Macromolecules **1999,** *32,* 3989−3993

Preparation Temperature Dependence and Effects of Hydrolysis on Static Inhomogeneities of Poly(acrylamide) Gels

Shin-ichi Takata, Tomohisa Norisuye, and Mitsuhiro Shibayama*

Department of Polymer Science and Engineering, Kyoto Institute of Technology, Matsugasaki, Sakyo-ku, Kyoto 606-8585, Japan

Received February 10, 1999; Revised Manuscript Received March 14, 1999

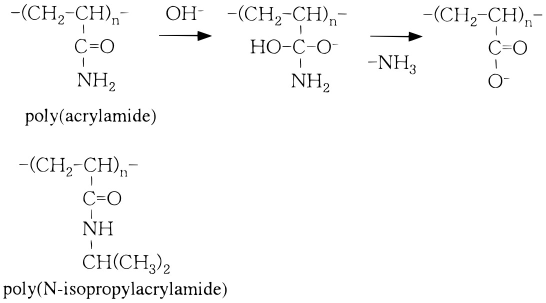
ABSTRACT: The concentration fluctuations of poly(acrylamide) (PAAm) hydrogels prepared at different preparation temperatures, T_{prep}'s, have been studied by dynamic light scattering (DLS). The ensemble average light scattered intensities obtained at the scattering angle of 90°, $\langle I \rangle_E$, was decomposed into two contributions: one from the static inhomogeneities, $\langle I_C \rangle_E$, and the other from thermal fluctuations, I_F. Contrary to the case of poly(*N*-isopropylacrylamide) (PNIPAm) gel, $\langle I \rangle_E$ was a decreasing function of T_{prep}. This indicates that these gels have opposite T_{prep} dependence with respect to the static inhomogeneities. However, the liquidlike properties of these gels, such as I_F and the cooperative diffusion coefficient, D, were found to be independent of T_{prep}, as they should be. It was also found by DLS and swelling experiments that, unlike PNIPAm gels, PAAm gels prepared by redox polymerization were subject to hydrolysis and changed to partially charged gels.

Introduction

Poly(acrylamide) (PAAm) gels are one of the most typical synthetic polymer gels, which have been widely used for the matrix of electrophoresis.[1] It is well-known, however, that a PAAm gel made by redox polymerization changes to a partially charged gel due to hydrolysis of its amide groups. This fact was demonstrated by Tanaka et al.,[2,3] who found a discrete volume phase transition in a PAAm gel, i.e., a discrete change in the volume of the gel by an infinitesimal change of the solvent composition (acetone/water mixture). Later, they recognized that the volume phase transition was due to hydrolysis of the PAAm gel.[4] That is, by aging an as-prepared PAAm gel in a highly basic condition, nucleophilic addition of OH^- takes place as shown in Scheme 1. This results in a substitution of $-CONH_2$ groups to $-COO^-$. As a result, a poly(acrylamide-*ran*-acrylic acid) weakly charged copolymer gel is made.[5] The charged groups introduced by hydrolysis generate a strong Donnan potential across the gel boundary, which leads to a discrete volume phase transition.[2] The degree of hydrolysis in PAAm linear polymers has been quantitatively studied by Zurimendi et al.[6] However, the importance of this effect on the physical properties of PAAm gels has often been ignored in the investigation of PAAm gels except for a few works, such as the work by Mallo et al.[7]

This type of hydrolysis scarcely occurs in poly(*N*-isopropylacrylamide) (PNIPAm) gels because the isopropyl group stabilizes the amide carbon against the attack of OH^-. The chemical stability of PNIPAm gels against hydrolysis is one of the reasons why PNIPAm gels have been often used to study the dynamics and the static inhomogeneities of gels.[8−11] It is now well-known that polymer gels have two types of concentration fluctuations, i.e., the static inhomogeneities and the dynamic fluctuations.[11−15] Hence, the scattered intensity is given by the sum of these two contributions, $I = I_C + I_F$, where I_C and I_F are the time constant (C) and time fluctuating (F) components of the scattered intensity originating from the static inhomogeneities and dynamic fluctuations, respectively. In our previous paper, we demonstrated that the static inhomogeneities in PNIPAm gels depend strongly on the gel preparation temperature, T_{prep}.[10] This fact was confirmed by measuring the ensemble average scattered intensity, $\langle I \rangle_E$, as a function of T_{prep}. Since PNIPAm aqueous solutions have a lower critical solution temperature (LCST)[16] and PNIPAm gels undergo a phase separation around 34 °C,[17,18] it is deduced that the higher T_{prep}, the more inhomogeneous the gel is. On the other hand, I_F and the cooperative diffusion coefficient, D, are found to be independent of T_{prep}. This observation is reasonable since the thermodynamic quantities, such as I_F and D, are not a function of T_{prep} but of T_{obs}, i.e., the temperature at observation. As a matter of fact, I_F and $1/D$ diverged by approaching the phase separation temperature.[19]

Scheme 1. (top) Chemical Structure of Poly(acrylamide) and Its Hydration Scheme; (bottom) Chemical Structure of Poly(*N*-isopropylacrylamide)



By taking account of these facts, we study, in this paper, the dynamics and polymerization/hydrolysis kinetics of PAAm gels in comparison with those of PNIPAm gels. Emphases are placed on the discussions about (1) T_{prep} dependence of the inhomogeneities, (2) the effect of hydrolysis on the static inhomogeneities as well as swelling behavior, and (3) the gelation kinetics of PAAm gels.

---

* To whom correspondence should be addressed.

10.1021/ma990188a CCC: $18.00

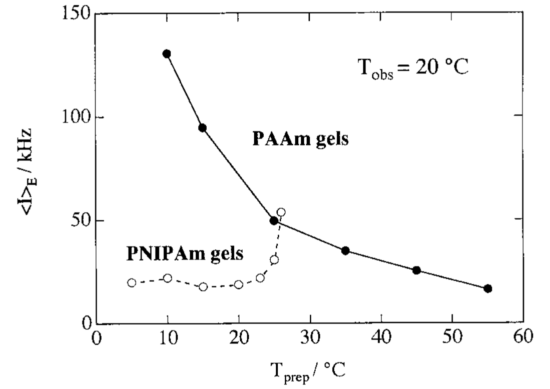


Figure 1. T_{prep} dependence of $\langle I\rangle_{\mathrm{E}}$ for PAAm (solid circles) and PNIPAm gels (open circles). The lines are drawn for the eye. The observation temperature, T_{obs}, was 20 °C.

Experimental Section

Samples. Two types of polymerization were employed to prepare PAAm gels, i.e., radical polymerization and redox polymerization. In both cases, acrylamide (AAm) and *N,N*′-methylenebisacrylamide (BIS) monomers were dissolved in distilled water, whose concentrations were 690 and 8.62 mM, respectively. The polymerization was initiated by adding 8 mM of *N,N,N*′,*N*′-tetramethylethylenediamine (TEMED; accelerator) and 1.75 mM ammonium persulfate. The polymerization was carried out in a 10 mm test tube at several temperatures in the range 10−55 °C for 20 h. Samples for the swelling measurement were made in a micropipet with 471 μm diameter at 20 °C. On the other hand, radical polymerization of PAAm was also conducted at 55 °C without adding TEMED in order to investigate the effect of TEMED on hydrolysis. For comparison, PNIPAm gels were also made by redox polymerization with the same manner from *N*-isopropylacrylamide monomers. The range of T_{prep}'s was limited to be 5−26 °C due to the LCST ($\approx$31 °C) nature of PNIPAm.

Dynamic Light Scattering (DLS). DLS measurements were carried out on a DLS/SLS-5000, ALV, Langen, Germany, with a 22 mW He−Ne laser (Uniphase, U.S.A.). A use of static and dynamic enhancers and a high-quantum efficient avalanche−photodiode detection system (ALV) led to an increase of photon counting rate by a factor of more than 50 times compared to a conventional system with pinholes and a photomultiplier, which was enough large for the evaluation of the cooperative diffuison of gels. All of the DLS measurements were conducted at $T_{\mathrm{obs}} = 20$ °C and at the scattering angle of 90°. Since the scattered intensity was strongly dependent on the sample position due to nonergodicity,[13] the measurement was repeated for 100 times for each sample by rotating the sample tube. The ensemble average scattered intensity was obtained by taking arithmetic average of the scattered intensities.

Swelling Measurement. The degree of swelling was measured by monitoring the diameter of the cylindrical gel, *d*, via an inverted microscope (TMD300, Nikon, Japan) coupled with an image processor (Algas 2000, Hamamatsu Photonics, Japan). The swelling degree was normalized with the mold diameter $d_0 = 471$ μm (micropipet).

Results and Discussion

Decomposition and Preparation Temperature Dependence. Figure 1 shows the T_{prep} dependence of $\langle I\rangle_{\mathrm{E}}$ for the PAAm gels (solid circles) and PNIPAm gels (open circles) observed at 20 °C. The lines were drawn for the eye. It is clearly shown that $\langle I\rangle_{\mathrm{E}}$ for PAAm gels decreases with increasing T_{prep}. Since $\langle I\rangle_{\mathrm{E}}$ is a measure of the spatial inhomogeneities in a gel, this indicates a suppression of spatial inhomogeneities in PAAm gels with increasing T_{prep}. In the case of PNIPAm gels, $\langle I\rangle_{\mathrm{E}}$ remains more or less constant for $T < 20$ °C but steeply increases with T_{prep} for $T > 23$ °C. This is due to the hydrophobic nature of PNIPAm gels. As a matter of fact, divergence of the scattered intensity around 34 °C was reported by light scattering[20] as well as small-angle neutron scattering experiments.[21] It is also well-known that PNIPAm aqueous solutions have a lower critical solution temperature (LCST) around 31 °C.[22,23] On the other hand, PAAm belongs to a polymer with an upper critical solution temperature (UCST)[24] because of the absence of any specific interaction with water compared with PNIPAm.

According to Tanaka et al.,[25] the intensity−time correlation function (ICF), $g^{(2)}(\tau)$, for a gel can be represented by a single-exponential function,

$$g^{(2)}(\tau) = \sigma_{\mathrm{I}}^{2} \exp[-2D_{\mathrm{A}}q^{2}\tau] + 1 \tag{1}$$

where D_{A} is the apparent diffusion coefficient, q is the magnitude of the scattering vector, and σ_{I}^{2} is the initial amplitude of the correlation function. The applicability of eq 1, i.e., the single-exponential behavior for ICF, is discussed elsewhere.[26] In the case of nonergodic media,[13] D_{A} varies randomly with sample position. More precisely, $g^{(2)}(\tau)$ is given by[14]

$$\begin{aligned} g_{\mathrm{p}}^{(2)}(\tau) - 1 = X_{\mathrm{p}}^{2} \exp(-2Dq^{2}\tau) +\\ 2X_{\mathrm{p}}(1 - X_{\mathrm{p}}) \exp(-Dq^{2}\tau) \end{aligned} \tag{2}$$

where D is the cooperative diffusion coefficient of the system. The subscript p denotes that the variable is position (p) dependent. The variable X_{p} denotes the ratio of the intensities from thermal fluctuations divided by that from total concentration fluctuations at a given sample position, p, and is given by

$$X_{\mathrm{p}} = I_{\mathrm{F}}/I_{\mathrm{p}} \tag{3}$$

By evaluating $D_{\mathrm{A,p}}$ at many sample positions, therefore, one can evaluate D and decompose the scattered intensity into those from static inhomogeneities, $\langle I_{\mathrm{C}}\rangle_{\mathrm{E}}$, and thermal fluctuations, I_{F}. The decomposition is attained by plotting $I_{\mathrm{p}}/D_{\mathrm{A,p}}$ vs I_{p}, i.e.,

$$\frac{I_{\mathrm{p}}}{D_{\mathrm{A,p}}} = \frac{2}{D}I_{\mathrm{p}} - \frac{I_{\mathrm{F}}}{D} \tag{4}$$

Note that $D_{\mathrm{A,p}} = D$ is the case for pure homodyne mode scattering ($I_{\mathrm{F}} = I_{\mathrm{p}}$), and $D_{\mathrm{A,p}} \approx D/2$ for pure heterodyne mode scattering ($I_{\mathrm{F}} \ll I_{\mathrm{p}}$). Figure 2 shows the decomposition plots of PAAm gels, from which I_{F} and D were evaluated. The range of I_{p} becomes smaller with increasing T_{prep}, although the slope ($2/D$) is roughly identical. This fact allows one to conjecture a lowering of speckles and invariance of D. In fact, Figure 3 indicates that the variations of (a) I_{F} and (b) D with T_{prep} are much smaller than that of $\langle I\rangle_{\mathrm{E}}$ (in Figure 1) for both PAAm and PNIPAm gels. Therefore, it is clear that the properties related to the liquid nature of a gel, such as I_{F} and D, do not change significantly with T_{prep}, while the static inhomogeneities is sensitive to T_{prep}.

Now, we discuss the universality of the initial amplitude of the correlation function (i.e., the normalized standard deviation of the scattered intensity at $\tau = 0$), σ_{I}^{2} $(=(\langle I^{2}(\tau{=}0)\rangle - \langle I(0)\rangle^{2})/\langle I(0)\rangle^{2})$. By substituting τ

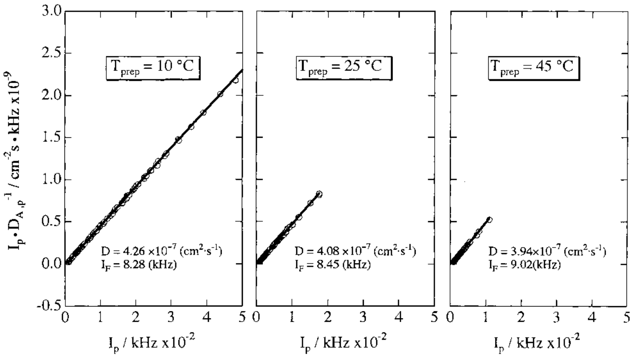


Figure 2. Decomposition plots for PAAm gels prepared at 10, 25, and 45 °C. The intercept and the slope give I_F/D and $2/D$, respectively, from which the cooperative diffusion coefficient, D, and the dynamic component in the scattered intensity, I_F, can be estimated.

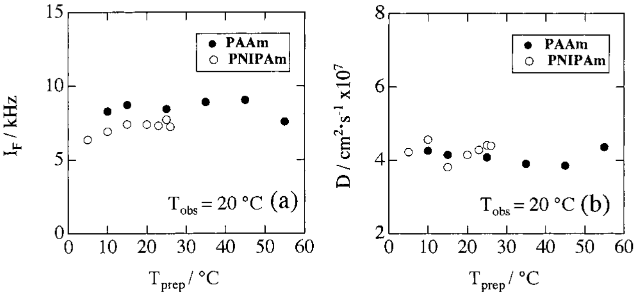


Figure 3. T_{prep} dependence of (a) I_F and (b) D for PAAm gels (solid circles) and PNIPAm gels (open circles).

= 0 in eq 2 and comparing with eq 1, one obtains

$$\sigma_I^2 \equiv \frac{\sigma_{I,obs}^2}{\beta} = X(2 - X) \qquad (5)$$

where $\sigma_{I,obs}^2$ is the observed initial amplitude and β is the experimental coherence factor. Therefore, a master relationship between σ_I^2 and X ($\equiv I_F/I_p$) is expected irrespective of the kind of gels, temperature, the degree of cross-linking, etc. In the case of PNIPAm gels having eight different cross-link concentrations, a well-correlated master curve was obtained, and 800 data points fell on a one master curve as shown in Figure 7 of ref 9. A similar master curve was obtained for PAAm gels observed at 10, 15, 25, 35, 45, and 55 °C. Note that the data points of 600 fall onto the theoretical curve given by eq 5 with much less deviations. The instrumental coherence factor β was estimated to 0.99 from a correlation function for a polystyrene latex, which is again much better than the case in the previous work (β = 0.88). These are entirely due to the improvement of the instrument, i.e., a better stability of the laser source and larger counting rates. The agreement between the experiment and the theory implies that the dynamic light scattering from a gel essentially consists of two contributions as discussed above: one from homodyne and the other from heterodyne modes. This kind of relationship holds, however, only for well-developed gels where the so-called "gel-mode" scattering[25] (i.e., a single-exponential type relaxation in $g^{(2)}(\tau)$) is observed. The criterion of the gel mode can be examined with the decay time distribution function as discussed elsewhere.[26]

As regards the comparison of static inhomogeneities between PAAm and PNIPAm gels, the experimental result is rather contradictory to our expectation since PNIPAm gels are believed to be more inhomogeneous than PAAm gels due to the following reasons: (1) PNIPAm gels carry strong hydrophobic groups which may lead to hydrophobic association of polymer chains in water. (2) The temperature range studied in this work is much closer to the phase separation temperature of PNIPAm gels (≈34 °C)[17,21] than that of PAAm gel (≈−17 °C).[24] Though the reason for this contradiction is not clear at this stage, this may be due to the difference in chemistry of the polymerization/cross-linking.

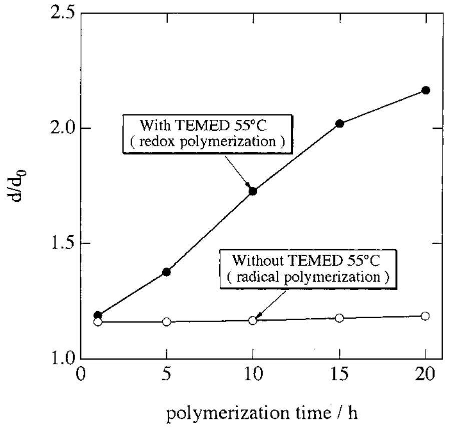


Figure 4. Polymerization time dependence of the swelling degree, d/d_0, for PAAm gels prepared with TEMED (redox polymerization, solid circles) and without TEMED (radical polymerization, open circles).

Effect of Hydrolysis. Mallo et al.[7] investigated the extent of hydrolysis for PAAm gels as a function of aging time and discussed the swelling degree and the cooperative diffusion coefficient. According to their results, the hydrolysis proceeds rather time linearly. Corresponding increases in the swelling degree and in the cooperative diffusion coefficient were also observed. In this section, we discuss the effect of hydrolysis by comparing two types of PAAm gels prepared by redox polymerization (with TEMED) by radical polymerization (without TEMED).

Figure 4 shows the swelling degree, d/d_0, as a function of polymerization time, where d is the radius of cylindrical gel at observation and d_0 is the radius of the mold. The solid circles indicate the values of d/d_0 for PAAm gels prepared at 55 °C with TEMED (redox polymerization) and the open circles denote those without TEMED (radical polymerization). It is clear from this figure that the gels in the presence of TEMED have a higher degree of swelling, and its tendency increases with increasing polymerization time. This eloquently indicates that the basic catalyst, i.e., TEMED, triggers hydrolysis of PAAm gels. Such an effect was not detected for PNIPAm gels. In the case of PNIPAm gels, the amide nitrogen is stabilized due to the presence of isopropyl group as already discussed in the Introduction. This is the reason for the clear difference in the swelling properties between the two systems.

Figure 5 shows aging time dependence of the swelling degree for PAAm gels prepared at different T_{prep}'s and aged in a reactor after completion of gel preparation in the presence of TEMED. Note that the aging was carried out at room temperature. Because of the presence of TEMED, d/d_0 increased with aging time. In addition, the higher the gel preparation temperature, T_{prep}, the higher the d/d_0 was. Since the hydrolysis itself is a chemical reaction, this result, i.e., the positive temperature effect of reaction, is quite reasonable.

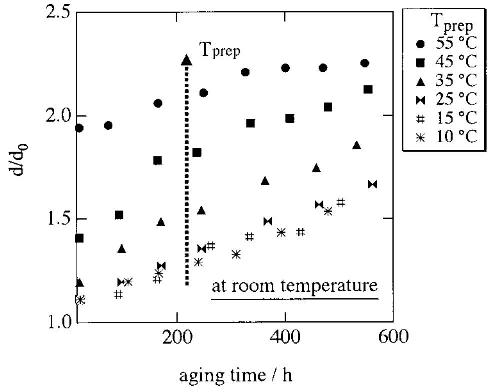


Figure 5. Aging time dependence of d/d_0 for PAAm gels prepared by redox polymerization at different temperatures. The swelling observation was made at room temperature.

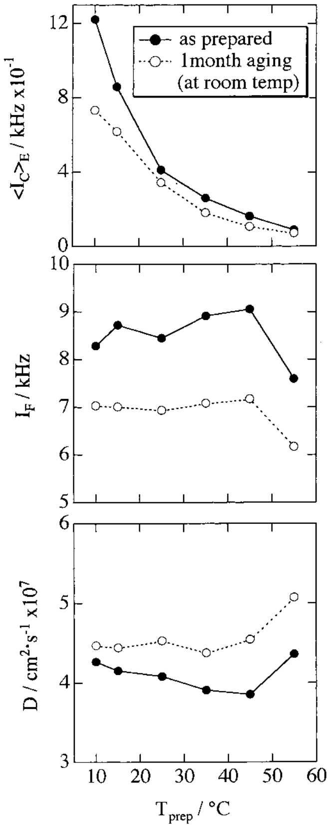


Figure 6. T_{prep} dependence of (a) $\langle I_C\rangle_E$, (b) I_F, and (c) D for as-prepared (solid circles) and 1 month aged PAAm gels (open circles).

The aging effect on the static inhomogeneities and dynamic fluctuations in PAAm gels is shown in Figure 6, where $\langle I_C\rangle_E$ ($\equiv\langle I\rangle_E - I_F$), I_F, and D of 1 month aged PAAm gels are plotted as a function of T_{prep} (open circles with dashed line). For comparison, those for as-prepared PAAm gels (the gelation time being 20 h) are also plotted with solid circles. It is clear from the figure that by aging, in other words, by progressive hydrolysis, both static inhomogeneities ($\langle I_C\rangle_E$) and dynamic fluctuations (I_F) decrease and the cooperative diffusion coefficient D increases (solid circles to open circles). The increase in D is consistent with the result reported by Mallo et al.[7] although the absolute values of D in their work are somewhat larger than those obtained here. This discrepancy may be ascribed to the difference in the TEMED concentrations. The TEMED concentration of the gels reported by Mallo et al. is about 30% larger than those studied here. This may promote a higher degree of hydration, resulting in an increase in D. Furthermore, the following fact should be noted. Mallo et al. underestimated D values (up to by the factor of 2) since they directly evaluated D_A without taking account of nonergodicity. Hence, if they had properly analyzed their data, the difference would have been larger. Our results are in good agreement with our previous observation as well as theoretical prediction.[27] The nonsystematic behavior in I_F and D at $T_{\mathrm{prep}} = 55$ °C is due to the fact that hydrolysis has occurred before the DLS experiment, and its effect appears in the measurement.

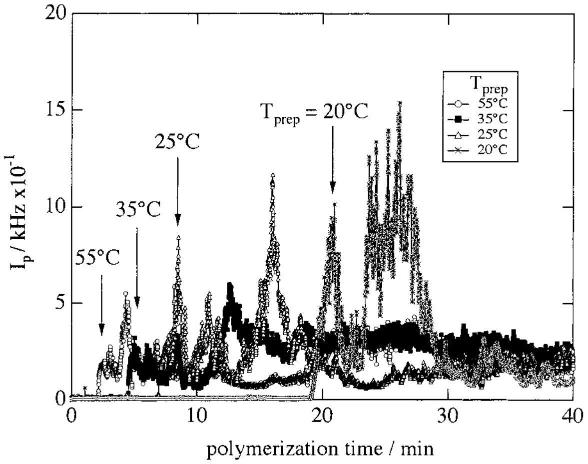


Figure 7. Variation of the scattered intensity observed at 90° during polymerization of PAAm gels in the presence of TEMED at various temperatures. The arrows with temperatures indicate the gelation threshold.

Kinetics of Gelation. According to our previous work,[28] the onset of gelation can be detected by a simple monitoring of the scattered intensity at a given angle. At the gelation point, at least in the case of radical polymerization of amide monomers, a pulselike scattered intensity rise is expected. More quantitatively, the gelation threshold can, in principle, be determined by an appearance of speckles or a power law behavior in ICF as extensively discussed elsewhere.[29]

In Figure 7, the preparation temperature dependence of gelation time is demonstrated, where the gelation threshold is indicated with arrows with polymerization temperature. This shows that the higher the T_{prep}, the faster the gelation is. The subsequent intensity fluctuations observed in each graph indicate formation and/or rearrangement of more complicated structure in the gel.[28] A comparison of the gelation time was also made for PAAm gels prepared with and without TEMED. It was found that the time required for gelation at $T_{\mathrm{prep}} =$ 55 °C was reduced from 30.6 min (without TEMED) to 2.2 min (with TEMED) (i.e., by a factor of 1/14). Thus, the effect of TEMED and of T_{prep} on the gelation kinetics can be quantitatively analyzed by this technique.

Conclusion

The preparation temperature, T_{prep}, dependence of the concentration fluctuations in PAAm gels has been investigated in terms of dynamic light scattering. The results show an opposite tendency between PAAm gels and PNIPAm gels. The ensemble average scattered intensity $\langle I\rangle_E$ from PAAm gels decreases with increasing T_{prep}, while that from PNIPAm gels increases. This was accounted for by the UCST (LCST) nature of PAAm (PNIPAm). The decomposition of $\langle I\rangle_E$ to the static ($\langle I_C\rangle_E$)

and dynamic components (I_F) revealed that I_F is invariant with respect to T_{prep}. A master relationship between the initial amplitude of the correlation function and the ratio of $I_F/I_p \equiv X_p$ was also found, where p indicates that the variable carrying the value is position dependent. This implies a universal relationship between σ_I^2 with X for gels exhibiting the so-called gel mode scattering.

It was found that the effect of hydrolysis is observed exclusively in PAAm gels prepared by redox polymerization. The higher the polymerization temperature and the longer the aging time, the larger the hydrolysis.

Acknowledgment. This work is partially supported by the Ministry of Education, Science, Sports and Culture, Japan (Grant-in-Aid, 09450362 and 10875199 to M.S.). Thanks are due to the Cosmetology Research Foundation, Tokyo, for financial assistance.

References and Notes

(1) Andrews, A. T. *Electrophoresis*; Clarendon Press: Oxford, UK, 1986.

(2) Tanaka, T. *Phys. Rev. Lett.* **1978**, *40*, 820.

(3) Tanaka, T. *Polymer* **1979**, *20*, 1404.

(4) Tanaka, T. *Sci. Am.* **1981**, *244*, 110.

(5) Miller, G.; Fenyo, J. C.; Selegny, E. *J. Appl. Polym. Sci.* **1980**, *25*, 627.

(6) Zurimendi, J. A.; Guerrero, S. J.; Leon, V. *Polymer* **1984**, *25*, 1314.

(7) Mallo, P.; Candau, S.; Cohen, C. *Polym. Commun.* **1985**, *26*, 232.

(8) Sato-Matsuo, E.; Orkisz, M.; Sun, S.-T.; Li, Y.; Tanaka, T. *Macromolecules* **1994**, *27*, 6791.

(9) Shibayama, M.; Norisuye, T.; Nomura, S. *Macromolecules* **1996**, *29*, 8746.

(10) Shibayama, M.; Takata, S.; Norisuye, T. *Physica A* **1998**, *249*, 245.

(11) Shibayama, M. *Macromol. Chem. Phys.* **1998**, *199*, 1.

(12) Bastide, J.; Leibler, L. *Macromolecules* **1988**, *21*, 2647.

(13) Pusey, P. N.; van Megen, W. *Physica A* **1989**, *157*, 705.

(14) Joosten, J. G. H.; McCarthy, J. L.; Pusey, P. N. *Macromolecules* **1991**, *24*, 6690.

(15) Panyukov, S.; Rabin, Y. *Phys. Rep.* **1996**, *269*, 1.

(16) Shild, H. G. *Prog. Polym. Sci.* **1992**, *17*, 163.

(17) Hirokawa, Y.; Tanaka, T. *J. Chem. Phys.* **1984**, *81*, 6379.

(18) Shibayama, M.; Tanaka, T. *Adv. Polym. Sci.* **1993**, *109*, 1.

(19) Shibayama, M.; Fujikawa, Y.; Nomura, S. *Macromolecules* **1996**, *29*, 6535.

(20) Sato-Matsuo, E.; Tanaka, T. *J. Chem. Phys.* **1988**, *89*, 1695.

(21) Shibayama, M.; Tanaka, T.; Han, C. C. *J. Chem. Phys.* **1992**, *97*, 6829.

(22) Heskins, M.; Guillet, J. E. *J. Macromol. Sci. Chem.* **1969**, *2*, 1441.

(23) Schild, H. G. *Prog. Polym. Sci.* **1992**, *17*, 163.

(24) Tanaka, T.; Ishiwata, S.; Ishimoto, C. *Phys, Rev. Lett.* **1977**, *38*, 771.

(25) Tanaka, T.; Hocker, L. O.; Benedek, G. B. *J. Chem. Phys.* **1973**, *59*, 5151.

(26) Norisuye, T.; Takeda, M.; Shibayama, M. *Macromolecules* **1998**, *31*, 5316.

(27) Shibayama, M.; Kawakubo, K.; Norisuye, T. *Macromolecules* **1998**, *31*, 1608.

(28) Norisuye, T.; Shibayama, M.; Nomura, S. *Polymer* **1998**, *39*, 2769.

(29) Norisuye, T.; Shibayama, M.; Tamaki, R.; Chujo, Y. *Macromolecules* **1999**, *32*, 1528.

MA990188A