Similarity in Linear Viscoelastic Behaviors of Network Formation and Degradation Processes

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The design of the network formation and degradation processes is required to control hydrogel’s life cycle. There have been many studies to optimize both processes of the hydrogel for biomaterials. The conventional studies based on the assumption that the network formation and degradation processes are equivalent. The premise of the similarity is not self-evident, which is difficult to investigate experimentally. It is because of the difficulty in the experimental determination of the connectivity of the network. In this study, we utilized the Tetra gel with slow degradation units to observe a continuous process from network formation to degradation of a single hydrogel. We measured the time-development of the linear viscoelastic properties during the network formation and degradation processes using a single system. The linear viscoelasticity at the same connectivity and the critical points for gelation and degradation well agreed. These results strongly suggest that the network formation and degradation go along the identical path toward the opposite directions. Based on our findings, we propose a simplified criterion for the gelation over the Winter-Chambon criterion based on the observation. These findings will help a basis for the sol-gel transition behavior of the polymer gels.

Key Words: Gelation / Viscoelasticity / Winter-Chambon criteria / Critical exponent / Tetra Gel

1. INTRODUCTION

Hydrogels show the mechanical softness and biocompatibility, and are promising materials for biomedical applications[1-4]. In the case of the biomedical field, the hydrogels are often utilized as an injectable device; hydrogels are formed and molded on-site. After the treatment, the hydrogels should be degraded and eventually disintegrated in the body. Therefore, it is required to control hydrogels’ whole life cycle from gelation to disintegration (reverse gelation). To design the hydrogels’ life cycle, the elucidation of the gelation and degradation mechanism is essential.

There have been many attempts to reveal and control the physical properties during gelation and degradation processes. In the field of polymer rheology, the gelation process is one of the essential topics; not only the gelation point but also the critical behaviors observed near the gelation point are of great interest[5-12]. In the pioneering works by Winter et al., the gelation point was determined as a critical point where the viscosity diverges to infinity using the dynamic viscoelastic measurement[13-19]. As theoretical approaches, the percolation model has been established under various lattices[20-22]. In the generalized percolation model, the gelation behavior is discussed using two probabilities that sites are occupied, and neighboring sites are bonded as variables. When comparing the experimental results and theories, the polymer (or monomer) concentration corresponds to the site-occupation probability, and the reaction conversion is equivalent to the bond connectivity.

As for the degradation process, numerous experimental studies have been carried out to control the degradability for practical applications[23-29]. Most of the studies assume that the gelation process and the degradation process are identical, and the only difference is the “direction”. This assumption is not self-evident, and the precise investigation of the similarity between the gelation and degradation processes were limited. This limitation is attributed to the difficulty in the experimental determination of the connectivity of the network.

In 2008, we succeeded in designing the novel gel system, Tetra gel[30-34]. The Tetra gel is formed by the crosslink-coupling between two types of symmetric tetra-armed prepolymers. The end groups are mutually reactive and prohibit the intramolecular self-biting reactions. The crosslinking modules are precisely synthesized, leading to the ideal polymer networks with uniform strand length and functionality. Besides, the connectivity of the Tetra gel is estimated using the spectroscopy. Therefore, Tetra gel is a promising
model system for understanding the physics of polymer networks.

In this study, we investigate the similarity between network formation and degradation processes by observing a continuous process from network formation to the degradation of a single hydrogel. For the purpose, we used a reaction between Tetra-polyethylene glycols with mutually reactive thiol and acrylate end groups (Tetra-PEG-SH and Tetra-PEG-ACR, Fig. 1). In the gelation regime, SH and ACR react and form a linkage between corresponding PEG arm ends. On the other hand, an ester bond linking ACR with the PEG backbone dissociates through the hydrolysis in the degradation process. To observe the continuous process from network formation to degradation, the system was set to the following (Fig. 1): (i) The rate of hydrolysis is set to be much lower than that of the thiol-ene reaction so that these processes are chronologically separated. (ii) The maximum fraction of connected arms was precisely set to just above the gelation point so that the degradation proceeded in a limited time range. We found the similarity between the gelation and reverse gelation processes throughout the analysis on the critical behavior. Furthermore, we propose a simplified criterion for the gelation over the Winter-Chambon criterion based on the observation.

2. EXPERIMENTAL SECTION

2.1 Samples preparation

Tetra-acrylate-terminated PEG (Tetra-PEG-ACR) and tetra-Thiol-terminated PEG (Tetra-PEG-SH) were purchased from NOF Co. (Tokyo, Japan). The molar mass of prepolymers ($M_n$), Tetra-PEG-ACR and Tetra-PEG-SH, were matched to each other ($M_n = 10$ kg mol$^{-1}$). Constant amounts of Tetra-PEG-ACR and Tetra-PEG-SH were dissolved into
the deuterated phosphate buffer (pH7.4, 100 mM). The concentration of PEG was set to be 7.5, 20, 40, and 60 g L⁻¹. We mixed two solutions with off-stoichiometric ratio \( r = \frac{\text{Volume of Tetra-PEG-SH solution}}{\text{Total solution volume}} \). Here, we set \( r = 0.305 \) to observe the sol-gel transition points in both the gelation and degradation processes within two weeks.

2.2 Dynamic viscoelasticity measurement

We measured the dynamic viscoelasticity. The mixing solutions were poured into the interstice of the double cylinder of a rheometer (MCR301; Anton Paar). The storage modulus \( G' \) and the loss modulus \( G'' \) were measured during the gelation and degradation processes. The applied strain, angular frequencies, and temperature were 0.01–100, 0.1–10 s⁻¹, and 25 ºC, respectively. Before the measurements, we confirmed that the strain was within the linear viscoelastic region.

3. RESULTS AND DISCUSSION

Tetra-PEG-ACR and Tetra-PEG-SH aqueous solutions were mixed, and the precursor solution was poured into the interstice of the double-cylinder of the rheometer. Figure 2 shows the time-evolution of the angular frequency \( \omega \)-dependence of \( G' \) and \( G'' \) during the gelation and degradation processes for the Tetra gel. Because the time for a measurement \((-10^2 \text{s})\) is short compared to that of the network formation and degradation reactions \((10^5 – 10^7 \text{s})\), each experiment is performed under a pseudo-equilibrium condition. As time developed, the following six characteristics were observed in chronological order:

1. \( G' \) was smaller than \( G'' \) showing the terminal flow behavior, \( G' \sim \omega^2 \) and \( G'' \sim \omega^1 \).
2. The slopes of \( G' \) and \( G'' \) decreased, and \( G' \) approached to \( G'' \).
3. \( G' \) increased and became more significant than \( G'' \) at low frequencies, and the \( \omega \)-independent \( G' \) was observed.
4. \( G' \) decreased and approached to \( G'' \) again.
5. \( G' \) became smaller than \( G'' \).

Apparently, the characteristics 1–3 are for the network formation, and 4 and 5 are for network degradation. Notably, throughout the observation, we did not observe the apparent gelation (and reverse-gelation) critical behavior, \( G' \sim G'' \sim \omega^β \), which is well known as Winter-Chanbom criterion \(^{13-19}\).

For comparison, we briefly introduce a typical time-evolutions of \( G' \) and \( G'' \) during gelation (Fig. 3). Here, gray colored box is the experimental window of the rheological measurements in this study. At the initial condition \((p = 0)\), the system shows the terminal flow behavior \((G' \sim \omega^2 \) and \( G'' \sim \omega^1 \)). (ii) Accompanied with the progress of the cross-linking reaction, the slopes of \( G' \) and \( G'' \) become small. This change reflects the broad terminal flow behavior of the polymeric clusters with a wide distribution in size. (iii) At \( p = p_c \), the terminal relaxation mode is infinitely delayed, and the well-known power law that \( G' \approx G'' \sim \omega^β \) is observed. This power law behavior originates from the internal mode of the critical cluster with a fractal structure. (iv) In the region \( p \) is just above \( p_c \), the plateau of \( G' \) is observed, while \( G'' \) still shows the similar scaling law with that in the critical state.

Fig. 2 Change in the angular frequency-dependence \( \omega \) of the storage \( G' \) and loss moduli \( G'' \) during the gelation and degradation processes. Circles and squares represent \( G' \) and \( G'' \), respectively.
The critical scaling is attributed to the relaxation of non-percolating clusters or simply hyperbranched structure in the gel\(^{19}\). (v) At \(p \gg p_c\), \(G'\) is also \(\omega\)-independent and \(G''\) is often below the lower experimental limit.

The results shown in Fig. 2 well agrees with this conventional behavior, although the \(\omega\)-region for each time point is limited. One of the reasons for this limitation is the evaporation of water inhibiting the experiments with various temperature range. Thus, time-temperature superposition is hardly applicable to the hydrogel system. Another reason is that each experiment should be performed in a limited period, below the lower experimental limit. The critical scaling is attributed to the relaxation of non-percolating clusters or simply hyperbranched structure in the gel\(^{19}\). (v) At \(p \gg p_c\), \(G'\) is also \(\omega\)-independent and \(G''\) is often below the lower experimental limit.

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regardless of network formation and degradation processes. In addition, we found almost identical rheological data in network formation and degradation processes (Fig. 6 (B)). These results strongly suggest that the network formation and degradation go along the identical path toward the opposite directions.

Finally, we propose a simplified criterion for the gelation over the Winter-Chambon criterion based on the observation (Fig. 7). Indeed, the observation of the Winter-Chambon criterion \( (G' \sim G'' \sim \omega^\beta) \) is difficult because of the drastic change in \( G' \) near the gelation point. In this study, we could not observe the criterion, although we tried. This difficulty makes it challenging to investigate the gelation and the critical behavior. Again, we note that \( G'' \) is insensitive to \( p \) in the vicinity of the gelation threshold shown in Figs. 2 and 3. This invariable \( \beta \) indicates that one can consider the exponent of \( G'' "near" \) the gelation point as the critical exponent (\( \beta_c \)).

Furthermore, one can determine the gelation point as a point that the exponent of \( G' (\alpha_c) \) is the same with the critical exponent of \( G'' (\beta_c) \). The gelation point is determined as the cross point of the fitting lines of \( a \) and \( b \) near the gelation point (Fig. 7). The gelation and reversed-gelation points are estimated to be \( 1.17 \times 10^4 \) s and \( 2.40 \times 10^5 \) s, respectively. The corresponding critical reaction conversions are 0.56 and 0.57, respectively. They are almost the same and agree with our previous reports.\(^{35-37} \). This modification in the criterion may help estimate the gelation point and the critical exponent more easily.

4. CONCLUSIONS

In this study, we measured the dynamic viscoelastic behaviors during the network formation and degradation processes using the Tetra gel with SH and ACR groups. Our major findings are summarized as follows. (i) the exponent against the angular frequencies of \( G' \) drastically changed from 1.7 to 0 and from 0 to 1.4, while that of \( G'' \) slightly altered around 0.6 near gelation threshold. These changes in the exponents reflect the retardation of the inner mode accompanied with the cluster growth. (ii) The viscoelastic spectra for Tetra gel with the same connectivity well agreed with each other. This result strongly suggests that the network formation and degradation go along the identical path toward the opposite directions. (iii) Based on our findings, we proposed a simplified method to determine the critical gelation point as a crossing point between the fitting lines of exponents for \( G' \) and \( G'' \). The modified criterion may help understand the gelation behavior in the near future.
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APPENDIX:
ESTIMATION OF REACTION CONVERSION

Figure 8 (A) shows the infrared spectroscopy results for the Tetra gel during the gelation process. Accompanied by the gelation’s progress, the peak intensity at 1710 cm\(^{-1}\) decreased, attributed to the C = C bond of ACR. The reduction of the peak intensity reflects the progression of the gelation reaction (shown in Fig. 8 (B)).

Figure 9 shows the time-development of the peak intensity from the C = C bond through the peak separation. According to our previous studies\(^{38-40}\), the Tetra gel’s gelation reaction obeys the second-order reaction. The reaction conversion is described as

\[
p(t) = 1 - \frac{1}{C_0 k_{gel} t + 1}
\]

where \(p(t), C_0\), and \(k_{gel}\) are the reaction conversion at a certain time, the concentration of the C = C bond at the initial state, and the gelation coefficient, respectively. The absorbance of the C = C bond, which is reversely proportional to the reaction conversion, is rewritten as

\[
ABS = \alpha C_0 \left(1 - \frac{1}{C_0 k_{gel} t + 1}\right)^{-1}
\]

where \(\alpha\) is the molar absorption coefficient. In Fig. 6, the fitting results by equation (2) are represented as a red line. The fitting was carried out using the data within a short time range to eliminate the hydrolysis effect. The values of \(k_{gel}\) were estimated to 8.33 L mol\(^{-1}\) min\(^{-1}\), which is independent of the polymer concentrations.

As for the degradation, the IR measurements for the Tetra-PEG-ACR was carried out shown in Fig. 10 (A). The peak at 1710 cm\(^{-1}\) decreased as time developed, which indicates that the hydrolysis proceeds. Figure 10 (B) shows the time-development of the peak intensity from the ACR unit. The absorbance decreased with increasing the time obeying the single exponential function. These results suggest that the dissociation of the ACR under pseudo-first-order kinetics. In the first-order reaction, the absorption is described as

\[
ABS = A \exp(-k_{deg} t)
\]
where $A$ and $k_{\text{deg}}$ are the absorption at the initial state and the degradation coefficient, respectively. The fitting result is shown in Fig. 10 (B) as a red line.

Figure 11 shows the relationships between $k_{\text{deg}}$ and $T^{-1}$. The estimated $k_{\text{deg}}$ linearly decreased with increasing $T^{-1}$, suggesting that the degradation reaction obeys the Arrhenius equation. From the fitting, the degradation coefficient at 298 K was estimated to be $7.5 \times 10^{-3}$ day$^{-1}$.

REFERENCES

1) Hoare TR, Kohane DS, *Polymer*, 49, 1993 (2008).
2) Langer R, Peppas NA, *AIChE Journal*, 49, 2990 (2003).
3) Peppas NA, *Curr Opin Colloid Interface Sci*, 2, 531 (1997).
4) Qiu Y, Park K, *Advanced Drug Delivery Reviews*, 64, 49 (2012).
5) Martin JE, Adolf D, Wilcoxon JP, *Phys Rev A*, 39, 1325 (1989).
6) Takahashi M, Yokoyama K, Masuda T, Takigawa T, *J Chem Phys*, 101, 798 (1994).
7) Huang C, Chen Q, Weiss RA, *Macromolecules*, 49, 9203 (2016).
8) Gasparoux J, Tixier T, Tordjeman P, *Phys Rev E* 75, 11802 (2007).
9) Horinaka J, Sakata T, Takigawa T, *Nihon Reoroji Gakkaishi*, 43, 169, (2015).
10) Kakiuchi M, Aoki Y, Watanabe H, Osaki K, *Nihon Reoroji Gakkaishi*, 29, 53 (2001).
11) Hossain KS, Nemoto N, Nishinari K, *Nihon Reoroji Gakkaishi*, 25, 135 (1997).
12) Matsumoto T, *Nihon Reoroji Gakkaishi*, 38, 745 (1994).
13) Scanlan JC, Winter HH, *Macromolecules*, 24, 47 (1991).
14) Winter HH, Morganelli P, Chambon F, *Macromolecules*, 21, 532 (1988).
15) De Rosa ME, Winter HH, *Rheol Acta*, 33, 220 (1994).
16) Izu A, Winter HH, Hashimoto T, *Macromolecules*, 27, 6883 (1994).
17) Winter HH, Chambon F, *J Rheol*, 30, 367 (1986).
18) Chambon F, Winter HH, *J Rheol*, 31, 683 (1987).
19) Borsali R, Pecora R, “Structure and Dynamics of Polymer and Colloidal Systems (Nato Science Series C: Mathematical and Physical Sciences)” (2002), Springer, Berlin.
20) Wang J, Zhou Z, Zhang W, Garoni TM, Deng Y, *Phys Rev E*, 87, 52107 (2013).
21) Xu X, Wang J, Lv JP, Deng Y, *Front Phys.*, 9, 113 (2014).
22) Stauffer D, Coniglio A, Adam M, *Adv Polym Sci* 44, 103 (2014).
23) Raman R, Hua T, Gwynne D, Collins J, Tamang S, Zhou J, Esfandiary T, Soares V, Pajovic S, Hayward A, Langer R, Traverso G, *Sci Adv*, 6, 1 (2020).
24) Lee KY, Bouhadir KH, Mooney DJ, *Macromolecules* 33, 97 (2000).
25) Zusiak SP, Leach JB, *Biomacromolecules* 11, 1348 (2010).
26) Lee KY, Bouhadir KH, Mooney DJ, *Biomaterials* 25, 2461 (2004).
27) Shih H, Lin CC, *Biomacromolecules* 13, 2003 (2012).
28) Griffin DR, Kasko AM, *J Am Chem Soc* 134, 12103 (2012).
29) Madl CM, Katz LM, Heilshorn SC, *ACS Macro Lett* 7, 1302
30) Sakai T, *Polym J* **46**, 517 (2014).

31) Sakai T, Matsunaga T, Yamamoto Y, Ito C, Yoshida R, Suzuki S, Sasaki N, Shibayama M, Chung U, *Macromolecules* **41**, 5379 (2008).

32) Sakai T, Akagi Y, Kondo S, Chung U, *Soft Matter* **10**, 6658 (2014).

33) Fujiyabu T, Yoshikawa Y, Chung U, Sakai T, *Sci Technol Adv Mater* **20**, 608, (2019).

34) Sakai T, *Nihon Reoroji Gakkaishi*, **47**, 183 (2019).

35) Sakai T, Katashima T, Matsushita T, Chung U, *Polym J*, **48**, 629 (2016).

36) Fujinaga I, Yasuda T, Asai M, Chung U, Katashima T, Sakai T, *Polym J* **52**, 289 (2020).

37) Katashima T, Sakurai H, Chung U, Sakai T, *Nihon Reoroji Gakkaishi* **47**, 61 (2019).

38) Kurakazu M, Katashima T, Chijiishi M, Nishi K, Akagi Y, Matsunaga T, Shibayama M, Chung U, Sakai T, *Macromolecules* **43**, 3935 (2010).

39) Nishi K, Fujii K, Katsumoto Y, Sakai T, Shibayama M, *Macromolecules* **47**, 3274 (2014).

40) Nishi K, Fujii K, Chijiishi M, Katsumoto Y, Chung U, Sakai T, Shibayama M, *Macromolecules* **45**, 1031 (2012).