hysteresis loops. The microenvironment of these three phases was observed by steady-state and transient fluorescence spectroscopy, which indicated the multiple phase behavior appeared as the result of the coexistence of hydrophilic and less-hydrophilic (hydrophobic) domains in the gel and their fraction varied depending on pH.

Keywords: Polymer gel; Phase transition; Fluorescence spectroscopy

1. Introduction

Polymer gels are known to have two phases: swollen and collapsed phases. Volume phase transition occurs between the two phases either continuously or discontinuously [1–3]. Recently, more than two phases were found in copolymer gels consisting of copolymers with randomly distributed positively and negatively charged groups. These phases were characterized by different degrees of swelling at a given pH: the gel can take different degrees of swelling depending on the route taken in the pH–temperature coordinate system. The number of multiple phases appearing in the coordinate system depends on the monomer composition and pH [4,5]. By studying gels that exhibit such a multiple-phase behavior, the criterion for a gel to show the multiple-phase behavior is considered. It is deduced that polymer molecules interact with each other through randomly distributed repulsive and attractive interactions. The latter should be hydrogen bonding plus either hydrophobic or electrostatic interactions. Among these interactions, it has been demonstrated experimentally and theoretically that hydrogen bonding plays an essential role in inducing a multiple-phase behavior [5–7].

Fluorescence methods, such as steady state spectroscopy, fluorescence anisotropy and fluorescence decay measurement have been shown to be quite effective in the investigation for the microscopic environment around a chromophore [8–11]. A number of studies have been reported on the use of excimer or exciplex formation in polymeric systems to examine local segment mobility, phase separation and polymer compatibility. The fluorescence depolarization method has been widely used to monitor molecular motion in polymers, molecular aggregates and biological systems. Although the fluorescence technique was widely used in polymer systems, a few studies have been reported on polymer gels [12–18]. The first fluorescence probe study on a polymer gel was carried out by Horie et al. to investigate the hydrophobicity and dynamic characteristics of cross-linked polystyrene with dansyl probe [12]. They have successfully applied the fluorescence depolarization technique to investigate the volume phase transition of acrylamide gel network induced by the change in solvent composition and/or pH. They revealed that the volume phase transition of polyacrylamide gels is caused by the change in the solvation of the macromolecular chains, which alters the intra- and intermolecular interactions and chain conformation. The rotational mobility of the probe attached to polymer network becomes infinite at the transition point due to the dynamic fluctuation of the network [13–18].

The multiple-phase behavior in polymer gels has so far

* Corresponding author. Tel.: +81-43-290-3409; fax: +81-43-290-3401.
E-mail address: annaka@galaxy.tc.chiba-u.ac.jp (M. Annaka).

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been studied by simple swelling ratio measurements as a function of pH, temperature, solvent quality and so on. Although these studies demonstrated essential roles of the fundamental interactions for triggering volume phase transitions in gels, they have not disclosed any microscopic view of the structure of these kinds of gels. In this paper, we will investigate the microscopic structure of dansyl-labeled dimethylacrylamide (DMAAm)–methacrylic acid (MAAc) copolymer gel by means of fluorescence spectroscopy in order to elucidate the nature of the multiple phases. DMAAm has hydrogen bond acceptor in the monomer unit. MAAc has both hydrogen bond donor and acceptor, and undergoes ionization. As a result, due to a combination of two kinds of hydrogen bonding and electrostatic interaction, DMAAm–MAAc copolymer gels are expected to exhibit multiple phase behavior. The dansyl group has been widely used as a fluorescence probe to study conformational transition in proteins and synthetic polymers [10]. This group has a special photophysical property that provides the local polarity and mobility of the microenvironment [10,19,20].

2. Experimental

2.1. Materials

MAAc, DMAAm, ethylenediamine and acryloyl chloride (Wako Pure Chem. Co.) were purified by distillation under reduced pressure before use. Tetrahydrofuran (THF) and dimethylformamide (Aldrich) were purified according to standard procedures. *N*,*N*′-methylenebisacrylamide (Nakarai Tesque Co.), ammoniumpersulfate, triethylamine (Wako Pure Chem. Co.) and dansyl chloride (Aldrich) were used without further purification.

2.2. Preparation of fluorescent probe monomer

N-(Dansylethyl)acrylamide monomer (DanEAAm) was prepared for incorporation into MAAc–DMAAm copolymer network [11,21].

N-(2-aminoethyl)-5-(dimethylamino)-1-naphyhalenesulfonamide. To a solution of ethylenediamine (4.63 ml, 69.3 mmol) in THF (300 ml) at 0°C was added dropwise dansyl chloride (1.87 g, 6.93 mmol) in THF (150 ml). The reaction was stirred at 0°C for 3 h, and 1 N KOH aqueous solution (10 ml) was added. After THF was evaporated, the aqueous layer was extracted with CH_2Cl_2 (50 ml × 4). The combined organic layer was dried with Na_2SO_4, and was evaporated to leave yellow-green oil (1.73 g, 92%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.51 (d, 1H, ArH), 8.29 (d, 1H ArH), 8.22 (d, 1H, ArH), 7.51 (m, 2H, ArH), 7.15 (d, 1H, ArH), 2.90 (m, 8H, $N(CH_3)_2$ and SO_2NHCH_2), 2.68 (m, 2H, CH_2NH_2).

DanEAAm. To a solution of *N*-(2-aminoethyl)-5-(dimethyl-amino)-1-naphyhalenesulfonamide (0.50 g, 1.7 mmol) in THF (50 ml) at 25°C was added acryloyl chloride (0.14 ml, 1.7 mmol) and triethylamine (0.24 ml, 1.7 mmol). The reaction was carried out for 12 h at 25°C under stirring. The precipitated salt was filtered and was washed with THF. The combined filtrate was evaporated, and the residue was chromatographed on silica gel (Kiesel gel 60, Merck) with $CHCl_3$ as eluent to provide light yellow solid (0.55 g, 93%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.50 (d, 1H, ArH), 8.25 (d, 1H, ArH), 8.18 (d, 1H, ArH), 7.47 (m, 2H, ArH), 7.13 (m, 1H, ArH), 6.60 (bs, 1H, CONH), 6.25 (bs, 1H, SO_2NH), 6.12 (d, 1H, vinyl), 5.88 (dd, 1H, vinyl), 5.48 (d, 1H vinyl), 3.35 (m, 2H, $CONHCH_2$), 3.03 (m, 2H, SO_2NHCH_2), 2.84 (s, 6H, $N(CH_3)_2$).

2.3. Gel preparation

Gels were prepared by radical polymerization: 3.47 g of DMAAm, 3.01 g of MAAc, 0.03 g of DanEAAm, 0.107 g of *N*,*N*′-methylenebisacrylamide (cross-linker), and 8.0 mg of ammoniumpersulfate (initiator) were dissolved in 5.0 ml of dimethylformamide and water mixture (60/40 in volume). The solution was polymerized in a capillary with an inner diameter 140 μm ($= d_0$) at 10°C for 24 h. After completion of gelation, the cylindrical gels were removed from the capillary molds and were washed with distilled water.

2.4. Swelling experiments

The gel was placed in a glass cell whose temperature was controlled within 0.1°C and was continuously flushed with water from a reservoir, in which the pH was controlled by adding HCl solution (to lower pH) or water (to increase pH) below pH 7. NaOH solution was used above pH 7. Equilibrium gel diameter, *d*, was measured under a microscope. To avoid the effect of carbon dioxide in air, all the experiments were carried out under nitrogen gas atmosphere. The temperature was controlled within ±0.1°C by circulating water from LAUDA RM-6B during the measurement.

2.5. Static and dynamic fluorescence measurements

Steady-sate fluorescence spectra and anisotropy were measured with a HITACHI F-4010 fluorescence spectrophotometer. Emission spectra were recorded by exciting at 345 nm. Excitation spectra were monitored at 460 and 530 nm [13]. The excitation and emission slits were set at 5 nm. Emission spectra were not corrected except for the deconvolution of spectra. Quantum yields of deconvoluted spectra were calculated by integration of peak areas of corrected spectra measured in wavenumber units using standard procedure. The anisotropy, *r*, were averaged for emission from 420 to 580 nm. Fluorescence decay measurements were made with HORIBA NAES-1100 single-photon-counting apparatus equipped with a hydrogen pulse lamp. The transient decays of dansyl emission excited at 345 nm were detected at 460 and 530 nm with TOSHIBA U350 and Y43 filters. The half-width of the lamp pulse was

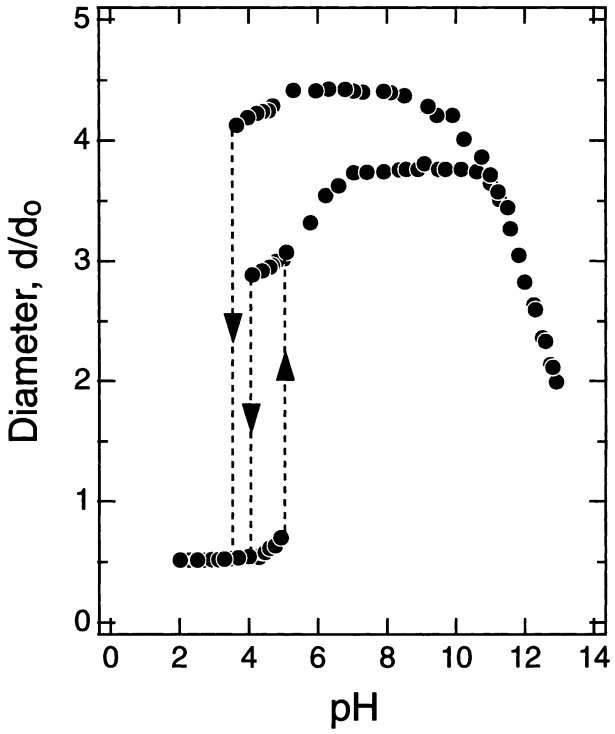

Fig. 1. Equilibrium swelling degree, d/d_0, of dansyl-labeled dimethylacrylamide–methacrylic acid (DMAAm–MAAc) copolymer gel as a function of pH at 20°C.

1.5–2.5 ns. The fluorescence decay curves were analyzed by using a nonlinear least-squares iterative deconvolution method. The temperature of the water-jacketed cell holder was controlled at 20 ± 0.5°C via a water circulating bath.

3. Results and discussion

3.1. Swelling equilibrium

Fig. 1 shows the equilibrium swelling degrees, d/d_0, of dansyl-labeled DMAAm–MAAc copolymer gel as a function of pH at 20°C, where d/d_0 is the linear swelling ratio evaluated by the gel diameter d normalized by the original diameter d_0. DMAAm–MAAc copolymer gel was found to undergo the multiple phase transition as shown in Fig. 1: the gel diameter, $d/d_0 = 0.51$ (phase051) at pH 2. Here, the three digits following the term 'phase' denote the linear swelling ratio in percentage with respect to the size at preparation. As pH was increased, the gel swelled discontinuously to phase220 at pH 4.9. If the pH was lowered from pH 4.9, the gel collapsed to phase054 discontinuously at pH 3.7. If, instead, pH was increased from 4.9, the gel changed its volume to phase376 continuously, and further increase of pH up to 12 caused the gel to shrink continuously. When pH was lowered, the gel swelled continuously to phase440. Further reduction of pH caused the gel to collapse back to phase051 discontinuously. These cycles were reproducible.

3.2. Steady-state fluorescence spectra

The fluorescence spectroscopy of dansyl derivatives has been studied extensively. It is relatively insensitive to quenching by oxygen and trace impurities. The absorption maximum is essentially independent of the medium [9–11,21].

Fig. 2 shows the fluorescence spectra of the dansyl probe attached to the network of DMAAm–MAAc copolymer gel at various pHs on both pH increasing and decreasing process. The dansyl probe exhibits dual fluorescence at 460 and 530 nm when the gel is in the swollen phase. The dansyl group is known to be sensitive to local hydrophobicity, polarity and mobility. When the dimethylamino group takes a coplanar conformation with a naphthyl group in the nonpolar or the hydrophobic microenvironment, λ_{em} of the dansyl group is about 430 nm, and when the dimethylamino group takes a twisted intermolecular charge transfer (TICT) state with a naphthyl group in the polar or the hydrophilic microenvironment, λ_{em} is about 580 nm [9,22]. The shift of λ_{em} from 430 to 580 nm is determined by the twisting angle and the speed of the dimethylamino group with the naphthyl group, which is induced by local hydrophobic interaction, polarity, viscosity and free volume [13,14,21–30].

Strauss and Vesnarver reported that there existed two isosbestic points at 268 and 304 nm in the absorption spectra of dansyl groups in copolymers consisting of maleic anhydride and vinyl alkyl ether, and the absorption between 368 and 304 nm was contributed from the protonated form and that above 304 nm was contributed from the unprotonated form of dimethylamino moiety of the dansyl group [10]. The acid dissociation constant, K_d, of the dimethylamino group is determined to be 3.9 under the presence of 0.5 M NaCl. The emission spectra are characterized by two bands with maxima at 336 and 580 nm which correspond to fluorescence from the excited states of the protonated and unprotonated forms, respectively, with excitation at 268 nm. In the present work, this absorption isosbestic point of the dansyl group due to the protonation of the dimethylamino moiety was not observed, and the excitation wavelength was set at 345 nm. Hu et al. observed the dual fluorescence in the dansyl-labeled weakly charged polyacrylamide gel due to the formation of TICT state. It is reasonable to consider that the observed emission at 460 and 530 nm for the gels are different origins from those observed by Strauss and Vesnarver. The excitation spectra of dansyl group attached to the DMAAm–MAAc copolymer gels monitored at 460 and 530 nm exhibited different ground state of the dansyl probe at each excited state [13,14]. The excitation spectra monitored at 460 and 530 nm at pH 8 on pH increasing processes are shown in Fig. 3. The observed dual fluorescence, therefore, indicates that there exist two emitting states for excited dansyl probes, which suggests the coexistence of hydrophilic and less-hydrophilic domains in the gel.

The fluorescence spectra of dansyl probe attached to the

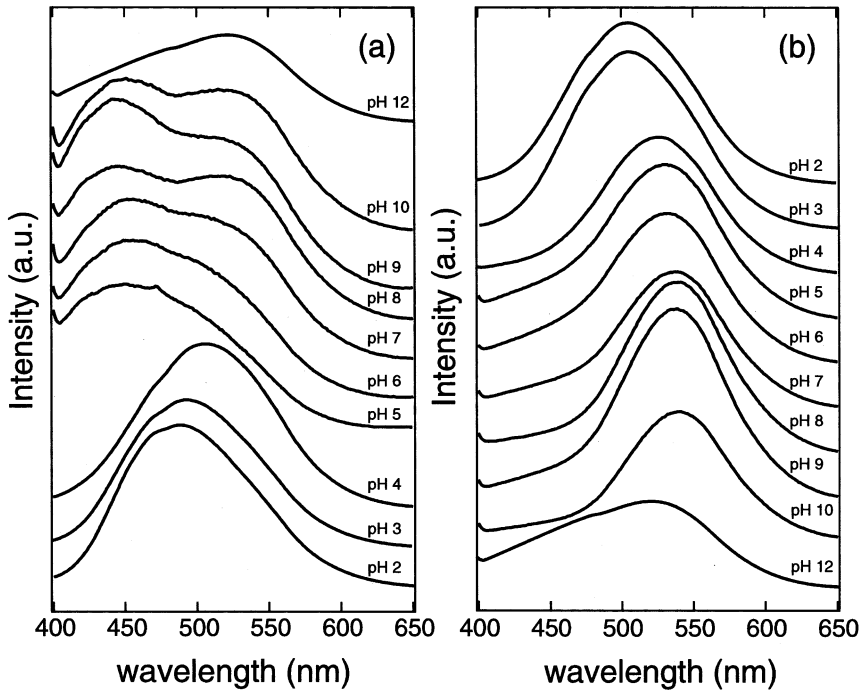

Fig. 2. Fluorescence spectra of dansyl attached to DMAAm–MAAc copolymer gel at 20°C on (a) pH increasing process and (b) pH decreasing process. The excitation wavelength is 345 nm.

DMAAm–MAAc copolymer gel can be resolved into two Gaussian peaks, TICT component and coplanar component. The fraction of TICT components, A_{TICT} is plotted as a function of pH in Fig. 4. The fraction of TICT component of DMAAm–MAAc copolymer gel increases with pH, which is related to the twisting motion of dimethylamino group around the naphthyl group. The change in the fraction of TICT component with pH is completely consistent with

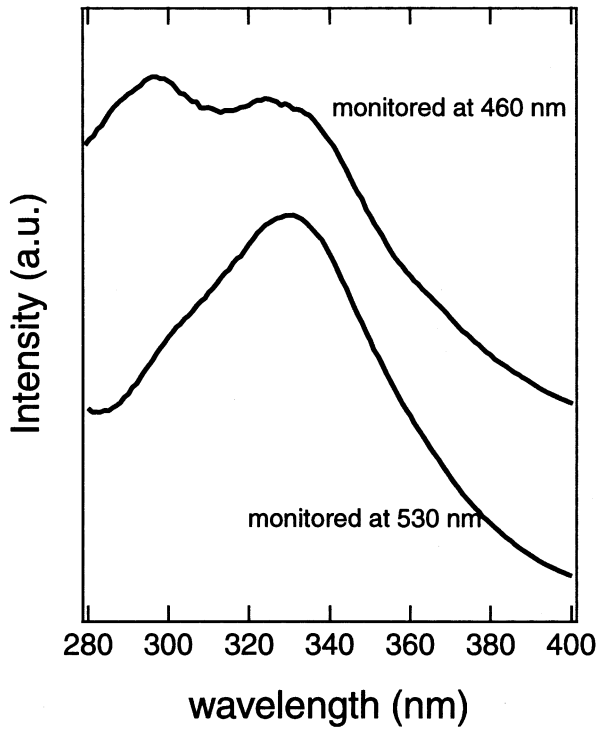

Fig. 3. The excitation spectra at pH 8 monitored at 460 and 530 nm on pH increasing process at 20°C.

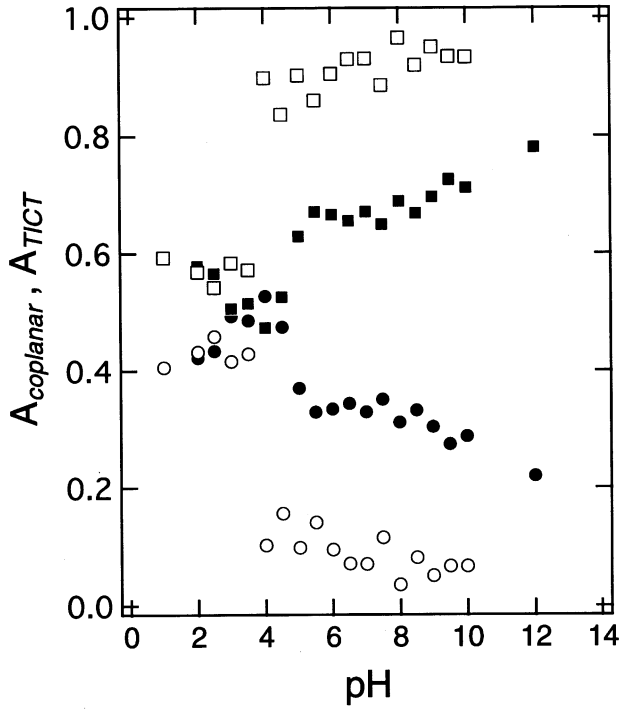

Fig. 4. The fraction of TICT component, A_{TICT} (■, □), and coplanar component, $A_{coplanar}$ (●, ○), as a function of pH. Solid symbol: pH increasing process, Open symbol: pH decreasing process.

the result obtained from the swelling ratio measurement; the fraction of TICT component on pH decreasing process has larger value than that on pH increasing process when the gel is in the swollen state: at pH 5.0, $A_{TICT} = 0.63$ at phase220 and $A_{TICT} = 0.90$ at phase440, and pH 8 $A_{TICT} = 0.69$ at phase376 and $A_{TICT} = 0.97$ at phase440. These results suggest that hydrophilic and less-hydrophilic domains coexist in the gel and their concentration vary depending on pH.

It is interesting to note that the TICT fraction exhibits anomaly in the vicinity of the phase transition. The density fluctuation and the nonequilibrium situation near the phase transition are considered to cause the rapid microscopic transition of the gel network between the solid-like state and the liquid-like state, and twisting motion of dimethylamino group is affected due to the rapid fluctuation of water rearrangement [14,18].

3.3. Fluorescence life time of the dansyl probe

The fluorescence lifetime of the dansyl group is a measure of the micropolarity and the microviscosity sensed by the label. A typical fluorescence decay profile with a residual is shown in Fig. 5 for the dansyl probe attached to DMAAm–MAAc copolymer gel at pH 8.0 on pH increasing process. Just as the steady state fluorescence spectra show two emitting states of dansyl probe attached to the gel, two emitting states are also indicated by the fluorescence transient decays shown in Fig. 6. In the present study, all fluorescence decay curves for DMAAm–MAAc copolymer gel could be satisfactory fitted with the double exponential function of Eq. (1).

$$I(t) = A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2) \quad (1)$$

On pH increasing process, the shorter decay time, τ_1, decreases with pH, while the longer decay time, τ_2, increases with pH. The amplitude of short and long lifetime components, $Q_i = A_i\tau_i / \sum A_i\tau_i$, are indicated in Fig. 7. These results are identical to the results of the pH dependence of the fractions of coplanar and TICT component in the steady-state fluorescence spectra. The shorter decay time, τ_1, corresponds to the TICT state where the dimethylamino group and naphthyl group in the dansyl probe are in a nonplanar excited state in a hydrophilic environment, while the longer decay time, τ_2, corresponds to the dansyl probe with a coplanar excited states in a hydrophobic environment. These results suggest that hydrophilic and less-hydrophilic domains coexist in the gel and their concentrations change depending on pH.

3.4. Dynamics of the dansyl probe

In order to monitor the changes in the rotational mobility of the dansyl group attached to the polymer chain, the fluorescence anisotropy ratio, r, was calculated from four polarized fluorescence spectra by using

$$r = (I_{VV} - GI_{VH})/(I_{VV} + 2GI_{VH}), \qquad G = (I_{HV}/I_{HH}) \quad (2)$$

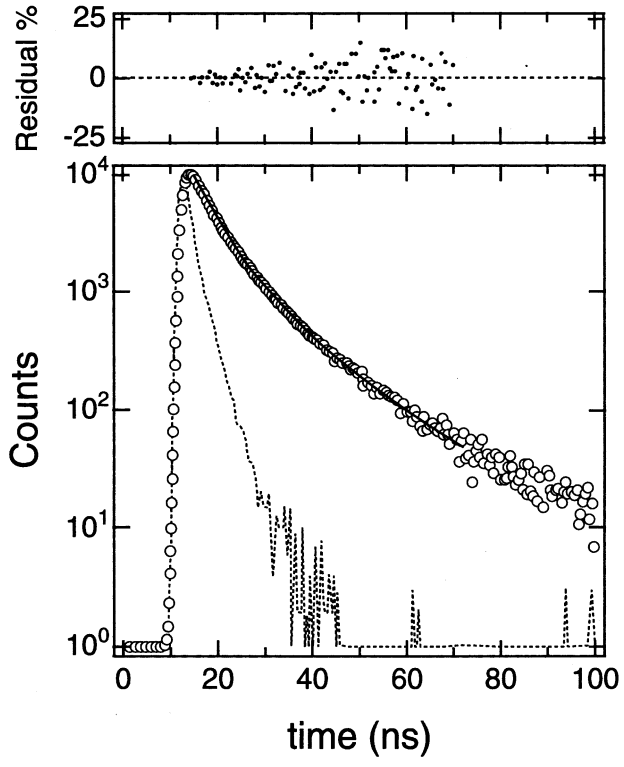

Fig. 5. Fluorescence decay and a residual of the dansyl probe attached to DMAAm–MAAc copolymer gel at pH 8.0 on pH increasing process.

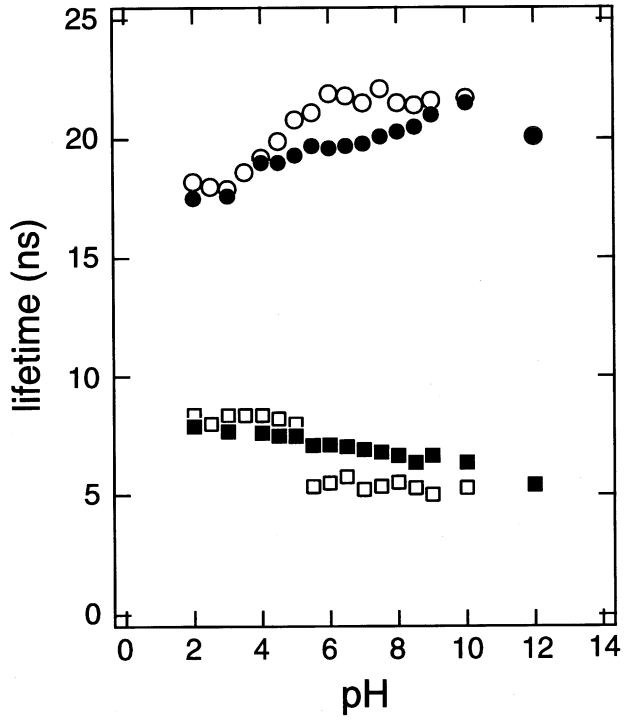

Fig. 6. Fluorescence lifetime of the dansyl probe obtained by using Eq. (1) for DMAAm–MAAc copolymer gel as a function of pH. (a) the short lifetime, τ_1 (■, □), (b) the long lifetime, τ_2 (●, ○). Solid symbol: pH increasing process, Open symbol: pH decreasing process.

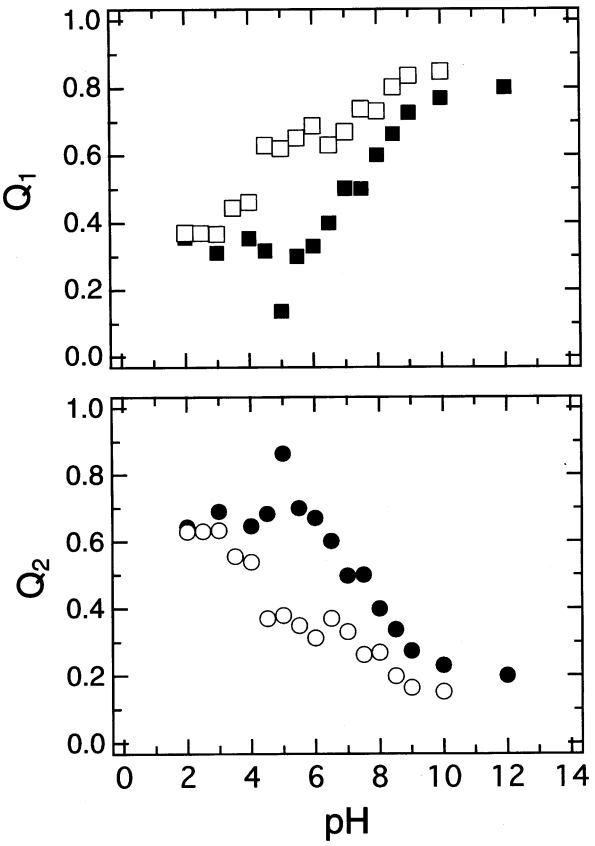

Fig. 7. The changes in the amplitude for (a) short lifetime component, Q_1 (■, □), and (b) long lifetime component, Q_2 (●, ○), of the dansyl probe as a function of pH. Solid symbol: pH increasing process, Open symbol: pH decreasing process.

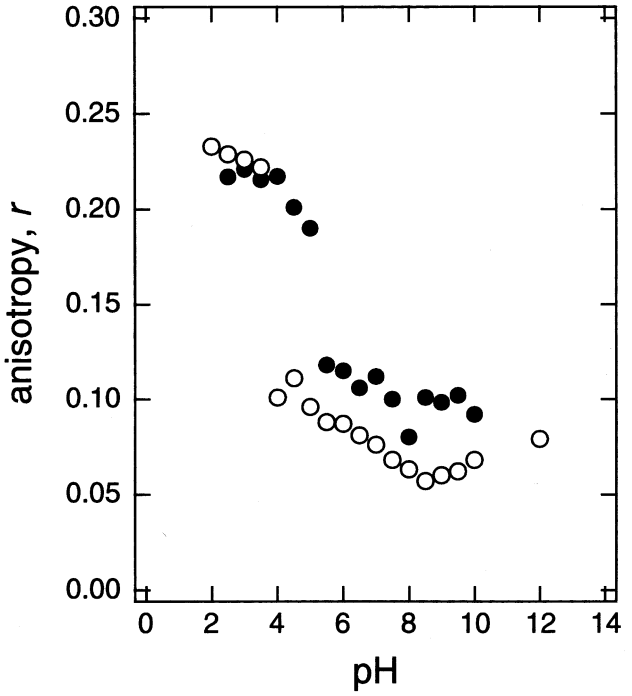

Fig. 8. Fluorescence anisotropy ratio, r, of dansyl probe attached to DMAAm–MAAc copolymer gel as a function of pH. The anisotropy were averaged for emission from 420 to 580 nm. Solid symbol: pH increasing process, Open symbol: pH decreasing process.

where I is the fluorescence intensity and the subscripts represent the orientation of polarizers (V is vertical, and H is horizontal) which are located for incident light (the first subscript) and for emitted light (the second subscript). The G value was used for correcting the depolarization characteristics of the grating-type monochromater. The anisotropy, r, were averaged for emission from 420 to 580 nm. Fig. 8 indicates the change in the anisotropy ratio of dansyl group attached to DMAAm–MAAc copolymer gel on both pH increasing and pH decreasing processes. The anisotropy ratio is rather small when the gel is in the swollen phase (0.099 for phase390 and 0.057 for phase450). In the collapsed gels, it increases dramatically to a maximum of 0.233 at pH 2.0.

The rotational diffusion coefficient, D_{ri}, of the dansyl probe can be calculated from the anisotropy ratio, r, with the lifetime τ_i (τ_1 and τ_2) on the basis of the Perin–Weber equation (Eq. (3)),

$$r_0/r = 1 + (k_B T/v\eta)\tau_i = 1 + 6D_{ri}\tau_i \quad (3)$$

where k_B is the Boltzmann constant, η is the viscosity of the solvent around the dansyl probe, T is the absolute temperature, v is the rotational volume of the dansyl probe, and r_0 is the limiting value of r in the medium where no rotation diffusion takes place or the Brownian motion is frozen. Hu et al. determined the value of r_0 to be 0.325 by extrapolation of r to infinite viscosity for the probe monomer with the dansyl group, DanEAAm, in water/glycerol mixture [13]. It is worthy to note that the anisotropy ratio, r, shown in Fig. 8 does not reach its limiting value, which indicates that the probe constrained in a collapsed gel experiences a high effective viscosity; it is still to undergo some rotational motion. The D_{r_1} and the D_{r_2} of the gel change similarly depending on pH, which are shown in Fig. 9a and b, respectively. The D_{r_1} and the D_{r_2} exhibit sharp changes in the vicinity of the phase transition points, and the rotational diffusion coefficients for pH decreasing process has larger values than those for pH increasing process. This change in the values of the D_{r_1} and the D_{r_2} is completely consistent with the results of swelling behavior and steady-state and lifetime measurement described above. These values for the different phases at the same pH are clearly distinguishable, indicating that each phase has a different local environment.

Coexistence of multiple phases is possible if they each correspond to a minimum in free energy. However, the lowest minimum is the stable equilibrium state and the others represent metastable phases. As the environment varies, the minima can cross, leading to a discontinuous phase transition. The fact that observed transitions always involve a discrete set of swelling degrees and none of the intermediate values, suggests that these swelling degrees correspond to separate free energy minima. Theoretically, multiple phases may be understood as a result of competition

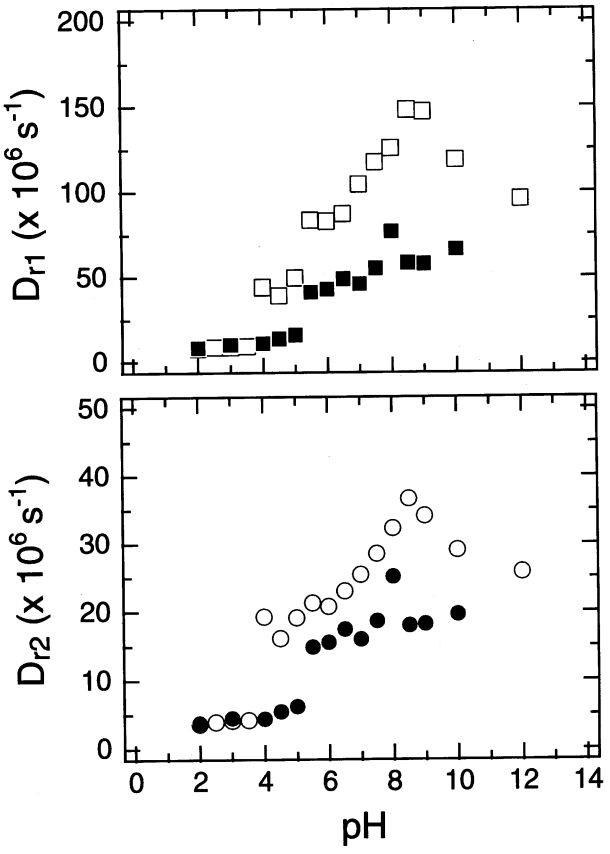

Fig. 9. Changes in the rotational diffusion coefficient, D_{r_i}, of dansyl probe attached to DMAAm–MAAc copolymer gel as a function of pH. D_{r_1} (■, □) and D_{r_2} (●, ○) are, respectively, calculated by using Eq. (3) with the values of lifetime τ_1, τ_2 and $r_0 = 0.325$. Solid symbol: pH increasing process, Open symbol: pH decreasing process.

among various factors. The mean field free energy consists of terms for rubber elasticity, osmotic pressure by counter ions, net charge repulsion and virial interactions [29–32]. These terms with different powers of polymer density can create free energy minima at two distinct densities. It is, therefore, necessary to introduce new order parameters in addition to the polymer density to predict more than three phases. Our previous studies indicate hydrogen bonding density ρ_{HB} may be a natural choice for a new order parameter [6,7]. Formation of hydrogen bonding is energetically favorable, but entropically undesirable since it restricts freedom of chain configurations. This competition can create two free energy minima for a fixed polymer density ρ. Since ρ_{HB} and ρ are coupled, the free energy $F(\rho, \rho_{HB})$ can have four minima in the $\rho-\rho_{HB}$ plane.

DMAAm and MAAc molecules can interact with each other through hydrogen bonding, and MAAc molecules can interact with each other through hydrogen bonding or ionic repulsive interaction. pH influences both hydrogen bonding and the degree of ionization and thus the electrostatic interaction. Recent studies have shown that the incorporation of a hydrophobic moiety into polyelectrolytes leads to a decrease in acidity or basicity [33–35]. One plausible explanation for this decrease in acidity or basicity is related to the dielectric constant surrounding ionizable groups. In the case of DMAAm–MAAC gel at phase051, since formation of hydrogen bonds makes the microenvironment less polar (hydrophobic), the dielectric constant surrounding the charges of carboxyl groups is lowered, which leads to lower the pK_a value. Although a swelling process give rise to an increase in the translational entropy of counter ions, the gel keeps its volume over a wide pH range (pH 5–10) at phase376. This may be due to the fact that some portion of the hydrogen bonds may keep associating and the gel network generates the spatial concentration fluctuation to maintain its volume, which leads to dual fluorescence from both polar and less polar domains. It is worth noting that although the gel maintains its macroscopic volume, the fraction of these domains changes depending on pH. Once the gel experiences pH 12, most of hydrogen bonds dissociate and the gel goes to the phase440.

The observed photophysical properties of DMAAm–MAAc copolymer gel, which exhibits multiple phase behavior, is considered to appear as a result of a combination of hydrogen bonding, hydrophobic interaction and electrostatic interaction between polymer segments. The combination of these interactions leads to the coexistence of hydrophilic and less hydrophilic (hydrophobic) domains in the gel, and their fraction in the system changes depending on the pH.

It is important to determine the local polymer order, if any, at each phase. Since the polymers in a gel are randomly cross-linked and finite in their length, there should be no symmetry breaking of the gel as a whole. Due to the competing interactions, some of the phases, in particular the denser phase, may be non-ergodic [36–38]. That is, the gel is trapped in a configuration corresponding to a local free energy minimum and is not allowed to experience all the other possible configurations with the same free energy. In this case, different cycles may trap the gel in different free energy minima. The ^{13}C NMR was performed on the copolymer gel in which three carbons of the MAAc are replaced by the isotope. Clear distinction between the spectra was observed for different phases at the same pH, which indicated that each phase has different local environment. More extensive study is needed, however, to identify the microscopic structure of the phases.

4. Conclusion

DMAAm–MAAc copolymer gel exhibits multiple phases as characterized by distinct degree of swelling: the gel can take one of the three different swelling values ($d/d_0 = 0.54, 3.9, 4.4$), but none of the intermediate values. Changes in microenvironments accompanied by pH-induced volume phase transition of the gel with dansyl probe are investigated by the fluorescence spectroscopy. The microenvironment of these three phases was observed by steady-state and

transient fluorescence spectroscopy, which indicated that the multiple phase behavior appeared as a result of the coexistence of hydrophilic and less-hydrophilic (hydrophobic) domains in the gel system and their fraction vary depending on the pH.

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