Article

Dilution Effect on the Cluster Growth near the Gelation Threshold

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Cluster growth process during the gelation is attractive from both scientific and biomedical point of views. Until now, there have been many attempts from theoretical and experimental approaches. However, the comparison of the experimental results with the theoretical prediction is ambiguous, because it is impossible to quench the gelation reaction and directly estimate the connectivity in experiments. In this study, we fabricated the states near the critical points using the Tetra-PEG gel system with off-stoichiometrically mixing. We estimated the connectivity directly using the NMR measurement. Using this system, we performed the DLS and viscosity measurements to discuss the cluster growth. The cluster size increase with approaching the gelation critical point showing the characteristic scaling relationship. The scaling exponent depended on the polymer concentration at preparation. At the high concentration region, the exponent agrees with the prediction of the percolation theory. While it downwardly deviates from the prediction in low concentration, which suggests the lattice approximation is failed below the overlapping concentration.

Key Words: Polymer gel / Gelation / Critical exponent / Cluster size / Tetra-PEG gel

1. INTRODUCTION

Hydrogels are the promising materials for biomaterials, because they are composed of 3D polymer network with a water. For the biomedical application, they are often utilized as the injectable gels: they are injected into the body before the gelation and solidified in vivo. This method is minimally invasive, because the dissection operation is not required. To design and fabricate injectable gels in predictable ways, the viscosity and cluster size growth process during the gelation reaction are important to understand.

On the other hand, investigation of network-forming processes has attracted a great interest in modern statistical physics. One of the most general theories to describe network-forming process is the percolation theory¹⁾. Especially, the mixed site-bond percolation²⁾ can be applied to understand processes such as the spreading of an infection, forest fires, and the sol-gel transition etc. Site-bond percolation is a natural generalization of pure site percolation and bond percolation. Until now, many experimental researches on the critical behavior of the sol-gel transition have been carried out to compare the model predictions with the experimental

results. Adam *et al.* quenched a chemical reaction near the sol-gel transition point and analyzed the relationship between the weight average molecular weight and z-average radius of gyration by static light scattering (SLS) measurements³⁾. They used polyurethane polymers synthesized by polycondensation reaction without solvent. They compared experimental results with the prediction of the Flory-Stockmayer theory⁴⁾ and the percolation theory. Martin measured the dependence of reaction conversion on the hydrodynamic radius around the gelation point for diluted irreversible polycondensates of tetramethoxysilicon and analyzed the critical exponents⁵⁾. The conventional theories predict that the maximum cluster size and the weight average molecular weight scaled with the power-law of the relative distance from the critical point ($(\epsilon = |p_c - p|/p_c)$). However, in previous researches, the reaction conversion was estimated from the reaction time assuming that the reaction proceeds ideally. Therefore, it was difficult to precisely compare the experimental result with the theoretical predictions.

Recently, we have succeeded in fabricating a near-ideal polymer network called Tetra-PEG gel⁶⁻⁸⁾. Our previous small angle neutron scattering and NMR studies suggest that Tetra-PEG gel has extremely small amount of spatial and connectivity inhomogeneity, compared with that of conventional gels⁹⁻¹²⁾. Studies of the mechanical properties of the

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Tetra-PEG gel demonstrated that the contribution from the trapped entanglements is negligible and that from the chemical crosslinks is dominant^{9, 10, 13}.

The advantages of the Tetra-PEG gels are follows: (1) the gelation reaction is precisely quenched, and (2) the reaction conversion can be estimated directly. Tetra-PEG gel is formed by A-B type crosslink coupling of two tetra-arm poly(ethylene glycol) units having mutually reactive amine and activated ester terminal groups. Combining tetra-PEG prepolymers at stoichiometrically imbalanced ratios, we can realize the quenched state during the gelation. Furthermore, the reaction conversion is measured by nuclear magnetic resonance (NMR) measurements from the ratio of the reacted amide bond peaks to those from unreacted amino bonds. In this study, we prepare the critical solutions near the sol-gel transition point at several polymer concentrations, and analyzed the ε -dependence of the cluster size. The cluster size is estimated by viscosity measurements and dynamic light scattering (DLS) measurements. We compare our results with conventional theories and discuss the growing process of clusters.

2. EXPERIMENTAL

2.1. Fabrication of the critical solution

Tetra-amine-terminated PEG (tetra-PEG-NH₂) and tetra-OSu-terminated PEG (tetra-PEG-OSu) were purchased from NOF co. (Tokyo, Japan). Here, -OSu stands for *N*-hydroxysuccinimide. The molecular weights (M_w) of tetra-PEG-NH₂ and tetra-PEG-OSu were 20k g/mol. We combined stoichiometrically imbalanced amount of tetra-PEG-NH₂ and tetra-PEG-OSu to control the reaction conversion at the final state. We prepared the samples at various values of polymer concentration ($C_0 = 40, 20, 13$, and 10 g/L). In order to find the critical gelation molar ratios, the molar ratios of tetra-PEG-OSu to total tetra-PEG (r) were varied at each concentration as shown in Table I. Tetra-PEG-NH₂ and tetra-PEG-OSu were dissolved in phosphate D₂O buffer (pH7.4) and phosphate-citric acid D₂O buffer (pH5.8), respectively. Two solutions were mixed, and retained for at least 12 h for the completion of the reaction.

Table I The polymer volume fraction of tetra-PEG prepolymers (ϕ_0) and the range of the molar ratio of tetra-PEG-OSu (r) of the critical solutions.

C_0 [g/L]	r
40	0.11, 0.13, 0.15, 0.17
20	0.17, 0.19, 0.21, 0.23
13	0.22, 0.24, 0.26, 0.28
10	0.27, 0.29, 0.31, 0.33

2.2. Nuclear magnetic resonance (NMR) measurement

The ¹H NMR spectra were measured by using a JEOL Alpha series (500 MHz) spectrometer. ¹H NMR (δ_{ppm} , D₂O): 3.57 (PEG, broad peak), 3.12 (t, 2H, CH₂CH₂CH₂NHCO), 2.99 (t, 4H, C₄H₄(OH)NO₂), 2.52 (t, 2H, CH₂CH₂CH₂NH₂), 2.11 (t, 2H, CH₂CH₂CH₂CH₂CH₂), 1.82 (m, 2H, CH₂CH₂CH₂NH₂), 1.65 (m, 4H, CH₂CH₂CH₂NHCO), 1.47 (m, 4H, CH₂CH₂CH₂CH₂CH₂), 1.21 (m, 2H, CH₂CH₂CH₂CH₂CH₂)

2.3. Dynamic light scattering (DLS) measurement

DLS measurements were carried out using ALV / CGS-3 goniometer system (ALV, Germany). A 22 mW He-Ne laser (wavelength, $\lambda = 632.8$ nm) was used as incident beam, and the scattering angle was 90°. Each measurement time was 30 s. The polymer concentration of the critical solutions was set to 1 g/L, and DLS measurements were performed at 25 °C.

2.4. Viscosity measurement

The intrinsic viscosity was obtained by measuring the viscosity at 25 °C using Ubbelohde-type capillary viscometer. The solution was diluted sequentially to the concentration (c_{diluted}) from the original one (c). The intrinsic viscosity $[\eta]$ and the Huggins constant (k') were evaluated using the Huggins¹⁴⁾ and Kraemer's¹⁵⁾ equations as follows:

$$\frac{\eta_{\text{sp}}}{c} = [\eta] + k'[\eta]^2 c \quad (1)$$

$$\frac{\ln \eta_r}{c} = [\eta] + k''[\eta]^2 c \quad (2)$$

where η_{sp} and η_r are the specific viscosity and relative viscosity defined by $\eta_{\text{sp}} = (\eta - \eta_s)/\eta_s$ and $\eta_r = \eta/\eta_s$, respectively. η and η_s are the viscosities of the sample and solvent, respectively.

3. RESULTS AND DISCUSSION

3.1. Relationships between polymer concentration and the critical reaction conversion

To determine the critical molar ratio (r_c) to form gel at each concentration, we tuned the molar ratio of mutually reactive tetra-arm polymers (r) and performed the dynamic viscoelastic measurements. Figure 1 shows the storage (G') and loss (G'') as a function of angular frequency (ω) at the polymer concentration of 40 g/L. The frequency-dependence of G' and G'' varied accompanied with an increment of r . At $r = 0.19$, G'' was higher than G' at all the frequencies and G'' was proportional to ω^1 , which indicates that the system is still

at sol state. At $r = 0.21$, the plateau region of G' was observed at low frequencies, suggesting that the system is already gelled and a solid. At $r = 0.20$, which is the intermediate value between them, G' and G'' agreed with each other and showed the same frequency-dependence ($\sim \omega^{0.69}$). This is consistent with the well-known Winter-Chanbom criteria¹⁶⁻²¹⁾. Using the dynamic viscoelastic measurements, we determined r_c to be 0.20 at the polymer concentration of 40 g/L. We performed the similar measurements at other concentrations to determine the values of r_c .

To discuss the relationships between the critical cluster size and the distance from the critical point, we converted the value of r_c into the reaction conversion (p_c). In order to estimate the values of p_c , we performed ^1H NMR measurements in D_2O . The reaction conversion was estimated from the ratio of the amide bonds to the unreacted amino groups as follows:

$$p = \frac{2b}{(a+b)(1+r)} \quad (3)$$

where a is the peak intensity of methylene protons of PEG ($\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2$; $\delta = 1.82$ ppm), b is the peak intensity of methylene protons of PEG ($\text{CH}_2\text{CH}_2\text{CH}_2\text{NHCO}$; $\delta = 3.12$ ppm), and r is the molar ratios of tetra-PEG-OSu. The detailed NMR spectrum and calculation was reported elsewhere²²⁾.

Figure 2 shows the critical reaction conversion against the polymer volume fraction (ϕ), which is estimated from the polymer concentration (c). The value of p_c decreased with increasing ϕ ; lower connectivity is required to percolate the system and vice versa. The prepolymers need to grow more to form a gel most probably due to the original occupancy is low at a low ϕ region. This condition is theoretically similar to the mixed site-bond percolation. However, the predicted values by the Monte-Carlo simulation^{23, 24)} shows the deviation from the experimental value especially at lower concentration. This is attributed to the failure of the assumption that the system is approximated to be a lattice at lower concentration region. The detailed on this deviation was reported elsewhere²²⁾.

3.2. ε -dependence of the cluster size near the sol-gel transition point

To discuss the growth of cluster size near the sol-gel transition point, DLS measurements were carried out for each sample diluted into the polymer concentration of 1 g/L, which was much lower than the overlapping concentration of clusters. Figure 3 showed the characteristic decay time distribution functions for the samples with various r for each

Fig. 1 The value of G' and G'' as a function of ω in the 40 g/L (circle; G' , triangle; G''). The dotted lines are the fitting curve showing the relationship, $G'(\omega) \sim G''(\omega) \sim \omega^{0.69}$.

Fig. 2 p_c as a function of polymer volume fraction (ϕ) (sol, cross; gel, circle).

initial polymer concentration. The peaks of the functions shifted right with increasing r . These results suggest that the cluster size increased with increasing r . We estimated the hydrodynamic radius (R_h) from the fast mode based on the CONTIN analysis.

We plotted the R_h against the relative distance (ε) from the critical reaction conversion ($\varepsilon = |p_c - p|/p_c$) at each concentration in Fig. 4. R_h increased with decreasing ε , suggesting that the cluster size grows towards the sol-gel transition point. All the data are scaled with ε as $R_h \sim \varepsilon^{-\nu}$. Notably the various physical properties obey the scaling rule at the vicinity of phase transition. Although the definition of ‘the vicinity of the gelation threshold is ambiguous, the scaling behavior observed in the gyration radius suggest that the system is near the gelation threshold.

The critical exponent (ν) decreased with decreasing c .

Fig. 3 Characteristic decay time distribution functions, $G(\Gamma^{-1})$ of the critical solutions prepared at (a) 40 g/L, (b) 20 g/L, (c) 13.3 g/L, (d) 10 g/L. ($r = r_c - 0.02$, circle; $r_c - 0.04$, triangle; $r_c - 0.06$, inverse triangle; $r_c - 0.08$, rhombus).

This data suggests that the growing process of clusters is steeper in higher polymer concentrations. Notably, the value of R_h was larger in lower concentrations; the clusters grow more in lower polymer concentrations.

The similar tendency was observed in the intrinsic viscosity. Figure 5 shows the representative plots of the reduced viscosity (η_{sp}/c_{dilute}) and the inherent viscosity ($\ln \eta_r/c_{dilute}$) against diluted concentration (c_{dilute}) for the sample prepared at 40 g/L and $r = 0.17$. The intrinsic viscosity was estimated to be 0.21 L g^{-1} as the intercept of eqs. (1) and (2).

Figure 6 shows $[\eta]$ against ε at each concentration. All the data are scaled with ε as $[\eta] \sim \varepsilon^{-\nu'}$, and ν' decreased with decreasing c . Because the intrinsic viscosity reflects the size of the clusters, these results are consistent with the DLS results.

We plotted the critical exponents (ν and ν') against c in Fig. 7. ν and ν' increased with increasing c in similar manners. These data suggest that the cluster growth near the critical point is more rapid at higher polymer concentrations.

The growth of polymeric clusters near the transition point was first described theoretically by Flory⁴⁾ and Stockmayer^{25, 26)}. The model is formulated on the Bethe

Fig. 4 The hydrodynamic radius (R_h) as a function of ε prepared at 40 g/L (circle), 20 g/L (triangle), 13.3 g/L (square), 10 g/L (rhombus). The dotted line is the fitting curve showing the relationship, $R_h \sim \varepsilon^{-7}$. The curves were shifted endways (factor A) to avoid overlap.

Fig. 5 Representative plots of the reduced viscosity (η_{sp}/c_{dilute}) and the inherent viscosity ($\ln \eta_r/c_{dilute}$) against diluted concentration (c_{dilute}) for the sample prepared at 40 g/L and $r = 0.17$.

lattice, and the intramolecular reaction is ignored. Sites within a cluster have equal reactivity, and excluded volume effects are not considered. The Flory-Stockmayer model predicts the scaling relations that $R_h \sim \varepsilon^{0.5}$ and $[\eta] \sim \varepsilon^0$.

On the other hand, Stauffer²⁷⁾ and de Gennes²⁸⁾ emphasized the importance of deviations from the solution of the Flory-Stockmayer model, and proposed percolation models on finite-dimensional lattices. In their works, the critical exponents for the viscosity and the hydrodynamic radius were predicted by applying the percolation model to electric networks or the Rouse dynamics. Especially for the intrinsic viscosity, the exponent was first predicted from the analogy to the electric network. The conductivity is influenced by the current flow, which is defined by the structure of the circuit. The current from a single wire tip depends on whether the tip point is a part of the infinite (percolated) conducting network or not. This picture has an analogy to the elasticity of the

Fig. 6 The intrinsic viscosity ($[\eta]$) as a function of ε for the samples prepared at 40 g/L (circle), 20 g/L (triangle), 13.3 g/L (square), 10 g/L (rhombus). The dotted line is the fitting curve showing the relationship, $[\eta] \sim \varepsilon^{-\nu'}$. The curves were shifted endways (factor A) to avoid overlap.

Fig. 7 Critical exponents (ν , ν') as a function of c (ν , triangle; ν' , circle).

polymer networks. This analogy was applied to the viscosity as well, where the viscosity varies with the conductivity in a random mixture of superconductors (infinite network fraction) and normal conductors (finite network fraction). This model provided the relation that $[\eta] \sim \varepsilon^{4/3}$ for two-dimensional percolation model.

Besides, the exponent ($= 4/3$) for the intrinsic viscosity can be predicted in the percolation model for the Rouse dynamics. Stauffer *et al.* predicted that the intrinsic viscosity can be determined by the cluster size and the number of the clusters, which is approximated as

$$[\eta] \propto \frac{\int_1^{\xi} s n_s \frac{R_s^z}{s} ds}{\int_1^{\xi} s n_s ds} \quad (4)$$

Here, n_s and s are the numbers of the clusters and units in one cluster near the gelation point, respectively. s_{ξ} is the number of the units in the maximum cluster. Near the gelation point, it is known that the gel system has the polydispersity and the

relationships between the number of the clusters, n_s , having s units is approximately described by the following scaling form, $n_s \propto s^{-\tau}$. This number of units, s , is also scaled by the distance from the critical point ε as

$$s \propto \varepsilon^{-(\beta+\gamma)} \quad (5)$$

β and γ are the critical exponents for the probability that a certain cluster is connected to the infinite network (gel fraction) and the average degree of polymerization, respectively. In the case of the Rouse clusters, where the hydrodynamic interaction is ignored, z equals to $1/\varrho + 2$. ϱ is the exponent of the relationship between the polymer size and the number of units. As a result, the intrinsic viscosity is scaled using ε as

$$[\eta] \propto \varepsilon^{\beta - 2\frac{\gamma+2\beta}{3}} \quad (6)$$

Under the three-dimensional percolation model, β and γ are predicted to be 0.45 and 1.7¹⁾. As a result, the same exponent ($= 1.3$) can be obtained.

The predicted critical exponents were summarized in Table II. Although the exponents of the percolation model depend on the characteristics of network, we here the above mentioned representative prediction for the Rouse dynamics.

Table II Comparison of the critical exponents with the model predictions.

Critical exponent	Flory-Stockmayer model	Percolation model for Rouse dynamics
ν	0.5	0.88
ν'	0	1.3

Here, the values of ν are close to the experimental values, while the values of ν' are totally different from the experimental ones. It is because ν' is defined to be the exponent for the ε -dependence of the “correlation length”, which is different from the cluster size especially above the overlapping concentration. In this study, we diluted the sample below the overlapping concentration. The use of the correlation length here is inappropriate.

Both the Flory-Stockmayer and percolation models did not mention about the concentration dependence of the critical exponents. On the other hand, our data showed the significant concentration dependence. Furthermore, the critical exponents at low concentration is close to the Flory-Stockmayer one, while those at high concentration is close to the percolation one, which seems to be a transition. The Flory-Stockmayer model assumes the Bethe lattice, and ignores the excluded volume effect. At the low concentration region, the excluded volume effect is generally dominant,

which is opposite tendency with our experimental results. Thus, it should be straightforward understood that the exponent just decreases with decreasing the concentration and deviates from the percolation model, because the lattice approximation fails at the low concentration region.

4. CONCLUSION

We fabricate the gelation critical solution using the Tetra-PEG gel system by off-stoichiometrically mixing of the precursor chains. The NMR measurement is performed to estimate the connectivity of the critical solution, and the DLS and viscosity measurements are carried out to evaluate the cluster size. The results showed that (1) the cluster size increased with approaching the gelation critical point; (2) the cluster growth obeyed the scaling relationship, and the exponent is dependent on the preparation concentration; (3) the exponent agrees with the percolation theory above the overlapping concentration, while it downwardly deviates from the prediction below the overlapping concentration; (4) this deviation reflects the failure of the lattice approximation, which is base for the percolation theory. These finding will help to design the novel injectable gels to consider the gelation process.

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