Dynamic Light Scattering Analysis on Critical Behavior of Cluster Size Distribution of Polyacrylamide and Agarose Solutions near the Sol-Gel Transition Point

Tomoyuki FUJII, Toshimasa YANO, Hitoshi KUMAGAI and Osato MIYAWAKI

Department of Applied Biological Chemistry, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113–8657, Japan

The dynamic light scattering (DLS) method was applied to measure the cluster radii of polyacrylamide and agarose solutions near the sol-gel transition point. In the case of polyacrylamide, primary clusters were formed first and secondary clusters on a larger scale were sequentially formed in the progress of the polymerization process. The z-average radius of the secondary cluster near the sol-gel transition point increased divergently with an increase in acrylamide concentration to the transition point. By fitting the data with the scaling law, the sol-gel transition concentration and the critical exponent were determined to be 1.53% and 0.84, respectively. The z-average radius of the agarose cluster also showed a tendency to diverge with an increase in concentration to the sol-gel transition point. With fitting by the scaling law, the sol-gel transition concentration and the critical exponent of the agarose solution were determined to be $4.5 \times 10^{-2}\%$ and 0.92, respectively, at 27.0°C. Thus, the percolation theory was proved to be effective to describe the cluster size distributions near the sol-gel transition point for both polyacrylamide and agarose solutions.

Gels are widely used in the food industry, the analytical field, and the medical field. Agarose is one of the typical natural gelling substances frequently used in these fields. In studies on the microstructure of agarose molecule, electron microscopy (Amsterdam *et al.*, 1975; Waki *et al.*, 1982; Sugiyama *et al.*, 1994) and x-ray diffraction (Arnott *et al.*, 1974; Foord & Atkins, 1989) revealed that molecules are in the form of double helices interconnected with side-by-side associations.

In the gelation process, it has been proposed that the sol-gel transition is analogous to the critical phenomena (De Gennes, 1979), such as magnetization phenomena near the Curie point. The gelation point is also believed to be a critical threshold: only a sol exists below the gelation point just as no magnetization exists above the Curie point. On the contrary, a gel exists above the gelation point and the magnetization is non-zero below the Curie point. The behavior of the magnetization near the Curie point is well described by the percolation theory (Stauffer & Aharony, 1992). In this connection, the macroscopic property of viscosity or elastic modulus of the gelation system has been measured, and the percolation theory has been successfully applied to the behavior of those macroscopic properties near the sol-gel transition point (Adam *et al.*, 1985; Tokita & Hikichi, 1987, Tokita, 1989; Yano *et al.*, 1993, Kumagai *et al.*, 1993; Kawabata *et al.*, 1996; Kjoniksen & Nyström, 1996a; Durand *et al.*, 1997). However, not only those macroscopic approaches but also a microscopic approach involving cluster-size distribution analysis is necessary to elucidate the mechanism of sol-gel transition.

The cluster size distribution has been analyzed by GPC technique. However, it is difficult to apply this technique without the breakdown of agarose clusters formed by non-covalent bonds. The dynamic light scattering technique is a non-destructive

method useful in polymer and colloid sciences (Chu, 1975). In this work, the dynamic light scattering method was applied to measure distributions of the hydrodynamic radii of clusters non-destructively. First, we investigated the critical behavior of the solution of polyacrylamide that was previously shown to be an appropriate model of the percolation theory (Yano *et al.*, 1993). Then, the critical behavior of agarose solution was analyzed and discussed from the viewpoint of scaling concept, and with reference to electron microscopy observations.

Theoretical

Percolation theory In the lattice model (Stauffer *et al.*, 1982, Stauffer & Aharony, 1992), each site of the lattice is occupied randomly with a probability p, independent of its neighbors; a cluster is thus defined as a group of neighboring occupied sites. Figure 1 shows the percolation networks obtained from this procedure. A collection of finite clusters is present for small p, whereas one infinite network exists for p close to unity. In the gelation process, a single monomer molecule represents the lattice site, and p implies the initial concentration of monomers. Polymers correspond to clusters and a gel corresponds to an infinitely large cluster. A sol-gel transition point is defined as a point at $p=p_c$. Thus a sharp phase transition takes place at an intermediate critical point, p_c, where an infinite cluster starts to appear. An infinite cluster means a network that extends from one end of the system to the other end as illustrated in Fig. 1. The scaling law describes a physical property, X, by a power form of a state variable, p, near a critical point as follows:

$$X \propto |p - p_c|^{-a}, \tag{1}$$

where a is a critical exponent.

The correlation length, ξ, is defined as the average distance of two sites belonging to the same cluster and is related to 'a radius of gyration' R_g:

E-mail: atomic@mail.ecc.u-tokyo.ac.jp

Fig. 1. Illustration of percolation network in lattice model.

$$\xi^2=\frac{\Sigma_s R_s^2 s^2 n_s}{\Sigma_s s^2 n_s},\qquad(2)$$

where s and n_s are the size of cluster and the number of clusters at a size s, respectively. The expression of the right-hand side of Eq. (2) is called a z-average of the squared cluster radius. The z-average of the cluster radius of gyration is thought to be similar to the correlation length ξ, which is described near the critical point by a power law of monomer concentration, C, as follows:

$$\xi\propto|C-C_g|^{-\nu}\text{ (sol-gel transition)},\qquad(3)$$

where C_g is sol-gel transition concentration, and ν is its critical exponent. The critical exponent of the correlation length in the percolation theory has been predicted to be about 0.88 by a computer simulation (Stauffer et al., 1982).

Experimental

Materials and sample preparation   A specified amount of acrylamide, 0.300 g of N,N'-methylene bisacrylamide, and 0.100

g of ammonium persulfate were dissolved in distilled water. Total weight of the sample solution was 100 g. The solution was filtered through a Millipore filter of 0.025 μm in pore diameter at a constant temperature of $40\pm0.1°$C. Polymerization reaction was started by adding 100 μl of 3-dimethyl-aminopropionitrile to the solution. Details of the preparation were described previously (Yano et al., 1993).

Agarose (Nacalai Tesque (Tokyo), Type SE) was dissolved in distilled water at 98°C, then cooled down to 27°C. Total weight of the sample solution was 100 g. The solution was held at $27\pm0.1°$C for 60 min, and analyzed at that temperature.

All the chemicals were obtained from Nacalai Tesque (Tokyo) and were of reagent grade. The distilled water was filtered through a Millipore filter with pore diameter of 0.025 μm.

Dynamic light scattering analysis   The dynamic light scattering method (Chu, 1975) has been used to measure the hydrodynamic colloidal particle size, which can be determined from the intensity of the fluctuations by the Brownian movement of the particles that occur over a short time interval. In this method, the light intensity $I(t)$ at a time t and that at a time $t+\tau$ are measured. From these two data, the autocorrelation function, $C(\tau)$, and the normalized autocorrelation function, $g^1(\tau)$, are calculated as follows:

$$C(\tau)=\langle I(t)\cdot I(t+\tau)\rangle,\qquad(4)$$

$$g^1(\tau)=\frac{C(\tau)-C(\infty)}{C(0)-C(\infty)}.\qquad(5)$$

As the sample, in general, is polydisperse, the correlation function is described by a distribution function:

$$g^1(\tau)=\int_0^\infty G(\Gamma)\exp(-\Gamma\tau)d\Gamma,\qquad(6)$$

where $G(\Gamma)$ is the normalized distribution function, and Γ is the relaxation rate which is related to the translational diffusion coefficient of the particle, D, and the magnitude of the scattering vector, Q.

$$\Gamma=D Q^2.\qquad(7)$$

D is related to the hydrodynamic radius R_h by the Stokes-Einstein equation and Q is related to wavelength of the incident light, λ, refractive index of the solution, n, and scattering angle, θ, as follows:

$$D=\frac{k_B T}{6\pi\eta R_h},\qquad(8)$$

$$Q=\frac{4\pi n}{\lambda}\sin\frac{\theta}{2},\qquad(9)$$

where k_B is Boltzmann's constant, T is absolute temperature, and η is solvent viscosity. The normalized size distribution function $G(R_h)$ is obtained from $G(\Gamma)$ by using Eqs. (7), (8), and (9). A z-average value of the hydrodynamic radius $\langle R_h\rangle_z$ is given by

$$\langle R_h\rangle_z=\int_0^\infty R_h G(R_h)dR_h.\qquad(10)$$

The autocorrelation function was measured with the Malvern 4700 photon correlation spectroscopy system, utilizing a 30 mW, 633 nm He-Ne laser (NEC). The scattering angle was 90°.

Electron microscopy   Agarose sample solutions of 10 μl at 27°C were deposited and mixed with 10 μl of 3% methylamine tungstate on an electron microscopy copper grid covered with a carbon film. The samples were then dried in air and were stained negatively. TEM measurements were carried out with a JEOL electron microscope (model JEM-2000EX).

Results and Discussion

Cluster radius distribution of polyacrylamide solution Polyacrylamide forms clusters by covalent bonds. Figure 2 shows the change of cluster radius distribution in the polymerization process at acrylamide concentration of 1.33% using the dynamic light scattering method. A peak at about 25 nm in the cluster radius appeared after 6 min of polymerization. At 10 min, another peak at about 50 nm in the cluster radius appeared in addition to the first one. The second peak grew larger than the first peak as the polymerization reaction progressed. The cluster radius of the second peak grew to about 150 nm after 89 min. This result suggests that the primary clusters were formed first, and the secondary clusters were sequentially generated in the polymerization of acrylamide. Kjoniksen and Nyström (1996b) reported that two relaxation modes exist in the gelation process of poly(vinyl alcohol) solution.

Figure 3 shows the time-courses of the z-average of hydrodynamic radius of the primary and the secondary clusters. The reaction was completed after 40 min.

Critical behavior of polyacrylamide solution near the sol-gel transition point Figure 4 shows the dependence of the z-average of hydrodynamic radius of the primary and secondary clusters 120 min after the beginning of the polymerization reaction. The z-average radius of the primary cluster was not dependent on the acrylamide concentration, and its value was about 25 nm, which is almost equal to the hydrodynamic radius of polyethylene oxide of 4×10^5 in molecular weight estimated from the relationship between molecular weight and hydrodynamic radius in water (Kuga, 1981). Polyethylene oxide is a representative molecular weight standard in GPC. On the other hand, the z-
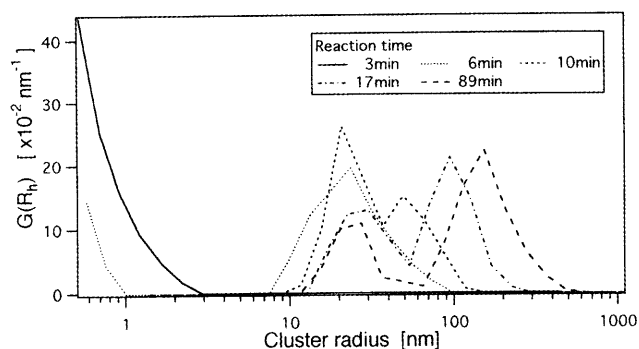
average radius of the secondary cluster near the sol-gel transition point increased divergently with increase in the acrylamide concentration to the sol-gel transition point. The z-average radius of the secondary cluster went up to about 270 nm at the acrylamide concentration of 1.43%. This result indicates that the secondary cluster plays an important role in the sol-gel transition of acrylamide.

Figure 5 shows the double logarithmic plot of the z-average radius of the secondary cluster vs. the deviation in concentration from the sol-gel transition point. Fitting the data with the scaling law, the sol-gel transition concentration and the critical exponent were determined to be 1.53% and 0.84, respectively. The sol-gel transition concentration previously evaluated from viscosity measurement (Yano et al., 1993) was 1.41%, which is not much dif-

Fig. 4. Dependence of the z-average hydrodynamic radius of the primary and secondary clusters on the acrylamide concentrations (Reaction time: 120 min).

Fig. 2. Changes of cluster radius distributions of polyacrylamide solution (C=1.33%).

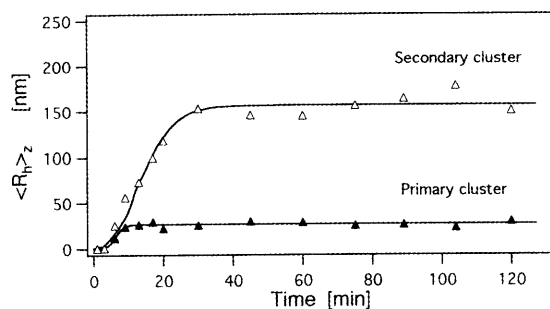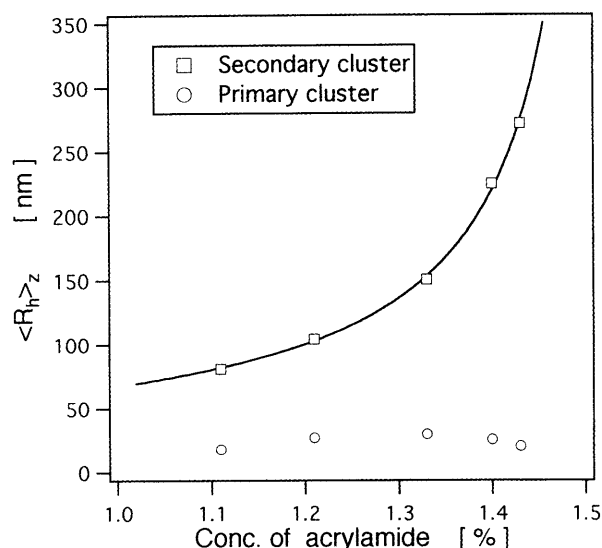**Fig. 3.** Time-courses of the z-average hydrodynamic radius of the primary and secondary clusters of polyacrylamide solution (C=1.33%).
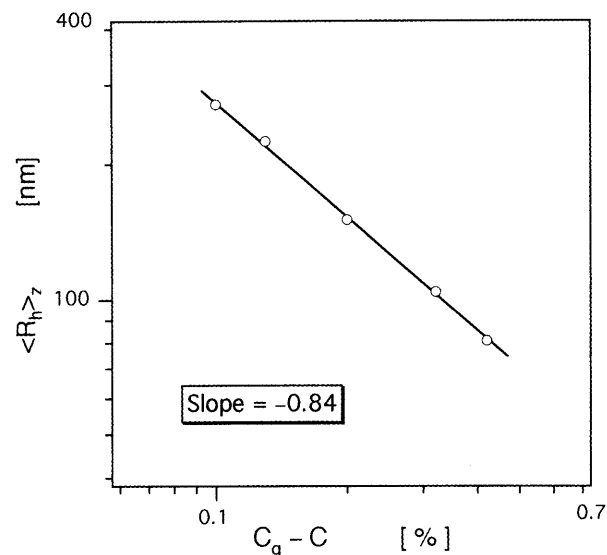

Fig. 5. Double logarithmic plot of the z-average hydrodynamic radius of the secondary cluster vs. the deviation in concentration from the sol-gel transition point (Reaction time: 120 min).
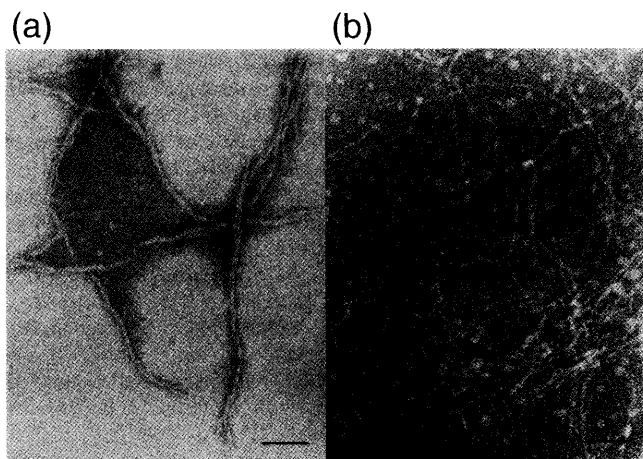
(a) **(b)**

Fig. 6. TEM images of agarose aggregates. (a) Agarose concentration: $3.1 \times 10^{-4}\%$; Bar: 50 nm, (b) Agarose concentration: $3.1 \times 10^{-3}\%$; Bar: 50 nm.
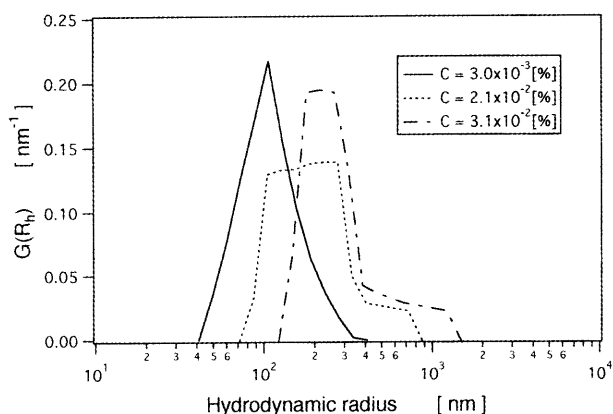

Fig. 7. Distributions of the hydrodynamic radius of agarose aggregates.

Fig. 8. Concentration dependence of the z-average hydrodynamic radius of the cluster of agarose solution (Cg=0.045%).

ferent from the present result. These results indicated that the behavior of the z-average radius of the secondary cluster could be described by the percolation theory near the sol-gel transition point.

Formation of agarose aggregates and gelation Figures 6(a) and (b) show TEM images of the agarose aggregates for agarose concentration of $3.1 \times 10^{-4}\%$ and $3.1 \times 10^{-3}\%$, respectively. Clear individual microfibrils were observed in Fig. 6(a). Agarose gel structure was reported to be a double helix by X-ray scattering on its gel by Arnott *et al.* (1974). In a neutron scattering study, Guenet *et al.* (1993) showed the existence of single-stranded helices in the sol state at 70°C. Dormoy and Candau (1991) observed the rod-like particles by electron microscopy in low-concentration solutions. The major aggregates of agarose in dilute solution were directly found to be double helical strands as shown in Fig. 6.

We can assume that, below the gelling concentration, various sizes of agarose aggregates were randomly formed depending on the agarose concentration, as shown in Fig. 6(b). Therefore, it seems reasonable that the percolation theory is applicable to the clusterization process of agarose. Considering the sol-gel transition of agarose, the agarose molecule plays the role of a unit that
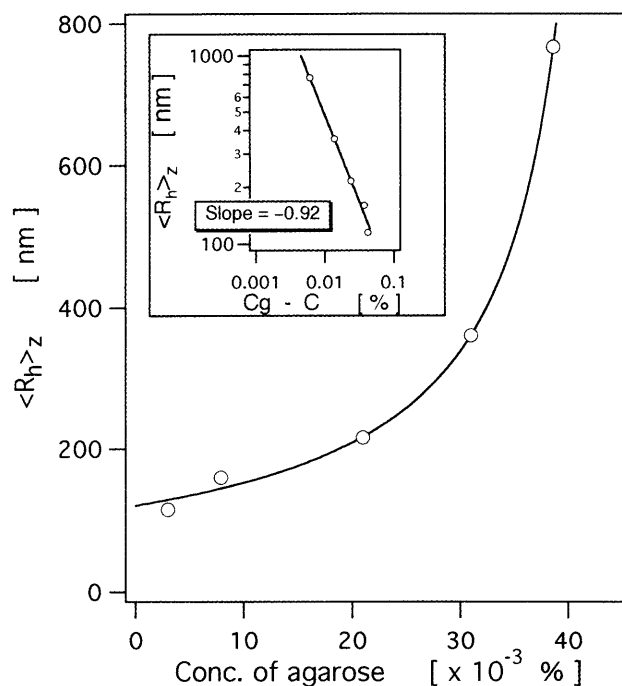
occupies a site of the lattice in the percolation theory.

Critical behavior of agarose solution near the sol-gel transition point Figure 7 shows the typical distribution of the hydrodynamic radii of the clusters of agarose at the temperature of 27.0°C. The distribution curves had a single peak which increased in size with an increase in the agarose concentration, thus indicating a tendency of agarose clusters to form a larger cluster with increased concentration.

Figure 8 shows the concentration dependence of the z-average hydrodynamic radius of the cluster of agarose in solution. The inset shows the double logarithmic plot of the z-average cluster radius vs. the deviation in concentration from the sol-gel transition point. The z-average cluster radius ranged from about 100 nm up to 1000 nm, and indicated a tendency to diverge when the concentration increased and approached the sol-gel transition point. It was possible to fit the data with the scaling law, and the sol-gel transition concentration and the critical exponent were determined to be $4.5 \times 10^{-2}\%$ and 0.92, respectively, at 27.0°C. This value of the critical exponent is close to the theoretical value, 0.88, of the correlation length by a computer simulation (Stauffer *et al.*, 1982). These results indicate that the percolation theory is applicable to the clusterization process in agarose solution with the coarse-graining scale much larger than the original molecular size, irrespective of the polydispersion of agarose.

In conclusion, the cluster radius distribution of both polyacrylamide and agarose could be measured using the dynamic light scattering method. The percolation theory was found to be applicable to the dependence of the z-average of the hydrodynamic cluster radius on the concentration near the sol-gel transition point. The critical exponent of the z-average radius, obtained from the scaling law, was close to the predicted value by a computer simulation.

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