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Polymer 44 (2003) 407–412

polymer

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Rheology characterization of sol–gel transition in aqueous alginate solutions induced by calcium cations through in situ release

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Received 6 June 2002; received in revised form 16 October 2002; accepted 21 October 2002

Abstract

Four alginate gels were prepared by in situ release of calcium cations from Ca-EDTA with gradual hydrolysis of D-glucono-δ-lactone (GDL) in 3 wt% of aqueous solutions of four alginate samples with different M/G compositions, molecular weights, and molecular weight distributions. The sol–gel transition was monitored by measuring the dynamic mechanical spectroscopy in the linear region with variation in the mole ratio f of Ca^{2+} to the carboxyl group in the alginate. f_{gel}, the critical value of f at the gel point, was determined according to the appearance of power law relaxation for the storage and loss moduli as proposed by Winter and Chambon. The critical exponent of relaxation moduli n for the power law was estimated from the loss tangent $\tan\delta = G''/G'$ at the gel point which is independent of angular frequency. f_{gel} for the two samples with higher M/G ratio is about 0.037 while that for the other two samples with lower M/G ratio is 0.049 and 0.060, suggesting that more Ca^{2+} cations are chelated into the 'egg-box' cavities formed by the G sequence to cross-link alginate chains with higher G content. The n value is higher than 0.5, showing that the Ca^{2+} induced alginate network is not perfect due to its low concentration.

Keywords: Alginate; Sol–gel transition; Gel point

1. Introduction

Alginic acid is a linear polysaccharide consisting of (1 → 4) linked β-D-mannuronate (M) and its C-5 epimer α-L-guluronate (G) residues (Fig. 1) arranged in a non-regular blockwise pattern along the linear chain and is mainly used as a gelling agent in numerous food and pharmaceutical applications [1–4]. The pK_a values of the M and G residues in 0.1 M NaCl are 3.38 and 3.65, respectively [5]. The chemical composition and sequence of the M and G residues depend on the biological source and growth and seasonal conditions [1,2]. There are three types of diad sequential blocks as MM, GG, and MG. These residues endow with the alginate chain different stiffness such as the mean square end-to-end distance per uronate residue for G component is 2.2 times larger than that for the M component [6].

The most attractive property of alginate is the gel formation induced by adding various divalent cations, except Mg^{2+} [7]. The G residue is in the alternate $^1C_4$ conformation and paired G-sequences making a buckled 2-fold structure as the shape of 'egg-box' form cavities to accommodate divalent cations in a chelate type of binding, which is considered as the intermolecular junction having arrays of site-bound cations [8,9]. The ability of alginates to form gels and gel strength strongly depend on the content of polyguluronate and also on the molecular weight and molecular weight distribution of alginates [5,7,10]. Until now, many studies have been devoted to reveal the gelation process and gel structure of alginates. Matsumoto and Mashiko investigated the influence of added salts on the viscoelatic properties of aqueous alginate solutions and argued that the interaction between alginate and metal cations did not work as the cross-linking points [10]. Matsumoto et al. also followed the gelation process in sodium alginate solutions induced by increasing polymer concentration without adding 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 [11,12].

By viscometry, Wang et al. [13,14] and Zheng et al. [15] investigated the critical phenomena of sol–gel transition in aqueous alginate solutions induced by divalent cations and

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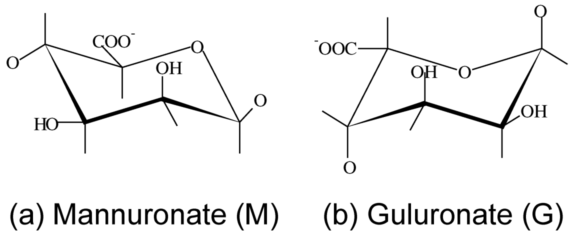
PII: S0032-3861(02)00771-1



(a) Mannuronate (M) (b) Guluronate (G)

Fig. 1. Monomer units of alginate: (a) Mannuronate (M); (b) Guluronate (G).

estimated the critical cation mole ratio f_c ($=f_{gel}$) of Ca^{2+} to the carboxyl group in the alginate at the gel point and the critical exponent k of relative viscosity η_{rel} [$\eta_{rel} \propto (f_c - f)^{-k}$]. Dynamic mechanic spectroscopy was adopted to monitor the structure change in Ca-alginate hydrogel with reaction time [5] and temperature [16]. The alginate gel was also observed with NMR to determine the interaction mode of different metal cations with definite residues during the sol–gel transition [17] and the localization of the Ca^{2+} ions [18]. The small-angle X-ray scattering (SAXS) data indicated the structure change in Ca-alginate gels with the alginate concentration, Ca^{2+} concentration, and the alginate composition [19,20].

However, a few studies concern the determination of gel point for the sol–gel transition in aqueous alginate solutions induced by chelating divalent cations [13–15]. It is difficult to extrapolate the solution viscosity to the gel point, where the viscosity becomes infinite, without ambiguous for a physically cross-linked system [21,22]. On the other hand, direct addition of aqueous Ca^{2+} ions was commonly used in preparing alginate gels. Addition of gel-inducing ions to high-molecular-weight alginate in aqueous solution results in macroscopically heterogeneous structures due to the rapid ion binding [23–25].

We have employed in situ release of Ca^{2+} from a Ca source of Ca-EDTA (ethylene-diaminetetra-acetic acid) to prepare homogeneous alginate gel samples to avoid the problem of Ca^{2+} concentration gradient in the gel. The process of sol–gel transition was characterized by viscoelasticity and the gel point was determined according to appearance of the power law for the dynamic moduli at the critical gel as proposed by Winter and Chambon [26,27]. The critical exponents have been estimated from the viscoelasticity data at the gel point.

2. Experimental

2.1. Alginate samples

Four sodium alginate samples 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 10,000) 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 and molecular weight distribution of samples were determined by GPC with a Waters apparatus using the elution of 0.1 M Na_2SO_4 aqueous solution and the standards of narrowly distributed PEO. The mole ratio of mannuronate (M) to guluronate (G) residues (M/G) and the mole fraction of GG, MM, and GM (MG) diad sequences F_{GG}, F_{MM}, and F_{GM} were determined by ^1H NMR according to Grasdalen [28,29] in D_2O of 14 mg/ml at 70 °C. The characterization results are summarized in Table 1.

2.2. Preparation of Ca-alginate gels by in situ release of calcium cations

We define the stoichiometric mole ratio $f = [Ca^{2+}]/[COO^-$ in alginate$]$ as a structure parameter which controls the gelation process with the assumption that the ratio f is proportional to the fraction of cross-links formed inter- and intramolecules. In order to prepare homogeneous Ca-alginate gels at room temperature, the method of in situ release of calcium cations from EDTA (EDTA, Tianjin Institute for Chemicals, standard reagent) chelate was adopted through lowering the pH with the slow hydrolysis of D-glucono-δ-lactone (GDL, Sigma) as described in the literature [5,20].

The chelating stable constant of Ca-EDTA decreases with a decrease in pH, which is high at pH = 7 while becomes negligibly small at pH = 4. The aqueous Ca-EDTA solution was prepared by adding required amount of EDTA and $CaCl_2$ (1:1 in mole) to deionized water, the pH was adjusted to 7 with 0.1 M NaOH solution. The amount of GDL required to induce pH = 4 after complete hydrolysis for 24 h at different Ca-EDTA concentrations was determined from calibration curves. The calibration curve was established by monitoring the solution pH variation with GDL concentration at a given Ca-EDTA concentration without alginate as shown in Fig. 2.

The same volumes of a concentrated alginate stock solution and a Ca-EDTA solution with different concentrations at pH = 7 were mixed to give a solution of alginate concentration $C = 3$ wt% and desired f values. Freshly prepared GDL aqueous solution was added to reach the amount of GDL determined from Fig. 2. All samples were

Table 1
Characterization results 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}
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
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

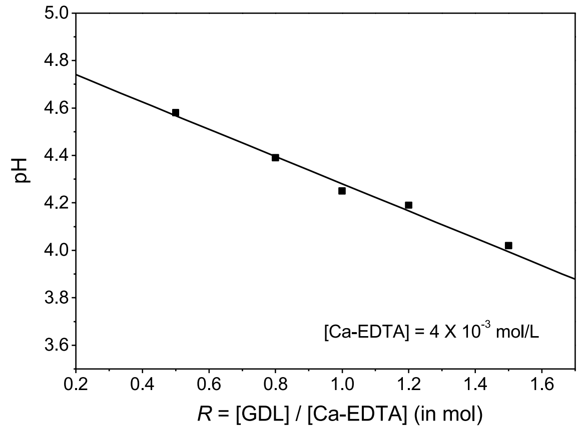


Fig. 2. The calibration curve for solution pH varying with the dosage of GDL when [Ca-EDTA] $= 4 \times 10^{-3}$ mol/l.

stirred for 15 min and incubated for 24 h before viscoelastic measurements.

2.3. Rheology measurement

Dynamic viscoelastic spectra were measured by a RFS-II rheometer (Rheometrics Ltd) with a cone-plate fixture. The diameter and angle of the cone were 50 mm and 0.04 rad, respectively. All measurements were carried out at 25 ± 0.1 °C.

3. Results and discussion

3.1. Viscoelastic behavior during sol–gel transition

If the investigated system is in the region of linear viscoelasticity, the absolute value of complex modulus, G^* should be independent of strain γ [30]. Fig. 3 is the plots of shear strain γ dependence of G^* for MHGH-1 sample in water with various f below and above gelation at 25 °C. The linear viscoelasticity can be observed over the γ range from 0.1 to 60%. G^* increases with increasing f because the alginate network is deduced and becomes more and more mature. Other three samples in water show the similar strain and f dependence of G^*. All of the reological characterization was carried out in the linear viscoelasticity range.

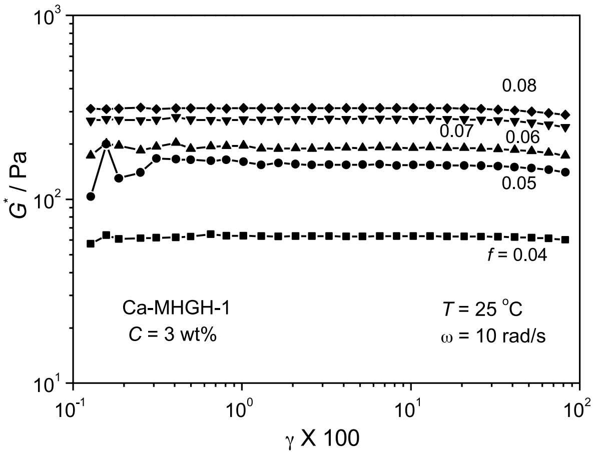


Fig. 3. Shear strain γ dependence of absolute value of complex modulus G^* for Ca-MHGH-1 of 3 wt% with indicated f values at 25 °C and $\omega = 10$ rad/s.

Figs. 4–7 show the angular frequency ω dependence of the storage and loss moduli G' and G'' of four aqueous Ca-alginate systems at various f. The data in each figure are vertically shifted by a factor of 10^a to avoid overlapping. The dynamic moduli of the systems increase with increasing f in the detected frequency region, similar to that reported by Matsumoto and Mashiko [10]. At low values of f, G' and G'' in low ω range are proportional to $\omega^{1.1-1.9}$ and ω^1, respectively, and G'' is always higher than G' in entire frequency range without any plateau appearing in G' vs. ω curves. These correspond to the characteristics of a viscoelastic fluid according to the Rouse-Zimm theory [30] due to the lack of enough Ca^{2+} to be coordinated with alginate chains to form three-dimensional networks. The deviation from $G' \propto \omega^2$ and $G'' \propto \omega^1$ predicted by the Rouse-Zimm theory may be attributed to the broad molecular weight distribution of the samples and the ω still far from the low-frequency limit. At higher values of f, G' becomes higher than G'' with a plateau appearing in the G' vs. ω curves in low frequency range. This indicates the formation of viscoelastic gels of low cross-linking

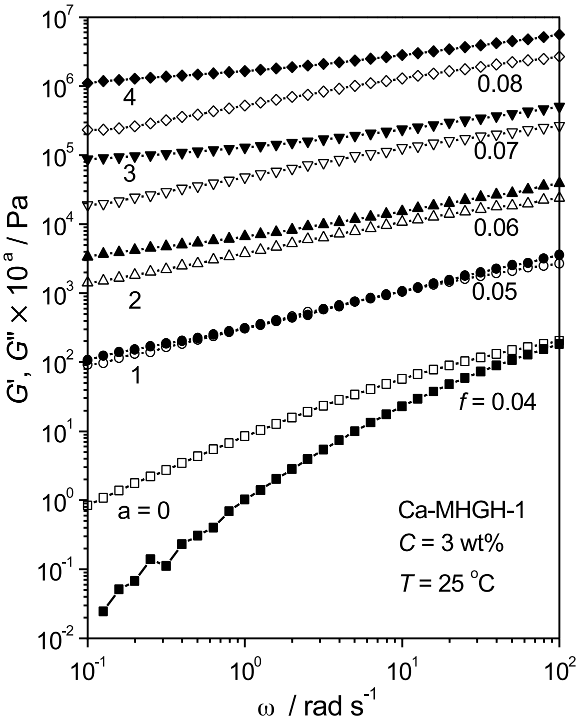


Fig. 4. Angular frequency ω dependence of storage modulus G' (solid symbol) and loss modulus G'' (open symbol) for Ca-MHGH-1 of 3 wt% with indicated f. The data have been vertically shifted by a factor of 10^a with given a to avoid overlapping.

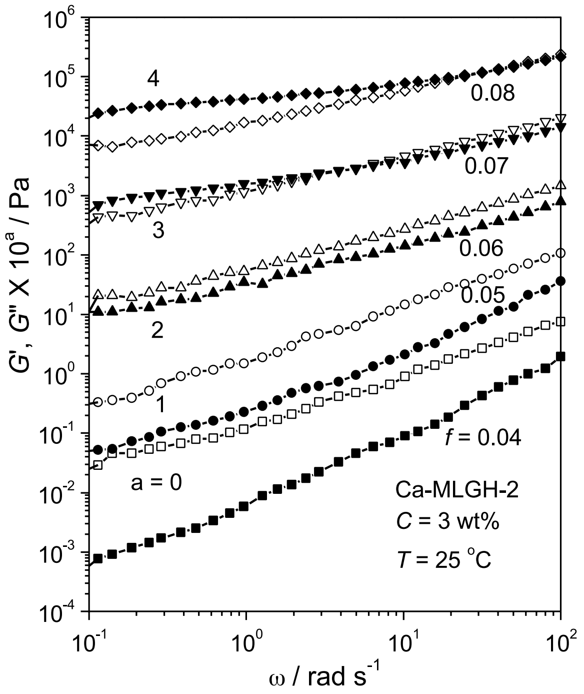


Fig. 5. Angular frequency ω dependence of storage modulus G' (solid symbol) and loss modulus G'' (open symbol) for Ca-MLGH-2 of 3 wt% with indicated f. The data have been vertically shifted by a factor of 10^a with given a to avoid overlapping.

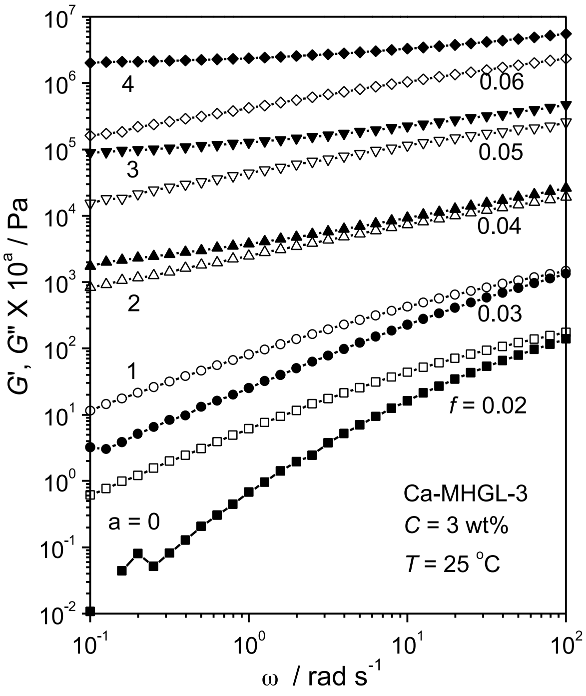


Fig. 6. Angular frequency ω dependence of storage modulus G' (solid symbol) and loss modulus G'' (open symbol) for Ca-MHGL-3 of 3 wt% with indicated f. The data have been vertically shifted by a factor of 10^a with given a to avoid overlapping.

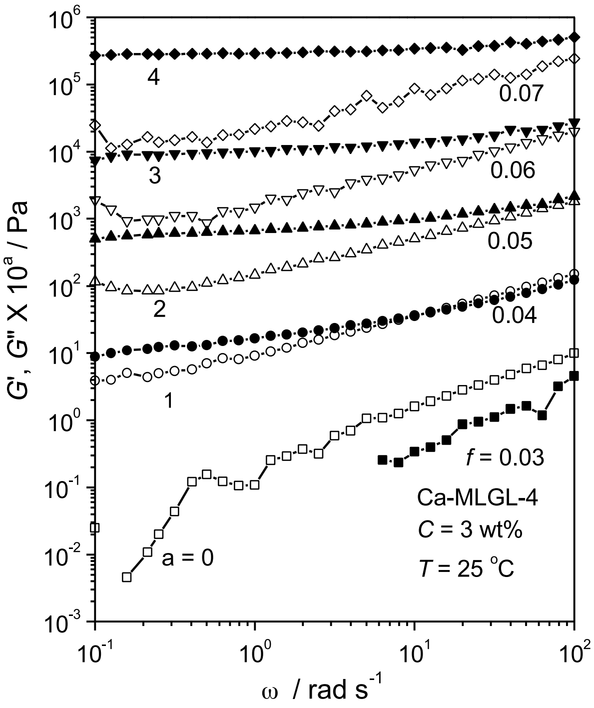


Fig. 7. Angular frequency ω dependence of storage modulus G' (solid symbol) and loss modulus G'' (open symbol) for Ca-MLGL-4 of 3 wt% with indicated f. The data have been vertically shifted by a factor of 10^a with given a to avoid overlapping.

density. The gels formed by in situ release of calcium cations are homogeneous and transparent.

At moderate values of f, there is a transition region from solution to gel, where ω dependence curve of G' becomes almost parallel to or even coincides with that of G'' over a wide frequency range, such as the curves of $f = 0.05$, 0.06, 0.04, and 0.04 in Figs. 4–7, respectively. The slope of these G' vs. ω curves is about 0.5, consistent with what predicted by Winter and Chambon for the power law of relaxation modulus during the sol–gel transition [26,27].

3.2. Critical phenomenon of sol–gel transition

The gel point for the present aqueous Ca-alginate system is defined as an f value, f_{gel}, where the viscosity becomes infinite and the equilibrium modulus appears. It is usually difficult to determine the gel point exactly for a physical gel because it has neither intrinsic size scale nor intrinsic time scale. As proposed by Winter and Chambon [26,27], the critical gel has the relaxation modulus $G(t)$ as

$$G(t) = St^{-n} \tag{1}$$

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 between moduli and frequency

$$G'(\omega) \propto G''(\omega) \propto \omega^{n} \tag{2}$$

This means that G' and G'' are parallel in log G vs. log ω

plots with the slope of n as seen in Figs. 4–7. Therefore, the tangent of the loss angle δ from the Kramers–Krönig relationship is independent of frequency at the gate point

$$\tan\delta = G''/G' = \tan(n\pi/2) \tag{3}$$

and ω independence of $\tan\delta$ provides a convenient criterion for determining the gel point.

Following this way, we determine the gel point and critical exponent n for the calcium alginate gels formed by in situ release of calcium cations in aqueous alginate solutions. As an example, Fig. 8 shows the $\tan\delta$ data obtained from Fig. 4 plotted against f, the mole ratio of the Ca^{2+} ion to the COO^- ion in the alginate, at several frequencies. The intersecting point, where $\tan\delta$ is independent of ω, gives the critical point values of f_{gel} and n as 0.049 and 0.562, respectively. The f_{gel} and n values for other aqueous Ca-alginate systems are evaluated similarly and listed in Table 2.

One can find from the table that f_{gel} varies with samples but the n is always larger than 0.5 for all four samples. These n values indicate that at the gel point the G'' is large than G' and the network structure is not perfect due to the low alginate concentration. The fact that the f_{gel} value for the samples MHGL-3 and MLGL-4 with higher M/G ratio is lower than that for the samples MHGH-1 and MLGH-2 with lower M/G ratio suggests that more Ca^{2+} cations are required to cross-link alginate chains with more G residues. It apparently contradicts the observation that the alginate with higher G content can form a gel stronger than that with higher M content [5,7,11,12]. Actually, this f_{gel} value reflects the point where the critical gel with infinite molecular size just begins to form, never being a completely cross-linked gel as reported in the literature. And the cross-linker formed by the fold G sequence of the egg-box shape can accommodate more divalent cations for one intermolecular junction. Therefore, more Ca^{2+} cations are chelated into the alginate network with higher G content when compared at the same cross-linking degree of the gel point.

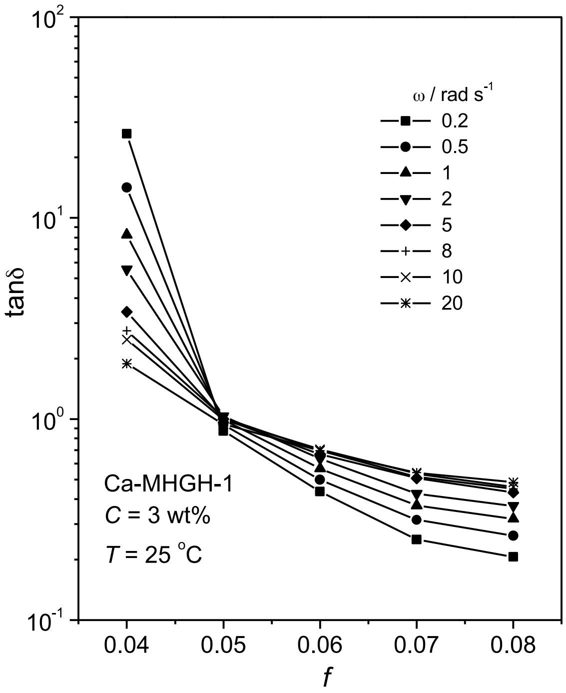


Fig. 8. $\tan\delta$ at indicated ω plotted against f for Ca-MHGH-1 sample of 3 wt% to determine the gel point f_{gel}.

Table 2
Gel point f_{gel} and critical exponent n for Ca^{2+} induced sol–gel transition of alginate

Samples	f_{gel}	n
Ca-MHGH-1	0.049	0.562
Ca-MLGH-2	0.060	0.689
Ca-MHGL-3	0.036	0.580
Ca-MLGL-4	0.037	0.620

For easy comparison, we plotted the storage modulus G' of these four Ca-alginate samples in gel region with the same f value of 0.06 in Fig. 9. For the Ca-MHGH-1 and Ca-MLGH-2 samples, the curves obviously incline against the angular frequency because the f is close to their critical value f_{gel}. While for the Ca-MHGL-3 and Ca-MLGL-4 samples, the relaxation is quite weak, showing almost horizontal curves due to f value much larger than their f_{gel}. When comparing within these two pairs, we find that the alginate with higher molecular weight can form the gel having higher G', indicating higher chain density between cross-linkers for samples with higher M_w.

From the definition of the critical gel, it is assumed that the fraction of sites that have been already joined in the cross-link to the total sites that can participate in the cross-link is p, while the critical value required for the critical gel is p_c. The relative distance from the gel point is

$$\varepsilon = |p - p_c|/p_c \tag{4}$$

According to de Gennes [31], the percolation theory is suitable to account for the viscoelasticity around the sol–gel transition [32,33]. The divergence of the zero shear

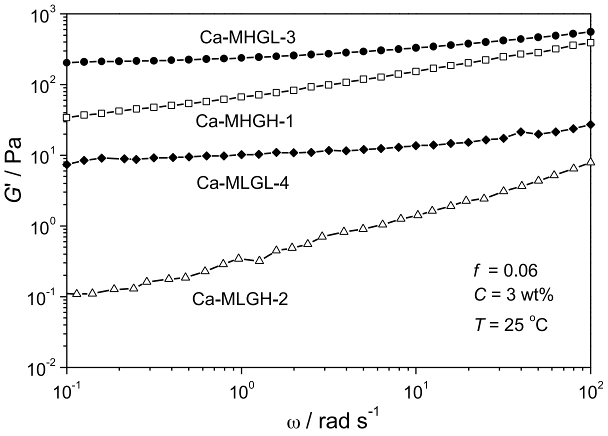


Fig. 9. Angular frequency ω dependence of storage modulus G' for indicated Ca-alginate gels of 3 wt% with the same f value of 0.06.

viscosity η_0 and equilibrium modulus G_e is described by [34]

$$\eta_0 \propto \varepsilon^{-k}, \quad p < p_c \tag{5}$$

$$G_e \propto \varepsilon^{z}, \quad p > p_c \tag{6}$$

where k and z are the critical exponents. Martin et al. [35] predicted that $k = 1.33$, $z = 2.67$, and

$$n = z/(k + z) \tag{7}$$

so that $n = 0.6675$, which is close to our experimentally estimated n values. Wang et al. [14] obtained $k = 0.90-0.97$from the f dependent curve of the relative solution viscosity, yielding $n = 0.748-0.734$. Zheng et al. [15] reported the k value of 1.27 for cupric cation induced gelation in aqueous alginate solutions, which yielded $n = 0.678$. Because only one alginate sample was used in their experiments, further research is needed to reveal the M/G dependence of the gel point and critical exponents at the sol–gel transition induced by Ca^{2+} cation.

Acknowledgements

The authors thank Prof. Y. Einaga of Nara Women's University, Japan for providing samples as gifts and National Natural Science Foundation of China (29725411) for financial support.

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