

Sol–Gel Transition in Aqueous Alginate Solutions Induced by Cupric Cations Observed with Viscoelasticity

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ABSTRACT: Sol–Gel transition was occurred in 2 wt% aqueous solutions of four alginate samples with different molecular weight M_w and the ratio M/G of repeat unit mannuronate (M) to guluronate (G) induced by adding cupric ions. The transition was monitored with dynamic moduli G' and G'' in the linear region of viscoelasticity and the gel point was determined according to the Winter's criterion where the loss angle $\tan \delta$ became independent of frequency ω . The mole ratio of Cu^{2+} to the carboxyl group in alginate at gel point, f_{gel} , was higher for the samples with lower molecular weight than those with higher molecular weight without obvious M/G dependence. This suggested there was no M/G selection of Cu^{2+} complex formation with alginate. The relaxation critical exponent n for alginate samples with higher G content was lower than 0.5, meaning $G' > G''$ for the critical gels with a denser network. The cupric ion number relevant to one alginate chain at the gel point, N_{gel} , was estimated from $f_{\text{gel}} M_n/M_0$ and reflected the gel elasticity.

KEY WORDS Alginate / Cupric Cations / Sol–Gel Transition / Critical Exponent / Dynamic Viscoelasticity /

Alginate is a natural polysaccharide consisting of β -D-mannuronate (M) and its C-5 epimer α -L-guluronate (G) residues (Figure 1) (1→4) linked in a nonregular blockwise pattern along the linear chain and is mainly used as a gelling agent in numerous food and pharmaceutical applications.^{1–4} There are three types of dyad sequential blocks as MM, GG, and MG. These residue sequences endow with the alginate chain different stiffness, *e.g.*, that the mean square end-to-end distance per uronate residue for G component is 2.2 times larger than that for the M component.⁵ The chemical composition and sequence of the M and G residues depend on the biological source and growth and seasonal conditions.^{1,2}

The most attractive ability of alginate is the gel formation induced by adding various divalent cations, except Mg^{2+} .⁶ The gelation behavior and gel strength of aqueous alginate solutions strongly depend on the content of guluronate residues and also on the molecular weight and molecular weight distribution of alginates.^{6–8} Matsumoto and Mashiko investigated the influence of added salts on the viscoelastic properties of aqueous alginate solution and argued that the interaction between alginate and metal cations did not work as the cross-linking point.⁸ Matsumoto *et al.* also followed the gelation process in sodium alginate solutions induced by increasing polymer concentration without any divalent cations and found the promoting effect to the gelation of chain stiffness, *i.e.*, the alginate with higher G residue content formed gel at lower concentration with more perfect structure.^{9,10} By viscometry,

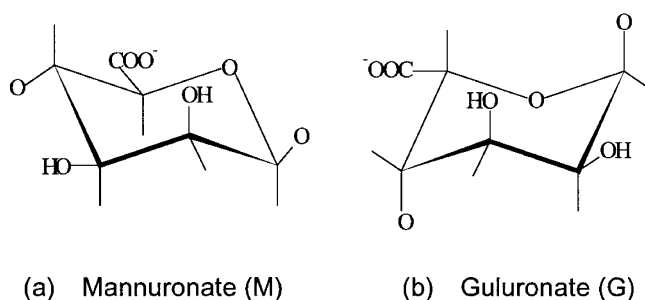


Figure 1. Monomer units of alginate: (a) Mannuronate(M) and (b) Guluronate(G).

Wang *et al.*^{11,12} and Zheng *et al.*¹³ investigated the critical phenomena of sol–gel transition in aqueous alginate solutions induced by divalent cations.

The most studied cation-induced gelation of alginate solutions is the aqueous system of Ca^{2+} -alginate. Dynamic mechanic spectroscopy was used to monitor the structure change in Ca^{2+} -alginate hydrogel.^{7,14} The binding mode of Ca^{2+} -alginate during the sol–gel transition was observed by nuclear magnetic resonance (NMR).^{15,16} Small angle X-ray scattering (SAXS) was adopted to detect the structure of Ca^{2+} -alginate gels.^{17,18}

An important application of alginate is to absorb heavy metals of Cu^{2+} , Co^{2+} , and Zn^{2+} from aqueous media such as ore leachates and industrial waste.^{19–21} Alginate gels will form with these cations in the solution and the Cu^{2+} -binding efficiency of alginate is affected by the acidity of the solution. The coordinative structure of Cu-alginate gels was observed with NMR,¹⁵ infrared (IR), electron spin resonance (ESR), and X-ray photoelectron spectroscopy (XPS).²² Up to

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now, few studies concern the determination of gel point and the critical behavior of sol-gel transition in aqueous alginate solutions induced by cupric ions.

Recently, we have determined the gel point of aqueous alginate solutions induced by calcium cations released *in situ* using rheological measurements at different Ca^{2+} concentrations²³ with the Winter's criterion,^{24,25} avoiding the extrapolation to the infinite viscosity. In the present work, we focused on the sol-gel transition of aqueous alginate solutions induced by Cu^{2+} addition with the same procedure.

EXPERIMENTAL

Alginate Samples

The same four sodium alginate samples used previously²³ produced by Kimitsu Chemical Industries Co., Japan were purified as follows: the aqueous solution of alginate samples about 5 wt% was first dialyzed in distilled water using cellulose tubular membranes (the cut-off molecular weight is 14000 ± 2000) until the conductivity of water outside became constant before and after refreshing. Then, the solution was filtered by a G2 acid-resisting filter and freeze-dried to produce purified dry samples. Molecular weight M_w and molecular weight distribution M_w/M_n of samples were determined by gel permeation chromatography (GPC) with a Waters apparatus, using 0.1 M Na_2SO_4 aqueous solution as the elution and narrowly distributed PEO as the standard. The mole ratio of mannuronate (M) to guluronate (G) residues (M/G), the mole fraction F_G and F_M of G and M, and the mole fraction of GG, MM, and GM (MG) dyad sequences F_{GG} , F_{MM} , and F_{GM} were determined by ^1H NMR according to Grasdalen's procedure^{26,27} in D_2O of 14 mg mL^{-1} at 70°C . The characterization results are summarized in Table I.

Rheology Measurements of Cu-Alginate Gels

We define the stoichiometric mole ratio $f = [\text{Cu}^{2+}]/[\text{COO}^- \text{ in alginate}]$ as a factor which controls the gelation process in alginate solutions with the assumption that the ratio f is proportional to the crosslink density formed inter- and intramolecularly. An alginate stock solution of 4 wt% was prepared by dissolution the sample in pure water, then 2 g of this solution was mixed with 2 mL cupric chloride solution of proper concentra-

tion to make an aqueous Cu-alginate system with a required f value. All sample systems were homogeneous after magnetic stirring for 30 min at room temperature and incubated at 10°C for 48 h prior to the viscoelastic measurement.

In order to reveal the sol-gel transition in aqueous alginate solution induced by cupric cations, its dynamic viscoelasticity were monitored with a Rheometrics RFS-II rheometer with a cone-plate fixture. The diameter and angle of the cone were 25 mm and 0.04 rad, respectively. All measurements were carried out at $25 \pm 0.1^\circ\text{C}$.

RESULTS AND DISCUSSION

Viscoelasticity of Aqueous Cu-Alginate during Sol-Gel Transition

The linearity of the viscoelasticity responding to stimuli should be confirmed before discussing the gel properties in the framework of classic viscoelastic theory. The shear strain γ dependence of complex modulus G^* for Cu-MHGL-3 sample in water shown in Figure 2 with various f below and after gelation at 25°C illustrates an example for the linearity because the absolute value of G^* is independent of strain γ over the γ ranging from 0.1 to 20%.²⁸ Other three samples in water at different f show the similar strain and f dependence of G^* during the sol-gel transition. All of the rheology measurements were carried out within the linear

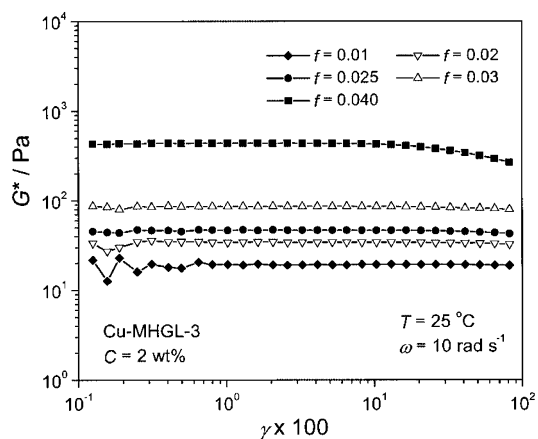


Figure 2. Shear strain γ dependence of absolute value of complex modulus G^* for Cu-MHGL-3 of 2 wt% with indicated f values at 25°C and $\omega = 10 \text{ rad s}^{-1}$.

Table I. Characterization of alginate samples

Sample	$M_w \times 10^{-4}$	M_w/M_n	M/G	F_G	F_M	F_{GG}	F_{MM}	F_{GM}
MHGL-3	323	12	1.85	0.35	0.65	0.10	0.40	0.25
MLGL-4	122	24	1.85	0.35	0.65	0.17	0.47	0.18
MHGH-1	349	14	0.91	0.52	0.48	0.44	0.40	0.08
MLGH-2	41	6	0.60	0.63	0.38	0.45	0.20	0.18

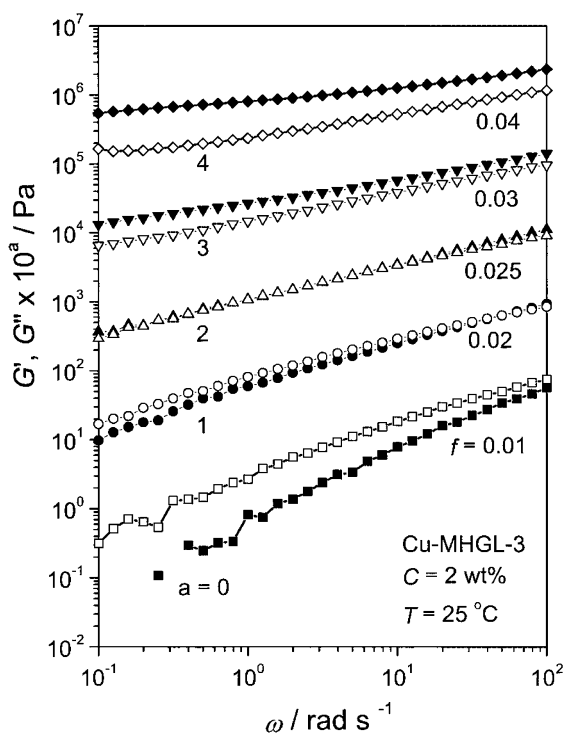


Figure 3. Angular frequency ω dependence of storage modulus G' (solid symbol) and loss modulus G'' (open symbol) for Cu-MHGL-3 of 2 wt% with indicated f . The data have been vertically shifted by a factor of 10^a with given a to avoid overlapping.

viscoelasticity region.

Figures 3–6 show the angular frequency ω dependence of the storage and loss moduli G' and G'' of the four aqueous Cu-alginate systems at various f . The data in each figure are vertically shifted by a factor of 10^a to avoid overlapping. Some G' values at low frequency with low f were too small to be measured accurately. The four alginate samples with different M_w and M/G ratios demonstrate a similar viscoelasticity feature in the gelation process induced by cupric ions. At low f values, G' and G'' in low ω range are proportional to $\omega^{1.1-1.6}$ and $\omega^{0.7-1}$ respectively, and G'' is always higher than G' in whole frequency range without any plateau appearing in G' vs. ω curves. This is the typical characteristic of a viscoelastic fluid according to the Rouse–Zimm theory.²⁸ Because the main purpose of this work is to investigate the sol–gel transition in the aqueous Cu-alginate system by interpolation, quite a few Cu^{2+} ions are added to induce this transition. In other words, the f value here is too high to observe the theoretical prediction of $G' \propto \omega^2$ and $G'' \propto \omega^1$. Broad molecular weight distribution of the samples is another reason for this deviation. At high f values, G' becomes higher than G'' and the G' vs. ω curves approaches to horizontal. This indicates the formation of viscoelastic gels with low crosslinking density. These newborn gels crosslinked by cupric cations are homo-

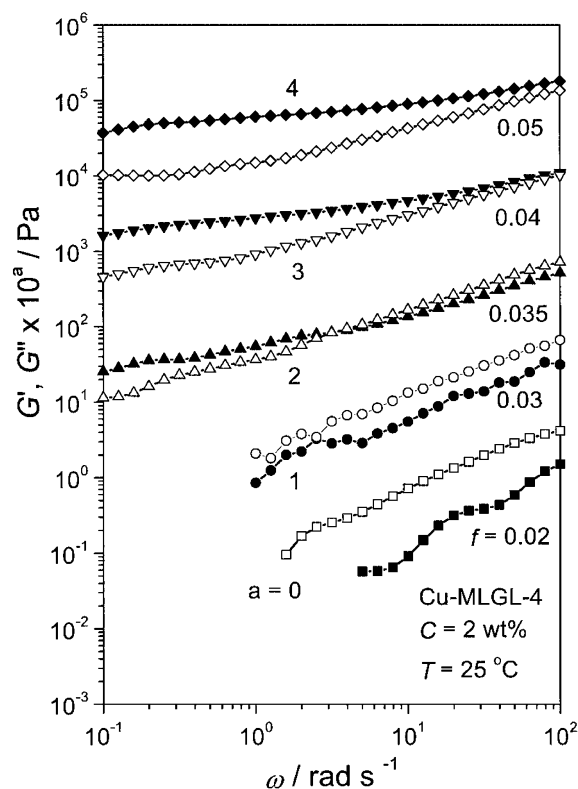


Figure 4. Angular frequency ω dependence of storage modulus G' (solid symbol) and loss modulus G'' (open symbol) for Cu-MLGL-4 of 2 wt% with indicated f . The data have been vertically shifted by a factor of 10^a with given a to avoid overlapping.

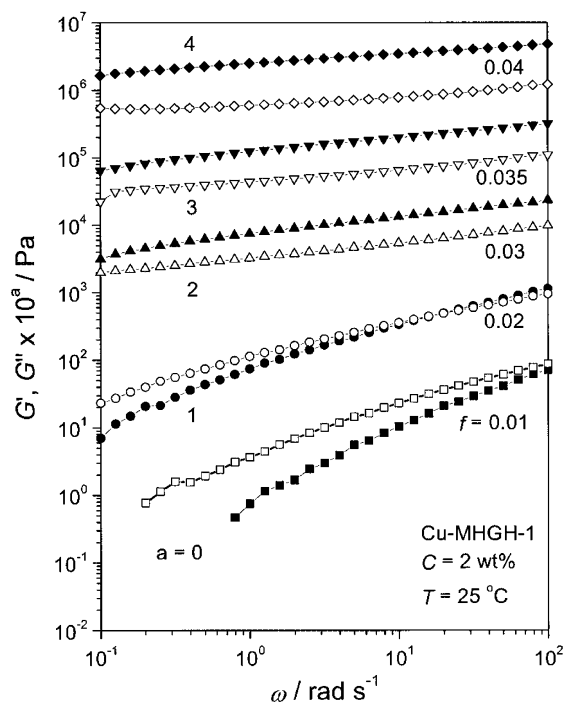


Figure 5. Angular frequency ω dependence of storage modulus G' (solid symbol) and loss modulus G'' (open symbol) for Cu-MHGH-1 of 2 wt% with indicated f . The data have been vertically shifted by a factor of 10^a with given a to avoid overlapping.

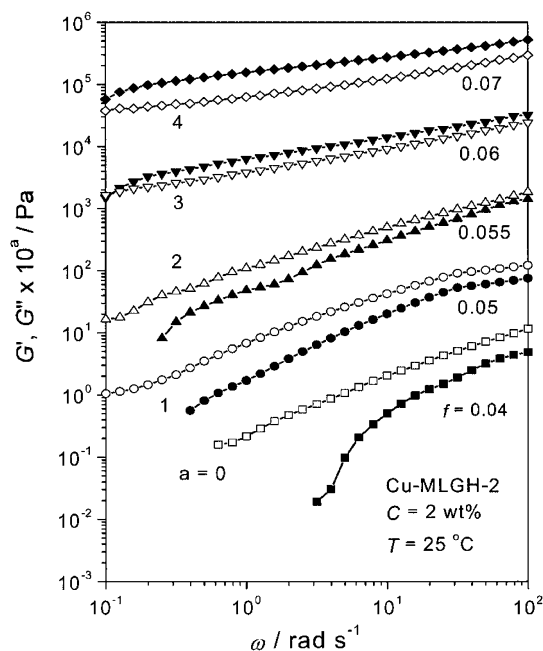


Figure 6. Angular frequency ω dependence of storage modulus G' (solid symbol) and loss modulus G'' (open symbol) for Cu-MLGH-2 of 2 wt% with indicated f . The data have been vertically shifted by a factor of 10^a with given a to avoid overlapping.

geneous and transparent.

At moderate values of f , there is a transition region from solution to gel, where ω dependence curve of G becomes parallel to or even coincides with that of G' over a wide frequency range such as the curves of $f = 0.025, 0.035, 0.02, \text{ and } 0.06$ in Figures 3–6, respectively. The slope of these G vs. ω curves at the indicated f is close to 0.5 as predicted from the power law of relaxation modulus at sol–gel transition.^{24,25} This power law behavior suggests that these critical gels possess the self-similar network structure over a wide spatial scale.^{29,30}

To follow the elasticity increase in the alginate solutions with crosslink effect of cupric ions, we plotted G at 1 rad s^{-1} against f in Figure 7 for these samples. It is obvious that the addition of Cu^{2+} ions increases the elasticity, suggesting the network formation at higher f values. By considering the logarithmic ordinate used for G' , the samples of MHGH-1 and MHGL-3 with higher molecular weight yield a much higher G at the same concentration of cupric ions.

Critical Phenomenon of Sol–Gel Transition

The gel point for the present aqueous Cu-alginate system is defined as an f value, f_{gel} , at which the macroscopic molecular network begins to form in the solution, consequently resulting in infinite viscosity and appearance of the equilibrium modulus. It is usually difficult to determine the gel point exactly for a physical gel from extrapolation either zero-shear vis-

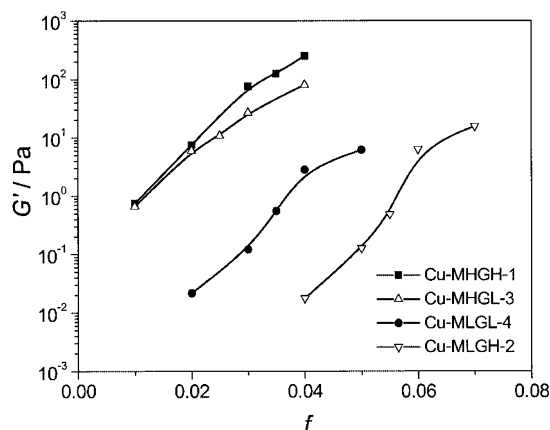


Figure 7. Storage modulus G' plotted against f for the four samples.

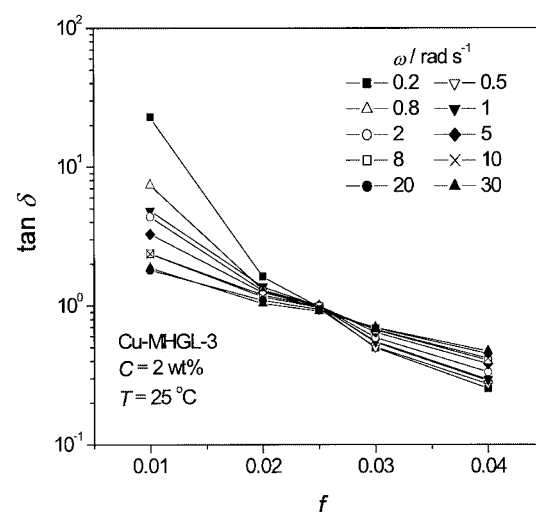


Figure 8. $\tan \delta$ at indicated ω plotted against f for Cu-MHGL-3 sample of 2 wt% to determine the gel point f_{gel} and critical exponent n .

cosity or intrinsic viscosity. Winter and Chambon proposed the relaxation modulus $G(t)$ for the critical gel as^{24,25}

$$G(t) = S t^{-n} \quad (1)$$

where S is the gel strength and n the relaxation exponent. Consequently, the dynamic mechanical properties at the gel point is given by a power law relation of ω

$$G'(\omega) \propto G''(\omega) \propto \omega^n \quad (2)$$

This means that G' and G'' are parallel in $\log G$ vs. $\log \omega$ plots with the slope of n at the gel point as shown in Figures 3–6. Therefore, the tangent of the loss angle δ can be derived from the Kramers-Krönig relation as

$$\tan \delta = G''/G' = \tan(n\pi/2) \quad (3)$$

Thus, ω independence of $\tan \delta$ provides a convenient method to determine the gel point.

In this way, we have determined the gel point and critical exponent n for the sol–gel transition in the aqueous alginate systems induced by cupric ions. Figure 8

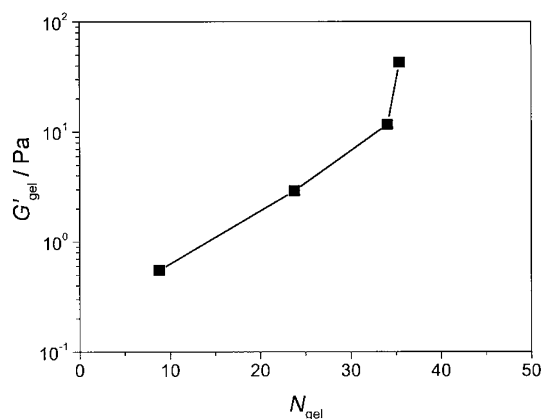
Table II. Gel point f_{gel} and critical exponent n for aqueous Cu-alginate

Samples	$M_w \times 10^{-4}$	M/G	f_{gel}	n
Cu-MHGL-3	323	1.85	0.025	0.50
Cu-MLGL-4	122	1.85	0.035	0.58
Cu-MHGH-1	349	0.91	0.028	0.29
Cu-MLGH-2	41	0.60	0.059	0.43

shows the f dependence of $\tan \delta$ obtained from Figure 3 at several frequencies as an example. The f_{gel} and n are evaluated from the intersecting point as 0.025 and 0.50, respectively. The f_{gel} and n values for other aqueous Cu-alginate systems are determined similarly and listed in Table II.

One can find from the table that the f_{gel} value is higher for the samples with lower molecular weight than that for the samples with higher molecular weight without obvious M/G dependence. According to the definition of the gel point, more Cu^{2+} cations are required to connect shorter alginate chains to form an infinite cluster having a similar structure to that formed with longer chains. This finding suggests that the complexation of alginate with Cu^{2+} cations depends on the alginate molecular weight but has no preferential composition selection for the M and G residues. This is contrary to the alginate gelation induced by Ca^{2+} ions, where more calcium cations are chelated by the alginate containing more G residues to achieve the same critical gel.²³ The gelation mechanism for Cu-alginate is different from that for Ca-alginate. In the former, four hydroxyl oxygen atoms, two from negatively charged deprotonated carboxyl group, and other two from uncharged carboxyl group, coordinate with one central Cu^{2+} ion to form the Cu-alginate complex.^{15,22} While in the latter, all function groups in guluronate residues cooperatively bind with Ca^{2+} ions.¹⁵ Therefore, the quantity of cupric ions relevant to one alginate chain is more important than total Cu^{2+} concentration in discussing the critical gel formation because the molecular weight should be taken into account.

Because M_n/M_0 is the number-average polymerization degree P_n (= number of carboxyl groups in one alginate chain) and f_{gel} is the Cu^{2+} number per carboxyl group required at the gel point, where M_n is the number-average molecular weight of alginate and M_0 the molecular weight of its repeat unit. The product $f_{\text{gel}} M_n/M_0$ equals to N_{gel} , the average quantity of Cu^{2+} ions corresponding to one alginate chain at the gel point. Figure 9 demonstrates the N_{gel} dependence of the storage modulus G'_{gel} estimated from Figure 7 at f_{gel} for the critical gel. That the G'_{gel} increases with N_{gel} confirms our idea that the number of Cu^{2+} ions relative to one alginate chain promotes the network formation for the

**Figure 9.** The average quantity of Cu^{2+} ions relevant to one alginate chain, N_c , dependence of the storage modulus G'_{gel} at the gel point.

aqueous Cu-alginate system.

On the other hand, the n value appears to depend on the M/G, which describes the perfectibility of the newborn network at the gel point. n for alginate samples with higher G content (MHGH-1 and MLGH-2) is lower than 0.5, showing $G' > G''$ already for these critical gels with a denser network. This is commonly ascribed to the high chain stiffness of the alginate sample with more G residues.^{6,7,9} The present n values are smaller than 0.678 estimated from the viscosity critical exponent k reported by Zheng *et al.*¹³ on only one sample.

Further investigations of polymer concentration dependences of the sol–gel transition in aqueous alginate solutions induced by either Ca^{2+} or Cu^{2+} cations are extremely important for understanding this gelation mechanism, which we will report in the following papers.

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