EDTA and pH-Sensitive Crosslinking Polymerization of Acrylic Acid, 2-Acrylamidoglycolic Acid, and 2-Acrylamide-2-Methyl-1-Propanesulfonic Acid

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ABSTRACT: Network formation was monitored by shear storage modulus (G') during free radical crosslinking polymerization to investigate the effects of pH and ethylenediaminetetraacetic acid (EDTA; a complex agent). Three types of acrylic monomers, acrylic acid (AAc), 2-acrylamidoglycolic acid (AmGc), and 2-acrylamido-2-methyl propanesulfonic acid (AmPS), were polymerized in the presence of a crosslinking agent. The ratio of crosslinking agent (methylene bis-acrylamide; MBAAm) to monomer was varied as: 0.583×10^{-3}, 1.169×10^{-3}, 1.753×10^{-3}, and 2.338×10^{-3}. G' of the hydrogel in crosslinking polymerizations of AAc and AmPS was effectively increased by addition of EDTA, which was not the case for the crosslinking polymerization of AmGc. The order of magnitude of G' differed based on the acidity of monomer. The maximum values of G' in crosslinking polymerizations of AAc, AmGc, and AmPS were ~20,000 Pa, 6000 Pa, and 400 Pa, respectively. G' varied linearly with the molecular weight between crosslinks (M_{wc}). pH and EDTA-complex affected the rate of intramolecular propagation during crosslinking polymerization. Our results indicated that G' was primarily affected by the following factors in the order: (1) acidity of monomer, (2) M_{wc}, and (3) physical interactions induced by pH and EDTA. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 41026.

INTRODUCTION

Gelation was first reported by Carothers[1] in 1931, who defined a gel as an infinitely large three-dimensional polymer network. Once the intermolecular linkage is formed between multifunctional monomers, all other unreacted monomers are bound to the polymer. In 1941, Flory[2] reported a number of possible cases for gel formation. He pointed out that if all the functionalities of two reacting species (e.g., two moles of glycerol and three moles of phthalic acid) react with each other, the polymer could not react with other molecules because of the absence of any reactive group. He presented a theoretical discourse on the probability of forming an infinite network, whereby one branched unit could be extended outward considering the termination and the ratio of the other unreacted monomer. Stockmayer[3] applied the condensation polymerization between –COOH and –OH developed by Flory to the crosslinking polymerization of symmetrical divinyl monomers.

Since then, hydrogels formed by polymerization of a hydrophilic monomer with a crosslinking agent has been particularly exploited as biomaterials and water absorbents. They were used as substrates for culturing neurons using the interaction on the cell surface.[4] Cushing et al.[5] reported the effectiveness of synthetic hydrogels in cell cultures and tissue engineering because of the consistent composition and predictable manipulation of their properties. Gilbert et al.[6] used a hydrogel substrate to culture self-renewable artificial muscle. Alexander et al.[7] utilized a humidity sensitive hydrogel in a dynamic actuation system with silicon nano columns. David et al.[8] improved the response time of hydrogel valves in a microactuator system. Owing to the swelling ability and the pH sensitivity of hydrogels,[9] these materials have also found application as super absorbent in disposable diapers[10–12] and in eroding reservoir devices in controlled drug-release systems.[13,14]

Crosslinked poly(acrylic acid) (PAAc) is one of most widely commercialized and valuable hydrogels. It exhibits pH sensitivity and adhesive properties. Copolymerization of AAc with *N*-isopropylacrylamide may be exploited in the design of a pH and thermo-sensitive device.[15–17] AAc is also highly water soluble, and the polymerization rate in water is fast.[18] PAAc hydrogels are widely applied in the biomedical industry,[19,20] and in

biosensors, microelectromechanical systems, coatings, and textiles.[21]

The crosslinked AmGc polymer resin is useful for waste or salt water treatment and can be used as an adsorbent for removal of trace heavy metal ions from saline aqueous solutions and sea water.[22] The crosslinked AmPS polymer has been widely utilized in membrane and cell cultures[23] and is also used in the fields of emulsion coatings, oil, adhesives, and in the medical industry.[24]

However, the use of hydrogels is still limited by a series of problems, the two most important of which are the heterogeneity and mechanical weakness when hydrogels are swollen in water. These features lead to inconsistent results in related studies. To resolve these issues, the gelation reaction must be understood and controlled. Catherine et al.[25] reported that alginate hydrogel was characterized by a non-uniform structure and insufficient mechanical strength for application to tissue engineering. In their study, the gelation rate was controlled by manipulation of the calcium ratio, reaction temperature, and initial alginate concentration. Swelling analysis showed that slower gelation generated a more uniform and mechanically stronger gel. Jeannine et al.[26,27] reported that the intramolecular cyclization reaction of the multi-vinyl monomer induced heterogeneity of the hydrogel. They also integrated the conventional kinetic model and the given reaction conditions such as monomer size, pendant birth time, and polymerization rate. The cyclization ratio varied during the gelation. The effect of pH, ionic strength, and dilution was another concern. The swelling ratio of the hydrogel was higher in more dilute solution, indicating that the crosslinking agent was less efficient in more dilute solution owing to the reduced probability of contact between the pendant molecule and other oligomeric radicals. The pH and ionic strength of the solution affected the interaction between the hydrophilic monomers and growing polymers, thereby increasing or decreasing the rate of network formation.

The mechanical properties of a hydrogel are directly related to the network formation. The G' value of hydrophilic linear polymers is little affected as the chain is propagated in water. However, in the case of crosslinked polymers, and especially those in which the infinite network molecule is formed, the elasticity increases remarkably; i.e., the G' value increases, as crosslinking polymerization progresses. G' has been regarded as an indicator of monomer conversion because all the unreacted monomers are bound to one gigantic crosslinked polymer during the course of polymerization. Maria and coworkers[28] determined that G' was proportional to the crosslinking density. The measured G' value was substituted into the rheokinetic equation to determine the rheological conversion. The obtained rheokinetic result was in good agreement with the experimental result of epoxy-phenol novolac curing. Sarmento et al.[29] measured G', the loss modulus (G''), complex viscosity, and phase angle for the gelation of a siloxane-poly(methyl methacrylate) hybrid. Their experiment showed that linear chains were formed during the initial stage of gelation followed by formation of the branched structure and three-dimensional network.

Therefore, to resolve the issues of heterogeneity and mechanical weakness of hydrogels, we focused on investigating the gelation rates and the effect of the concentration of the crosslinking agent on G' under several reaction conditions. Crosslinking agent at four different concentrations, three pH values (pH 2, pH 6, and pH 10), and EDTA as a complex agent were used in crosslinking polymerization of three types of acid monomers – AAc, AmGc, and AmPS. Generally, the crosslinking polymerization of AAc was the faster and resulted in the higher G' values than those of AmGc and AmPS. The effect of pH was different based on the degree of crosslinking and the type of monomer. For all samples, M_{wc} was derived from the conversion rate of the monomer and the concentration of MBAAm at 60 min of polymerization. The plot of G'_{t60} versus M_{wc} was close to linear. The results of this study indicate that the intermolecular reactions induced by hydrogen bonding, electrostatic repulsion, ion shielding, or the screening effect (chelation) can also contribute to gel formation.

EXPERIMENTAL

Materials

AAc (anhydrous 99%), AmGc (monohydrate 96%), and AmPS (99%) were purchased from Aldrich Chemical. MBAAm was purchased from Fluka, and EDTA was purchased from Sigma. Ammonium persulfate (APS) (98+%, A.C.S. reagent) was purchased from Sigma–Aldrich. Potassium phosphate monobasic and sodium hydroxide were purchased from Mallinckrodt. Hydrochloric acid (Reagent A.C.S.) was purchased from Fisher Scientific. All these chemicals were used as received. Chemical structures of three monomers, MBAAm, and EDTA were presented in Figure 1.

Polymerization and Conversion Rate

Hydrogels were prepared by crosslinking free radical polymerization. Initial solution consists of monomer (AAc, AmGc, or AmPS), MBAAm, EDTA, and solvent (deionized water (W) or pH solution). As shown in Table I, the molar ratios of MBAAm to monomer were 0.583×10^{-3}, 1.169×10^{-3}, 1.753×10^{-3}, and 2.338×10^{-3}, respectively. Solid wt % was fixed to about 23% to reduce the termination reaction during the gelation. Before corresponding ingredients were poured into petri dishes, and then placed onto oven kept at 60°C, solution was stirred and deoxygenated by bubbling with nitrogen gas for 20 min. When the temperature of solution reached to 60°C, initiator APS solution (5 mL) was added, and kept the reaction complete. Polymerization time was 60 min for all samples. After the polymerization, weights of samples were measured to get the swelling ratio, and then thoroughly washed with chilled acetone/W co-solvent five times to remove unreacted monomer and water-soluble fraction of the polymer. Samples were dried in a vacuum oven at 80°C for two days, and weights remained constant were measured.

Gravimetric conversion rate measurement was carried out. The equivalent amount of monomer was calculated based on the weight of each sample and the weights % of monomer and MBAAm initially charged.

$$X_c = \frac{(W_2 - W_0) - (W_1 - W_0) \times C_{initiator}}{(W_1 - W_0) \times C_{monomer}} = \frac{W_P}{W_m} \quad (1)$$

where W_0 is the weight of empty dish, W_1 is the weight of dish after the initial sample solution was dropped in, and W_2 is the weight of dish after drying. $C_{initiator}$ and $C_{monomer}$ were given by
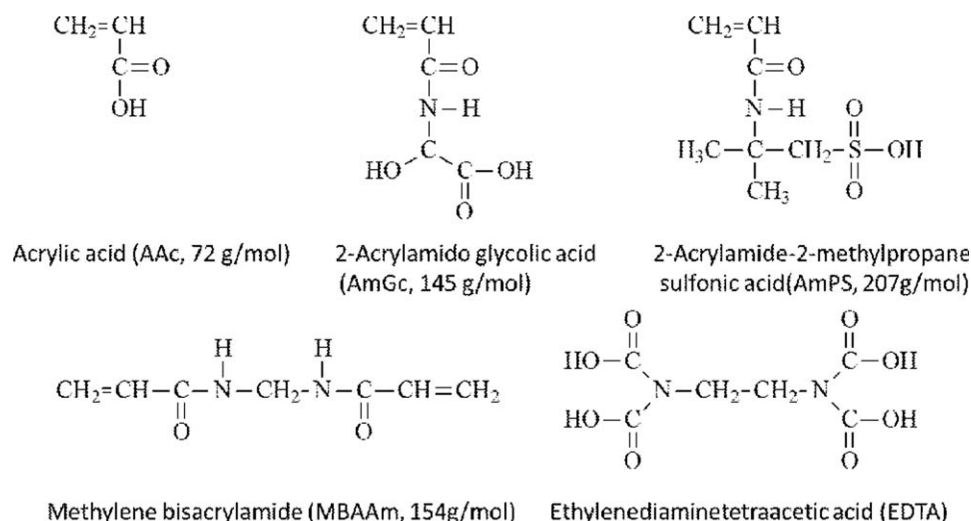
Figure 1. Three acrylic monomers, a crosslinking agent, and a complex agent used in the crosslinking polymerization at 60°C.

the weight ratio of the corresponding ingredients (initiator and monomer) charged and the weight of total charge. W_p is the weight of polymer and W_m is the weight of initial monomer. It was assumed that the conversion rate of MBAAm was 100%.

pH Solution and Titration

Three pH solutions were first prepared to examine the effect of pH on the gelation behavior. To prepare solutions (non-buffers) of pH 2 and pH 10, diluted stock solutions of HCl (pH 2, $0.1M$) and NaOH (pH 13, $0.1M$) were mixed to reach the desired pH. pH 6 solution (buffer, $0.1M$) was prepared by mixing 291 mL of NaOH solution ($0.1M$) and 500 mL of KH_2PO_4 solution ($0.1M$).

In Figure 2, pK_a of AAc, AmGc, and AmPS were obtained with the titration. $0.1M$ solutions were prepared by dissolving AAc

(7.2 g), AmGc (14.5 g), and AmPS (20.7 g) to 1000 mL of W, respectively. Then, pH measurement was followed by dropping 2 mL of NaOH ($0.1M$) to 25 mL of each monomer solution. This procedure was repeated until the 24 mL of NaOH solution was added.

Rheological Measurements

Measurements of the rheological properties were performed on the gelation for 60 min as the time sweep mode in a double-gap cylindrical geometry (DG27, MCR 301, Physica, Anton Paar). The gap between the cylinder and the bottom was kept at 2 mm. Shear strain (amplitude gamma) was 1%, and frequency was 1 Hz. Temperature, which was fixed at 60°C, was controlled by a water bath controller (Julobo, F25). The amount of initial gel solution poured into the DG27 was 7 mL. After temperature

Table I. Ingredients Used for Crosslinking Polymerizations of AAc, AmGc, and AmPS

Code[a]	MBAAm (mg)	Monomer			APS (mg)	Water (mL)[c]	EDTA (mg)[d]
		AAc (mL)[b]	AmGc (g)	AmPS (g)			
AAc1	2.83	2.16			13.44	8	7.93
AAc2	5.66	2.16			13.44	8	7.93
AAc3	8.49	2.16			13.44	8	7.93
AAc4	11.32	2.16			13.44	8	7.93
AmGc1	1.42		2.26		6.72	8	3.97
AmGc2	2.83		2.26		6.72	8	3.97
AmGc3	4.25		2.26		6.72	8	3.97
AmGc4	5.66		2.26		6.72	8	3.97
AmPS1	0.99			2.25	4.69	8	2.77
AmPS2	1.98			2.25	4.69	8	2.77
AmPS3	2.97			2.25	4.69	8	2.77
AmPS4	3.95			2.25	4.69	8	2.77

[a] Molar ratios ([MBAAm]/[Monomer]) x 10^3 equal to 0.583(1), 1.169(2), 1.753(3), 2.338(4).
[b] Density of AAc is 1.05 g/cm³.
[c] Solid wt % is 23% ((wt of monomer)/(wt of monomer + wt of water)).
[d] EDTA was added only to the crosslinking polymerization of monomer + EDTA samples.
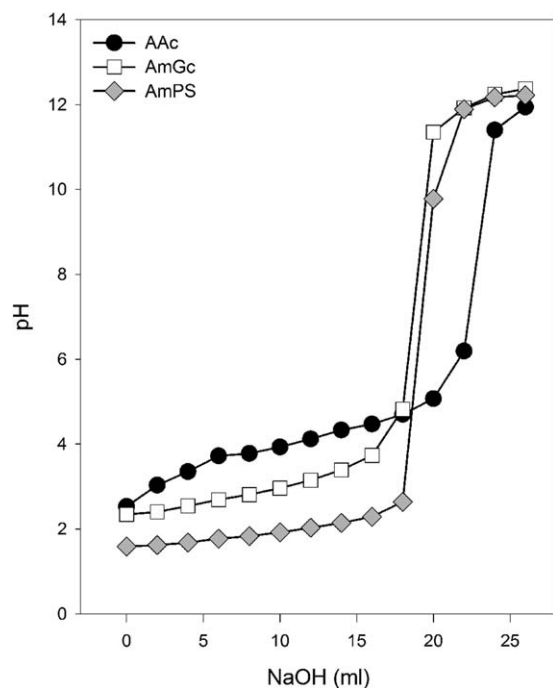
Figure 2. pH titration curves of acrylic monomers.

reached to 60°C, 1 mL of initiator solution was added. Concentration ratios of each ingredient were presented in Table I. The time gap between the addition of initiator solution and the starting time of measurement was 15 s. A total of 30 points were scanned with a time interval of 2 min between each point.

RESULTS AND DISCUSSION

Network Formation

The profiles of the G' vs. polymerization time, and the damping factor vs. G' plots were similar for all of the evaluated samples. Three different phases in Figure 3(a), and two different phases in Figure 3(b) were observed which shows the curve for the crosslinking polymerization of AAc2 ([MBAAm]/[AAc] = 1.169 $\times 10^{-3}$) at pH 6 as a typical example. The G' values were zero up to 6 min, then increased sharply followed by a slowed increase by the beginning of phase 3. Damping factors were not observed for phase 1.

The hydrogel is generated by crosslinking polymerization of the hydrophilic monomer. If this type of monomer is polymerized

without a crosslinking agent, a hydrophilic linear polymer such as PAAc is produced. PAAc is water-soluble, and there is greater correlation between the degree of polymerization and the viscosity rather than the elasticity. Malkin et al.[30] used the viscosity as a polymerization index, where the viscosity was reported as a function of the degree of polymerization (average chain length) and the concentration. The relationship between viscosity and degree of polymerization is supported by the Mark–Houwink–Sakurada equation, $[\eta] = KM_v^a$, where K is a constant, M_v is the viscosity average molecular weight, and a is the constant (0.5 for an entangled coil polymer, and 1.0 for an extended rod-shaped polymer).[31] It is inferred that during phase one, all of the polymers, which are linearly propagated or branched, are water-soluble.

At the end of phase one, G' increases significantly. This indicates that network formation occurs in phase 2. The gel point is defined as the initial time of network formation and it corresponds to the formation of an infinite cluster of reacted bonds.[32] The infinite cluster means that the ends of the large crosslinked polymer meet at least two external boundaries of the reactor. The formation of an infinite cluster makes the polymer a continuous phase and the solvent a discontinuous phase in two-dimensional geometry. Therefore, the reactions between monomers and linear polymers dissolved in the solvent are more limited; instead, the unreacted or growing molecules are primarily bound to the crosslinks or the continuous infinite clusters. These phase transitions and the formations of new crosslinks are believed to increase G'.

The increase of G' is curtailed by the beginning of phase three. In this phase, it is assumed that the formation of new crosslinks is not dominant, but the extension of existing crosslinks and intramolecular crosslinking or termination of the infinite crosslinked polymer increases G'. At the end of phase 2, the surface of the PAAc hydrogel in AAc2, which was taken as the typical example, was flat as shown in Figure 4. However, after 30 min of phase 3, when the intermolecular reactions occurred to induce extension and tightening of the network, the surface became rough. Valery et al.[33] also attributed the steady increase of G' in this phase to network extension, explained in terms of the fact that G'' was constant, and the hydrogel prepared in this phase did not heal when it was damaged.

The structures of the hydrogels formed in phases 2 and 3 are different. Akira et al.[34] pointed out the formation of two
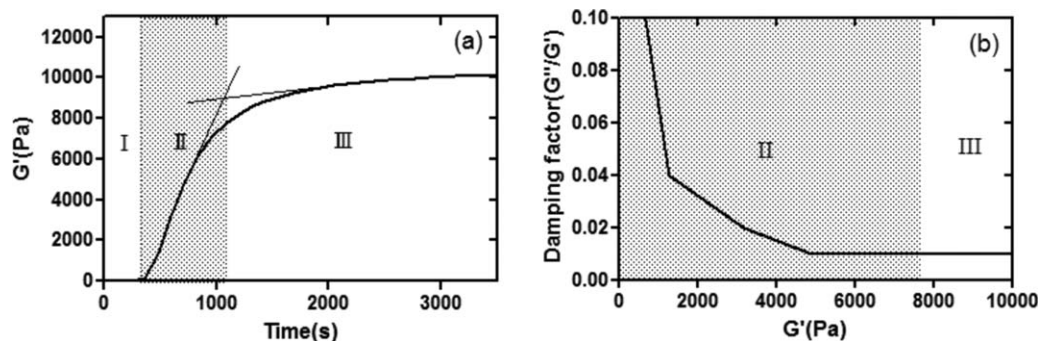

Figure 3. (a) G' and (b) damping factors in three phases of free radical crosslinking polymerization (AAc2 at pH 6).
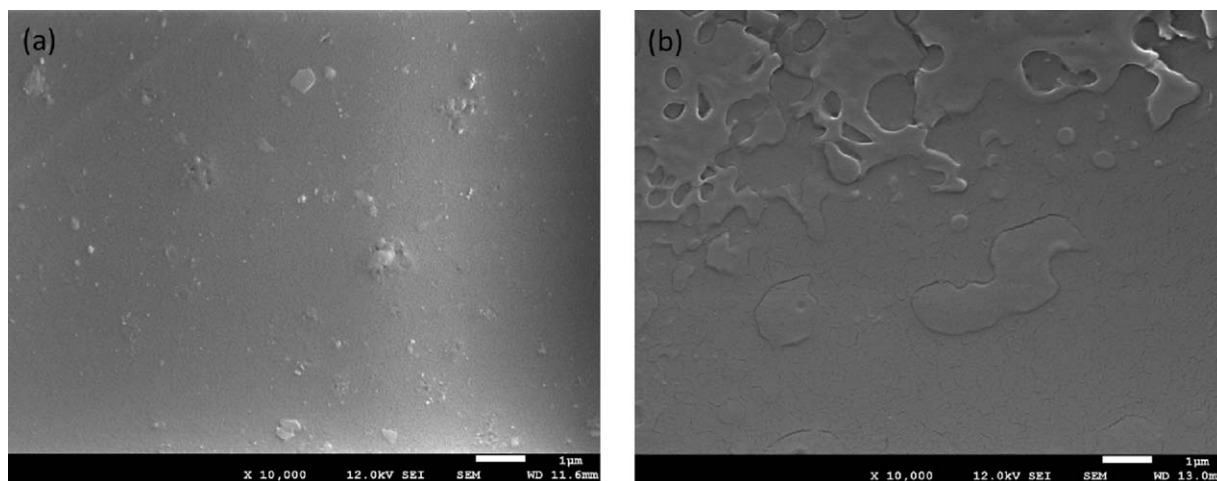
Figure 4. Representative scanning electron microscopy images of hydrogel during the crosslinking polymerization of AAc2 ([MBAAm]/[AAc] = 1.169 × 10^{-3}) at W, (a) at the end of phase 2, (b) at the end of phase 3.

extreme structures in the network, i.e., ideal network polymer region (shell) and microgel regions (cores). They postulated that the ideal network was produced by intermolecular propagation with monomers and intramolecular cyclization, and the microgels were formed via intramolecular crosslinking and intermolecular crosslinking with prepolymers (or separated network clusters). The increase of G' in phase three was mainly attributed to microgels formed. Intramolecular crosslinking resulted in more dense parts. This was assumed to increase G' in phase three, and the intermolecular crosslinking with prepolymers was believed to extend the network, further increasing G'.

Recently, we reported the swelling rates of polystyrene bead-filled and unfilled PAAc hydrogels prepared with different concentrations of MBAAm.[35] Several different swelling rates – rigid cores coexisting with water-absorbing shell, water-absorbing cores and water-saturated shell, and water-saturated cores and shell – were observed as the dried gel reached the equilibrium swelling state at W, which were explained in terms of the different crosslinking densities of the cores and the shell. Nicolas and coworkers also evaluated the swelling steps by an acoustic impedance method, where each step was characterized by a difference in the transmission.[36] Arifuzzaman et al.[37] reported that the different ordered structure was formed because of the different mechanical instabilities at different parts of polyelectrolyte hydrogel during its heterogeneous swelling. They pointed out geometrically the significant mismatch of swelling degree between the outer and inner parts, and the swelling resulted in the local stress of hydrogel.

Kinetic evaluation of crosslinking polymerization is difficult. A number of reactions are possible, and the rate and the conditions of each reaction are not distinct. The critical chain length when the linear polymer reacts with the crosslinking agent must also be known. During monomer initiation, three different monomeric radicals, Monomer•, MBAAm• with one double bond, and MBAAm•• are produced. The intramolecular termination or cyclization rate of the two radicals propagated from MBAAm in crosslinking polymerization is higher than the intermolecular termination in the linear polymer because of the

closer distance in the former, even if the monomer concentration is excessively high.

For propagation and termination, the concentrations and the propagation constants of the different types of radicals, monomer, or MBAAm, and the propagation with the radical reduction should be investigated in real-time for the duration of polymerization for kinetic evaluation of all of the reactions. Therefore, as the easiest way to monitor the network formation, G' was measured. The variation of G' resulting from network formation was initially evaluated for the first hour of polymerization. Secondly, as a simplification, the conversion rate at 60 min of polymerization was obtained under the assumption of 100% conversion of MBAAm in each sample, and the M_{wc} and G' were obtained by assuming proportionality to the conversion rate to circumvent the complicated polymerization kinetics.

G' in the Linear Polymerization of AAc

G' was observed to increase during the linear polymerization of AAc, and increased more steeply with the addition of EDTA. Figure 5(a) and (b) shows the behaviors of G' and damping factor during the respective polymerizations of AAc and AAc with EDTA for 60 min.

Gelation of the propagating linear polymer chains occurred because of chain entanglement. The gel point (the crossover of the elastic G' and viscous G'' moduli) was observed at 1200 s for the polymerization of AAc and 630 s for the polymerization of AAc+EDTA. According to Ponton et al.[38] and Neilson et al.[39], the gel point can be induced in linear polymerization and in polymer fluids because of the presence of entanglements. Entanglement is intensified when the molecular weight exceeds M_c (the critical molecular weight of entanglement). M_c varies according to the type of polymer, and is a function of the chain length. The length of backbone chain at M_c at which the branch unit is excluded is, however, constant regardless of the type of polymer. The initial entanglement occurs when the degree of polymerization for the backbone chain reaches 600.[40] If this is applied to AAc polymerization, the M_c of PAAc is 43200 g/mol. Based on the data in Figure 5, the M_c of PAAc was achieved at
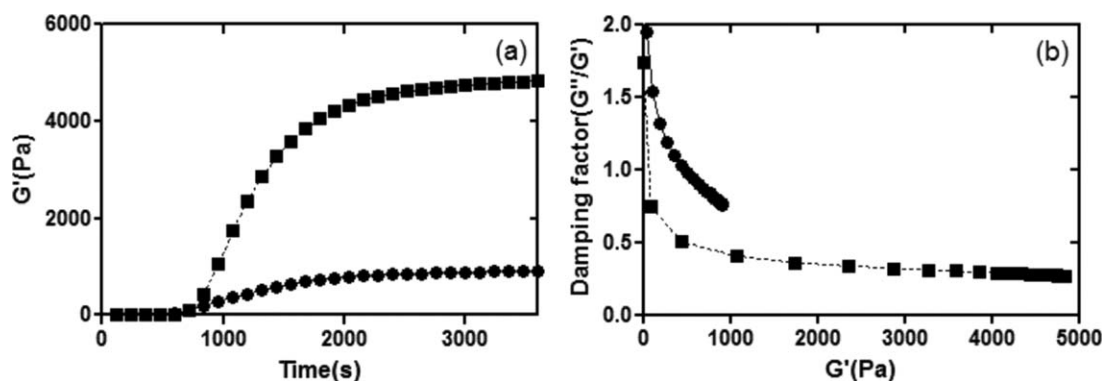
Figure 5. (a) G' and (b) damping factors, in the linear polymerizations of (-•-) AAc and (-■-) AAc with EDTA.

480 s, and the M_c of PAAc+EDTA was attained at 600 s of polymerization.

Interestingly, the respective time lags between the gel point and the initial entanglement were 720 s and 30 s for PAAc and PAAc+EDTA. Addition of EDTA delayed the entanglement during AAc polymerization and promoted crosslinking polymerization when the molecular weight exceeded M_c. The gel point indicates the point at which a large infinite molecular assembly is created by the crosslinking polymerization. Herein, the gel point was induced by entanglement or physical attractive interactions.

The respective G_{t60} values were 914 Pa and 4839 Pa for the PAAc and PAAc+EDTA polymerizations. This five-fold increase achieved with the addition of EDTA was attributed to electrostatic repulsion between the carboxylic groups of AAc. EDTA

dissolved in water has four $-COO^-$ groups bonded to two tertiary amines. EDTA is widely used to trap metal ions such as Ca^{2+} or Mg^{2+}, or applied to terminate enzyme activity, where the metal ion is trapped between the carboxyl groups and nitrogen.[41]

Crosslinking Polymerization of AAc

The G' values were monitored during the low crosslinking polymerization (AAc1) and the high crosslinking polymerization (AAc4). The highest G'_{t60} was 6732 Pa (EDTA) in AAc1, and 18950 Pa (EDTA) in AAc4. The respective G'_{t60} values for linear polymerization, AAc1, and AAc4 at W, were 914 Pa, 3674 Pa, and 10,690 Pa. With formation of the network in AAc1, and AAc4, the G'_{t60} value was 4-fold and 11-fold higher than achieved with linear polymerization. Figure 6(a) shows the
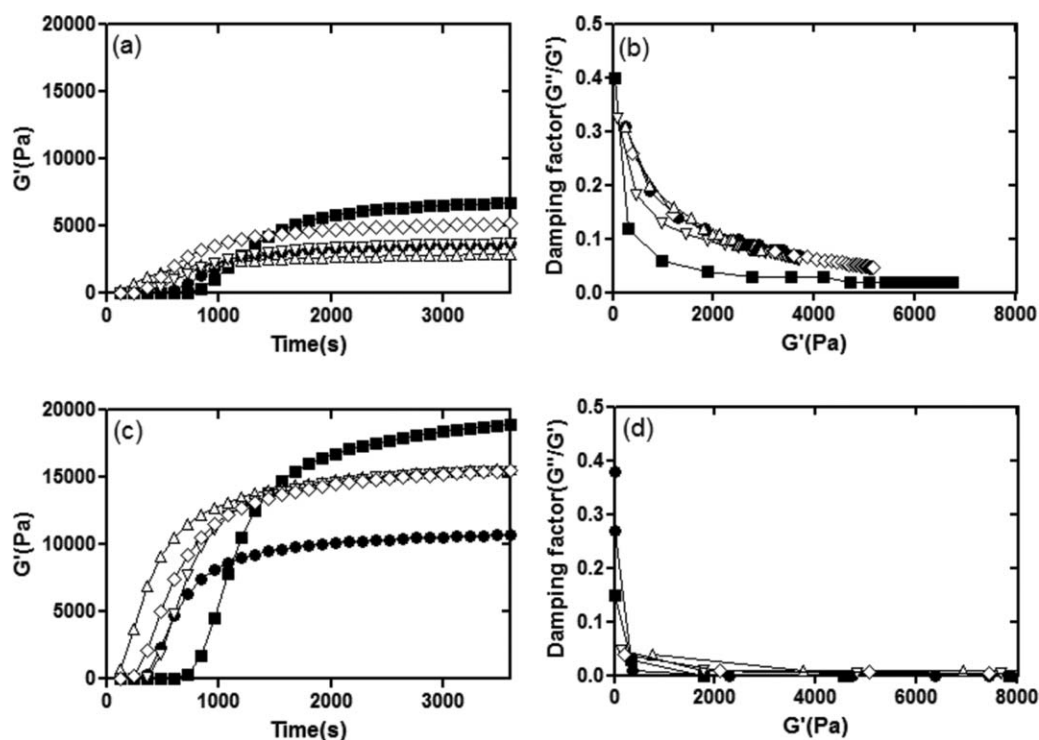

Figure 6. G' and damping factors in the crosslinking polymerization at (-•-) W, (-■-) EDTA, (-△-) pH 2, (-▽-) pH 6, and (-◇-) pH 10, (a) G' of AAc1, (b) damping factors of AAc1, (c) G' of AAc4, and (d) damping factors of AAc4.
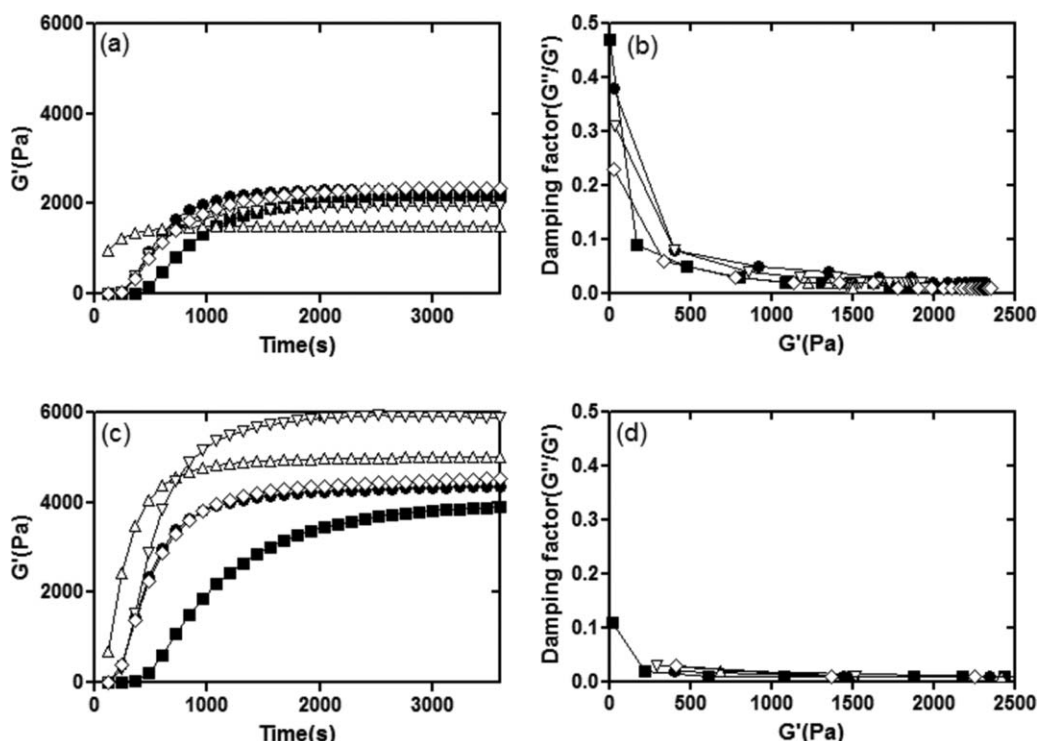
Figure 7. G' and damping factors in the crosslinking polymerization at (-•-) W, (-■-) EDTA, (-△-) pH 2, (-▽-) pH 6, and (-◇-) pH 10, (a) G' of AmGc1, (b) damping factors of AmGc1, (c) G' of AmGc4, and (d) damping factors of AmGc4.

effects of EDTA and pH on AAc1. Based on the conditions used, the G'_{t60} values of the polymer ranked in the order: EDTA $>$ pH 10 $>$ pH 6 $\approx$ W $>$ pH 2.

For the role of EDTA in the crosslinking polymerization, it is postulated that the ionized carboxyl groups of EDTA increased the repulsion of AAc. EDTA would capture and reduce the mobility of NH_4^+ generated from decomposition of the initiator. At W, the anionic carboxyl groups are in excess because pK_a of AAc is 5.0 as shown in Figure 2. These anions can repel each other, but in the absence of NH_4^+ capture by EDTA, the NH_4^+ may shield $-COO^-$ of AAc, thereby decreasing the repulsions.[42]

The G' in AAc1 was sensitive to pH, being lower at pH 2 than at pH 6 and pH 10. In the low pH range, especially below pK_a, hydrophobic hydrogen bonds are formed between the carboxylic groups of AAc. This bonding shortens the distance between AAc monomers. The monomeric double bond is activated by attack by other radicals, and the probability for these activated oligomeric radicals to react with neighboring monomers formed by hydrogen bonding is increased. As shown in Figure 6(a), the curve at pH 2 began to increase earlier than any other curves, indicative that the crosslinking reaction starts more quickly at this pH. However, the G'_{t60} was lower at pH 2 than at pH 6 and pH 10, and W. The formation of the monomer cluster via hydrogen bonding may induce a higher rate of termination of the propagating chain radicals or inefficiency of MBAAm, and is thought to induce the lower G'.

Damping factors in AAc1 as presented in Figure 6(b) were lower as the G' was higher. At pH 2, 6, 10, and W, it was not much different, but decreased at EDTA at the same G'. Without

the formation of the infinite network, G'' was dominant in the ratio of G''/G' in phase 1. Due to the low concentration of MBAAm, oligomeric radicals need to propagate more to meet the pendant double bonding than in AAc4.

For damping factors in AAc4, the length of phase two was shorter than in AAc1 as shown in Figure 6(d). Those were generally below 0.05 at 2000 Pa. In AAc1, those were over 0.1 at 2000 Pa except at EDTA.

The increase of G'_{t60} was generally proportional to the concentration of MBAAm. The G'_{t60} values at W, EDTA, and pH 10 increased about three-fold with a four-fold increase in the concentration of MBAAm.

The polymers had about three different values of G'_{t60} in AAc4. A low G'_{t60} value of 11,000 Pa was observed for W; a medium G'_{t60} value of 15,000 Pa was observed for pH 2, pH 6, and pH 10; and a high G'_{t60} of 19,000 Pa was observed for EDTA.

Thus, the addition of EDTA augmented G' in both AAc1 and AAc4. The effect of pH on G' was significant in AAc1, but not in AAc4. The reactions of MBAAm, and termination or intramolecular propagation such as the cyclization, in particular, were thought to be affected by pH and the ionic strength (W and pH 6) in crosslinking polymerization of AAc.

Crosslinking Polymerization of AmGc

G'_{t60} in AmGc1 (low crosslinking polymerization) was in the range of 1500–2350 Pa. With the change of pH values, G' was lowered. EDTA was not effective to increase G'. As shown in Figure 7(a), G' was the highest at W and pH 10, and lowered as the pH value was decreased. Figure 7(b) shows that damping
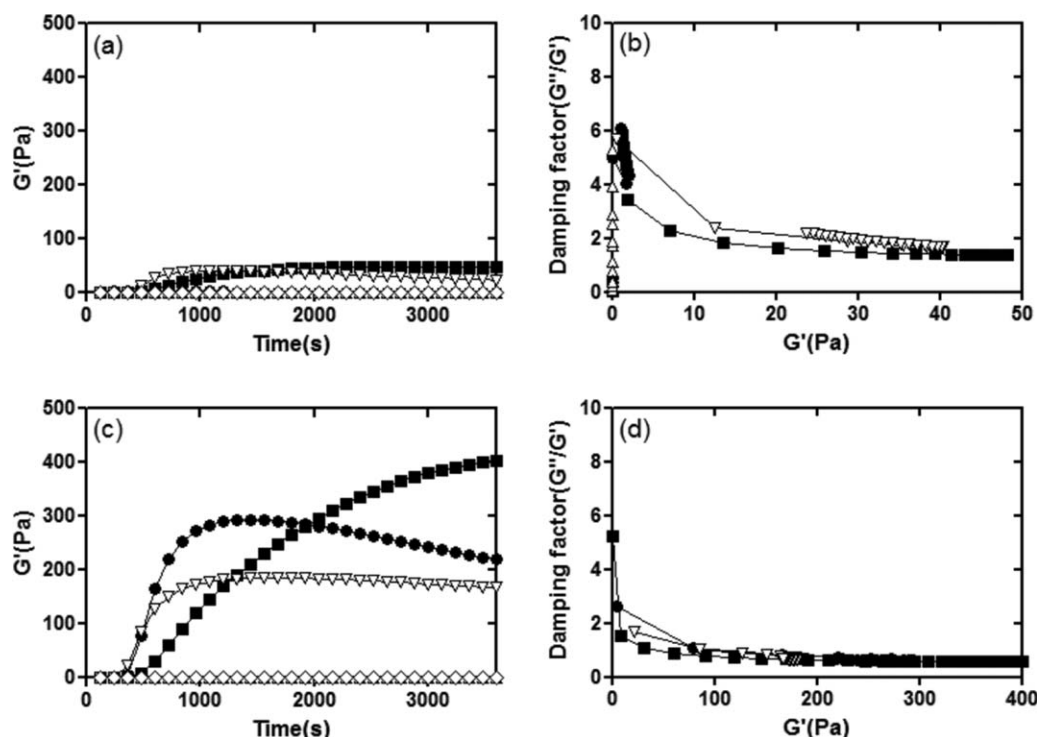
Figure 8. G' and damping factors in the crosslinking polymerization at (-•-) W, (-■-) EDTA, (-△-) pH 2, (-▽-) pH 6, and (-◇-) pH 10, (a) G' of AmPS1, (b) damping factors of AmPS1, (c) G' of AmPS4, and (d) damping factors of AmPS4.

factors in AmGc1 are not over 0.5, and lowest values are observed at pH 10.

G'_{t60} in AmGc4 (high crosslinking polymerization) was between 3900 Pa and 5870 Pa. In Figure 7(c), G' was lowered at EDTA than at any other conditions. G' was increased from pH 2 to pH 6, but decreased from pH 6 to pH 10. Damping factors in AmGc4 were below 0.05 as G' was over 300 Pa as shown in Figure 7(d).

For AmGc1, G'_{t60} was highest in the order: $W \approx$ pH 10 > EDTA $\approx$ pH 6 > pH 2. EDTA has similar structure and chain length with AmGc, but AmGc has the carbon-carbon double bonding on the backbone chain. Physical interaction of $-COO^-$s on both molecules could interrupt the chain propagation. In AmGc4, G'_{t60} was highest in the order: pH 6 > pH 2 > pH 10 $\approx$ W > EDTA. In both AmGc1 and AmGc4, G's were very similar at W and at pH 10. At pH 6, G' in AmGc1 was lower, but was higher than at pH 10 and W.

General increase of G' affected by EDTA and pH was different from that of AAc. AmGc ($pK_a \approx 3.7$) is the stronger acid than AAc. Many interactions of intra- and inter-molecular scales were expected in the pH solutions because of the hydrophilic groups on its branch-amide group, hydroxyl and carboxyl groups. It could be proved by using the molecular modeling tool that intramolecular hydrogen bonding was possible between $-COOH$ and $-C=O$ at pH 2, and the chelation of Na^+ between $-COO^-$ and $-C=O$ at pH 10. Electrostatic repulsion with negative charges of other molecules by these $-COO^-$ and O^- could occur only at pH 6. In Figure 7(c) for AmGc4, G' at pH 6 was the highest. However, in Figure 7(a) for AmGc1, it was the

highest at pH 10. In AmGc1, it was inferred that the chelation was not occurred. We assumed that intermolecular interactions were more possibly occurred instead of intramolecular hydrogen-bonding or hydrogen-chelation. But, in AmGc4, the higher concentration of MBAAm provided more rigidity to the backbone chain, and so the chance to form the intramolecular chelation was higher. In other words, intermolecular interactions in AmGc1, and intramolecular interaction in AmGc4 were dominant owing to the chain flexibility and mobility. Hydroxyl group of AmGc was oriented to opposite direction to the carboxyl group, and its ionization was thought to contribute only the hydrophilicity of AmGc.

Crosslinking Polymerization of AmPS

For G', AmPS hydrogel was much lower than AAc and AmGc. Maximum G's were 20,000 Pa in AAc, 6000 Pa in AmGc, but only 400 Pa in AmPS as the strongest acid monomer ($pK_a \approx$ 2.4).

In AmPS1, increase of G' was observed, but it was not significant as shown in Figure 8(a). For the 60 min polymerization, G' increased until 10–13 min, and gradually decreased. In case of EDTA, G' increased slowly, and reached to the highest value, about 50 Pa. At pH 2 and 10, increase of G' was not observed.

Damping factors in AmPS1 could not be lower than 1. The lowest value was observed at EDTA, and it was 1.37. Figure 8(b) shows decreasing G' and increasing damping factor at the end ($G' \approx 40$ Pa) of pH 6 curve.

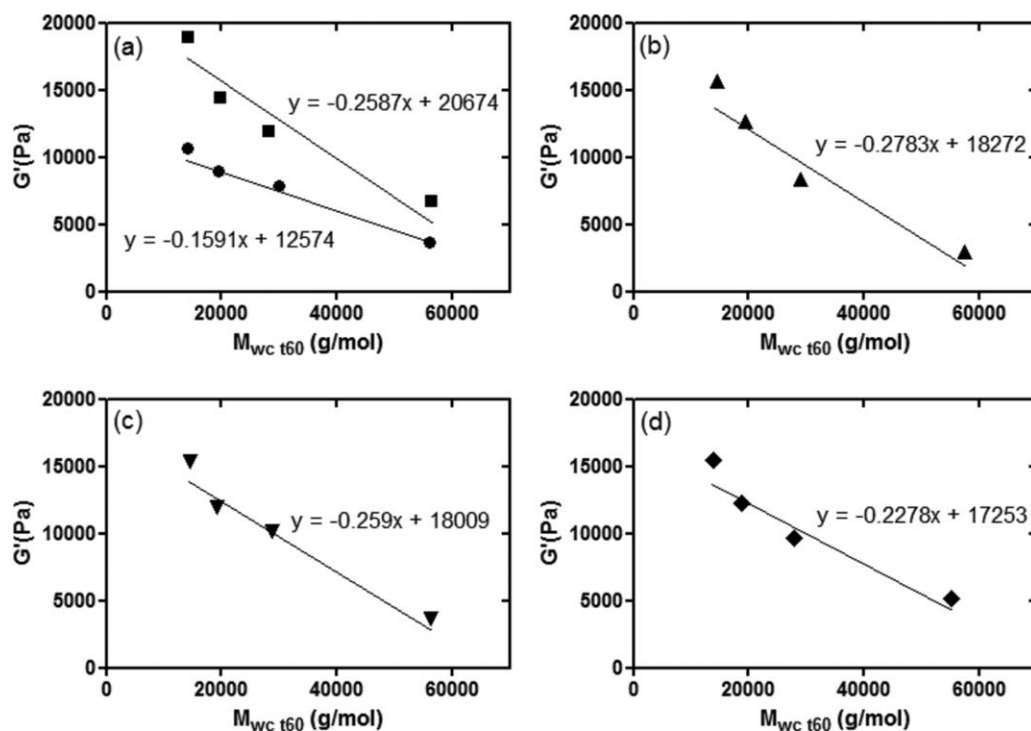G' in AmPS4 was presented in Figure 8(c). Generally, G' was higher as MBAAm concentration was increased. G' at W

Figure 9. G' as a function of M_{wc} in 60 min crosslinking polymerization of AAc at (a) (-•-) W and (-■-) EDTA, (b) (-▲-) pH 2, (c) (-▼-) pH 6, and (d) (-◆-) pH 10.

increased most significantly, and was higher than those of pHs. But it was still lower than that of EDTA at 60 min polymerization.

Figure 8(d) shows the damping factor in AmPS4. Differently in AmPS1, the range of each curve was far over 50 Pa, and the damping factors were generally lower. At pH 6, it was 1.7–5.7 in AmPS1, but 0.66–1.68 in AmPS4.

Based on damping factors during the crosslinking polymerization, two important characteristics of AmPS gel were estimated. First, AmPS gel was not 'true-gel', but 'weak-gel'. Kaoru and coworkers[43] reported the relationship between the damping factor and G' dependence on the frequency of globulin gels. If the damping factor is higher than 0.1, gel is called as 'weak-gel', and if it is lower than 0.1, it is 'true-gel'. For the weak-gel, G' and G'' change significantly as a function of frequency. G' decrease steeply with increasing shear strain owing to the structure deformation. In the meanwhile, G' and G'' were not so sensitive to the frequency in the true-gel. For the all samples, the lowest damping factor was 0.56 at EDTA in AmPS4. In AAc and AmGc, damping factors were much lower than 0.1 after G' increased. Therefore, it could be first estimated that AmPS was a weak-gel, and AAc and AmGc gels were true-gels even if we did not measure G' and G'' as a function of the frequency.

Second, this type of weak-gel showed different G' behavior in phase three. As previously described, the formation of dense region, and the extension by intermolecular crosslinking occurred in phase 3, which resulted in the cores–shell structure of hydrogel. Both the intramolecular crosslinking and the

intermolecular crosslinking with prepolymers contributed the formation of core structures, and intermolecular crosslinking extended the network increased G'. G' in AmPS in phase 3 was decreased. The possible assumption is that the structure deformation by the shear force during the measurement is occurred. However, to support this assumption, further studies such as the frequency sweep measurement for the weak-gel should be conducted.

Dependence of G' on M_{wc}

In crosslinking polymerization, it has been reported that G' changes exponentially as a function of the polymer concentration, that is, G' is proportional to C_p^m, where C_p is the polymer concentration, and m is a constant.[43–46] In those studies, the m values ranged from 2 to 3.5. It was proposed that the value of m depended on the ratio of monomer and coagulant. Valery et al.[33] also reported that m was less than 2 when the concentration of gelatin was increased above 10%, and m was over 2 when it was below 3%. Thus, we thought that G' might also be dependent on the concentration of MBAAm as a crosslinking agent such as a coagulant in gelatin, which is intimately related to the rate of network formation. In our study, polymer concentration at 60 min polymerization was over 20wt %.

The G'_{t60} values decreased almost linearly as M_{wc} increased. This behavior was observed for all samples. For the crosslinking polymerization of AAc in Figure 9, the highest increase in G'_{t60} was achieved by addition of EDTA. For the crosslinking polymerization of AmGc (Figure 10), the G'_{t60} values decreased less sharply to M_{wc}. The highest G'_{t60} value of 5870 Pa was achieved
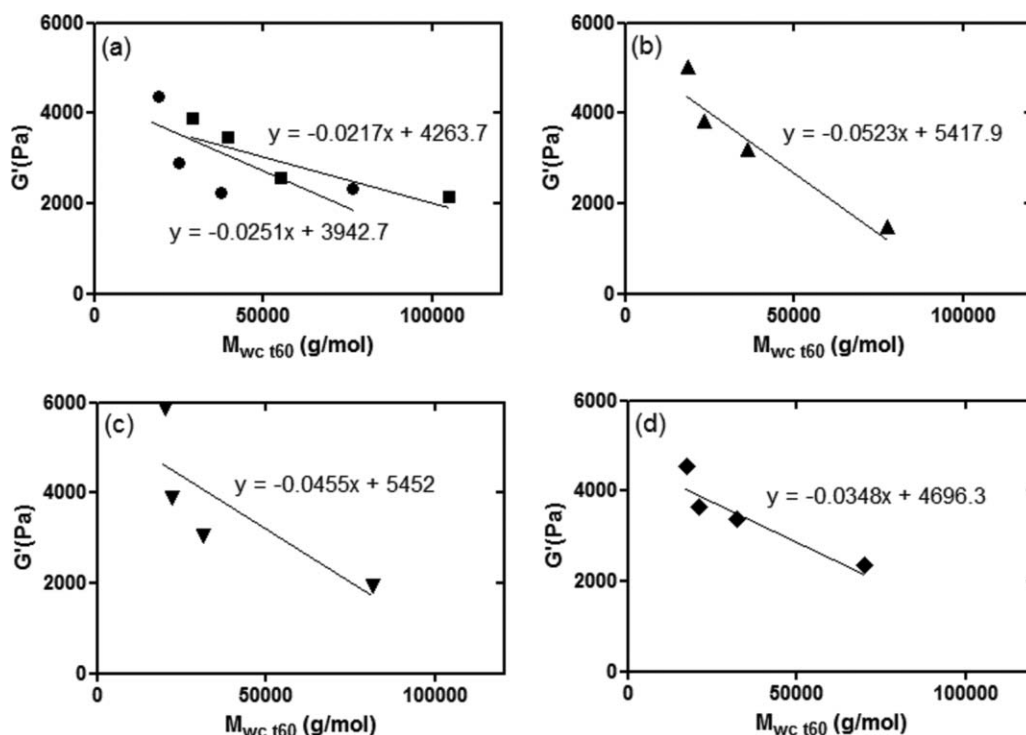
Figure 10. G' as a function of M_{wc} in 60 min crosslinking polymerization of AmGc at (a) (-•-) W and (-■-) EDTA, (b) (-▲-) pH 2, (c) (-▼-) pH 6, and (d) (-◆-) pH 10.

at pH 6 when the M_{wc} was 20,003 g/mol. For the crosslinking polymerization of AmPS, gelation point was not observed in AmPS1. The G'_{t60} values remained below 400 Pa, as shown in Figure 11.

The correlation of G'_{t60} and M_{wc} with the conversion rate (X_{ct60}) was interpreted in terms of the slopes in Figures 9, 10, and 11, for crosslinking polymerizations of AAc, AmGc, and AmPS, respectively.

The X_{ct60} values of AAc were over 90%. X_{ct60} was obtained after washing and drying the final product. The conversion rates before washing were 1–4% higher than the X_{ct60}s (not presented). This may indicate that the ratio of linear polymer to total converted polymer was below 4%. No defined trend

between X_{ct60} and M_{wc} was observed. M_{wc} was calculated from X_{ct60} using the equation presented below.

$$M_{wc} = \frac{1}{2} \times \frac{[\text{Monomer}]}{[\text{MBAAm}]} \times X_{ct60} \qquad (2)$$

where [Monomer] = (weight of initial monomer)/(molecular weight of monomer), and [MBAAm] = (weight of initial MBAAm)/(molecular weight of MBAAm). This equation may not describe M_{wc} exactly. However, it was assumed that the inefficiencies of MBAAm (increasing M_{wc}), and the monomer (looping and dangling, decreasing M_{wc}) were offset, resulting in a closer approximation to the real M_{wc}.

The slopes of the plots of M_{wc} and X_{ct60} in crosslinking polymerization of AAc are shown in Figure 9. The strongest
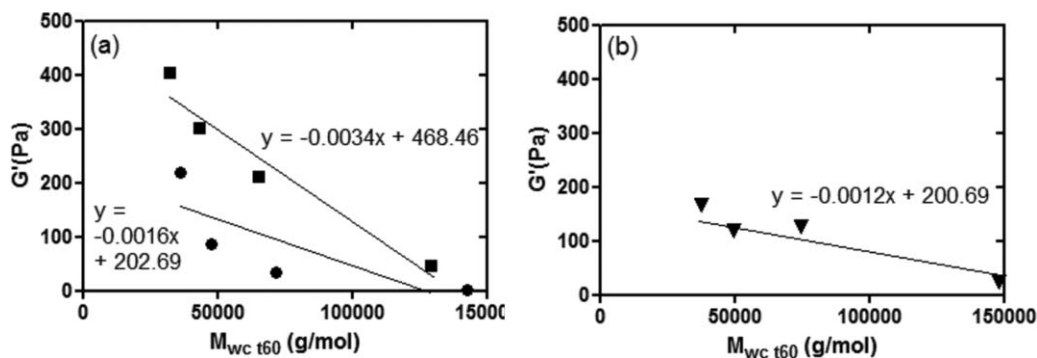

Figure 11. G' as a function of M_{wc} in 60 min crosslinking polymerization of AmPS at (a) (-•-) W and (-■-) EDTA, and (b) (-▼-) pH 6.

dependence of G'_{t60} on M_{wc} was observed at pH 2. As pH was increased, the slope was decreased. The slope at EDTA was −0.2587, and the intercept was 20,674. The lowest slope was observed at W, −0.1591.

For the crosslinking polymerization of AmGc, the higher X_{ct60} and M_{wc} were achieved in the presence of EDTA, but the G'_{t60} values were not significantly different to those of other sample as shown in Figure 10. X_{ct60} at EDTA was about 87%, but for others X_{ct60} was about 60%. M_{wc} at EDTA was also higher than at W and pHs. Due to the similar structure of AmGc and EDTA, it was thought that their carboxyl groups could form the intermolecular interactions. This was thought to induce the low polymerization rate, but the higher M_{wc}. The slope of the M_{wc} and X_{ct60} plot ranged from −0.025 when polymerization was performed at W to −0.0523 at pH 2.

The G'_{t60} value obtained via M_{wc} of AmPS in Figure 11 was the lowest among the three types of acrylic monomers, and G' did not increase at pH 2 and pH 10. The slope ranged from −0.0012 for polymerization at pH 6 to −0.0034 for the polymerization at W. This deviation was much lower than observed in crosslinking polymerizations of AAc and AmGc.

In Figures 9, 10, and 11, G'_{t60} was dependent on M_{wc}, but for the same M_{wc}, G'_{t60} was not consistent because the polymerization was conducted in different conditions. During the crosslinking polymerization of AAc, the M_{wc}s of 14,168 and 13,983 achieved at W and at pH 10 resulted in G'_{t60} values of 10,690 Pa and 15,500 Pa, which means that G' is not simply a function of the polymerization rate.

The results of the G' measurements can be explained based on the fact that the intramolecular propagations such as cyclization reactions (primary and secondary) of the propagating pendant groups had an impact on G'. As explained previously, the ideal network polymer (shell) may be formed via two reactions: (1) intermolecular propagation with the monomer, and (2) intramolecular cyclization. In the former case, the propagating network chain reacts with a monomer or a crosslinking agent.

Even though the molar concentration of the crosslinking agent was very low (i.e., about three orders of magnitude lower than the concentration of the monomer) and the polymerization rate was not much different from that of linear polymerization of the same type of monomer, increasing the concentration of MBAAm induced a major increase of G'.

The latter would play a more important role in increasing G' during phase 2. Phase 2 is the period of network formation. Intramolecular cyclization is faster than intermolecular propagation with the monomer. The rate of increase of G' during phase 2 was proportional to the concentration of MBAAm, but not exactly linearly. Thus, we propose that several reaction parameters such as pH, ionic strength, and the addition of EDTA may affect the intramolecular propagations, such as cyclization.

With the addition of EDTA, linear polymerization progressed to a greater extent than under the other conditions of polymerization, and the G' values were much higher, except for the crosslinking polymerization of AmGc. As shown in Figure 5, G' increased in the presence of EDTA, not having the vinyl groups, during AAc polymerization without MBAAm. Thus, we assumed that EDTA primarily affected the linear polymerization of AAc, with little effect on the intramolecular propagation.

In addition to EDTA, it was thought that other conditions affected the reaction between the propagating polymers extending from MBAAm, such as the cyclization. Propagation of the oligomeric chains from MBAAm is dependent on pH and the ionic strength. It is plausible that these propagating chains may undergo physical attractive interactions with –C=O of the amide group if the carboxyl group is not negatively charged.

Consequently, the dependence of G'_{t60} on M_{wc} was most affected by the type of monomer based on the intrinsic reactivity of the monomer and not much on the molecular size. The pH and ionic strength affected the intramolecular propagation (such as the cyclization) resulting in differences in the dependence of G'_{t60} on M_{wc}, but to a lesser extent than the type of monomer.

The slopes of the plots of G'_{t60} versus M_{wc} for the crosslinking polymerization of AAc were −0.159 to −0.278. The dependence of G'_{t60} on M_{wc} was not pronounced for the crosslinking polymerization of AmGc as much as for that of AAc (the range of slope was −0.022 to −0.052), and the dependence of G'_{t60} on M_{wc} was more decreased in the crosslinking polymerization of AmPS (the range of slope was −0.001 to −0.003). As the concentration of MBAAm increased, the rates of network formation and intramolecular propagation increased, and both effects increased G'_{t60}.

The dependence of G'_{t60} on M_{wc} was the highest at pH 2 in AAc and AmGc, and at EDTA in AmPS. The difference in the slope of the plots of G'_{t60} versus M_{wc} for each type of monomer was mainly due to the difference in the concentration of MBAAm, with additional effects from pH. EDTA as a complex agent was thought to mainly affect the linear polymerization rate.

CONCLUSIONS

Herein, the effects of the concentration of the crosslinking agent, pH, and the addition of a complex agent on the network formation of acrylic monomers were evaluated to control the rate of crosslinking polymerization, which may be intimately related to the heterogeneity of hydrogels. Network formation occurred in three phases. In the first phase, all of the polymers were water-soluble and linear polymerization was dominant. In the second phase, network formation was initiated and an initial increase of G' occurred prior to achieving the M_c. In the third phase, intramolecular crosslinking induced tightening of the polymer, and intermolecular crosslinking resulted in extension of the network. The core structure was formed by intramolecular crosslinking.

The gel point was observed without the aid of a crosslinking agent in AAc linear polymerization. Addition of EDTA as a

complex agent effectively accelerated the polymerization, and induced an increase in G'.

The maximum G' values of 20,000 Pa, 6000 Pa, and 400 Pa were, respectively, achieved during crosslinking polymerizations of AAc, AmGc, and AmPS. This difference was not attributed to the monomer size, but rather to the monomer reactivity and acidity. Higher values of G' were achieved by polymerization of weaker acid monomers.

The higher values of G' were obtained according to the following order based on the conditions: EDTA > pH 10 > pH 6 $\approx$ W > pH 2, during AAc1, and the order: EDTA > pHs 10, 6, 2 > W, during AAc4. The effect of pH on G' lessened as the concentration of the crosslinking agent was increased. Higher values of G' were achieved under the conditions: W $\approx$ pH 10 > EDTA $\approx$ pH 6 > pH 2, during AmGc1, and pH 6 > pH 2 > pH 10 $\approx$ W > EDTA, during AmGc4. It was proposed that the intramolecular hydrogen bonding at pH 2, and the intramolecular chelation at pH 10 resulted in a lower G' than achieved at pH 6 during AmGc4. This was not observed in AmGc1. AmPS, the strongest acidic monomer, did not form a network during AmPS1. However, the gel point was observed during AmPS4, giving rise to 'weak-gels' with a damping factor significantly higher than 0.1, but lower than 1.0. The effect of the crosslinking agent on the polymerization was much more significant than the effect of physical interactions such as hydrogen bonding or the electrostatic repulsion force.

The dependence of G' on M_{wc} was expressed as the slope of a plot of these parameters. The slope ranged from -0.159 to -0.278 for the crosslinking polymerization of AAc, -0.022 to -0.052 for the crosslinking polymerization of AmGc, and -0.001 to -0.003 for the crosslinking polymerization of AmPS. Dependence of G'_{t60} on M_{wc} was the highest at pH 2 for the crosslinking polymerizations of AAc and AmGc, and at EDTA for the crosslinking polymerization of AmPS.

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