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Critical behavior at sol–gel transition in gellan gum aqueous solutions with KCl and $CaCl_2$ of different concentrations

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ABSTRACT

The sol–gel transition in aqueous gellan gum solutions with added KCl or $CaCl_2$ upon cooling was investigated using rheology measurements. The gelation temperature T_{gel} (gel point) and the relaxation critical exponent n were successfully determined according to the Winter's criterion. With increasing salt concentration, T_{gel} increased while n decreased. The scaling for the zero shear viscosity η_0 in the sol phase and the equilibrium modulus G_e in the gel phase were established against the relative distance ε ($\varepsilon = |T - T_{gel}|/T_{gel}$) from the gel point, which gave the critical exponents k and z. No universal critical exponent n was found for this sol–gel transition because this gelation belonged to the cross-linking of existing macromolecules and the concept of random connectivity failed. The fractal dimension d_f calculated from n without hydrodynamic interaction nor excluded volume suggested a denser filling structure in the critical gel at higher salt concentration.

1. Introduction

Gellan gum is a microbial polysaccharide produced by fermentation of the organism *Pseudomonas elodea* and composed of tetrasaccharide repeating units: 1,3-β-D-glucose, 1,4-β-D-glucuronic acid, 1,4-β-D-glucose, and 1,4-α-L-rhamnose (Jansson, Lindberg, & Sandford, 1983; O'Neill, Selvenderan, & Morris, 1983). Native gellan contains a substitution of an L-glycerate group at C_2 and a partial substitution of acetate group at C_6 of 1,3-β-D-glucose. The commercial gellan gum product has been completely de-esterified by alkali treatment and the deacetylated gellan gum has lower sol–gel transition temperature and stronger gel strength than the native one (Chandrasekaran & Thanilambal, 1990; Mazen, Milas, & Rinaudo, 1999; Morris, 1995; Rinaudo, 2001). In the presence of cations, aqueous gellan gum solutions at very low concentration change into hydrogels upon decreasing temperature. By comparing with other polysaccharide gels, the gellan gum gel is heat-resistant and less pH-sensitive (Mazen et al., 1999). Therefore, gellan gum has been widely used in food industry as a thickening agent (Morrison, Sworn, Clark, Chen, & Talashek, 1999; Omoto, Uno, & Asai, 1999). Moreover, gellan gum is one of the most promising *in situ* gelling polymers in the human body and applicable for biomedicine technology, such as drug delivery vehicles and protein immobilization media (Coviello et al., 1998; Jerome, 2007; Nanjawade, Manvi, & Manjappa, 2007).

The gelation of gellan gum in aqueous solutions has been intensively investigated up to now and a two-step process has been found for this gelation. The first step is the conformation change from random coil to double helix and the second step is aggregation of the double helices to form junction points, which results in gelation eventually (Chandrasekaran, Millane, Arnott, & Atokins, 1988; Chandrasekaran, Puigjaner, Joyce, & Arnott, 1988; Crescenzi, Dentini, Coviello, & Rizzo, 1986; Grasdalen & SmidsrØd, 1987; Nijenhuis, 1997; Ogawa, Takahashi, Yajima, & Nishinari, 2005; Robinson, Manning, & Morris, 1991). The gelation mechanism of gellan gum solutions depends on the nature of cations and the divalent cation promotes the gelation much more effectively than the monovalent cation. The monovalent cations shield the electrostatic repulsion and benefit the aggregation of double helix. While, the divalent cations form ionic bonds directly with the carboxylic acid groups at different gellan chains, resulting in the aggregation of the double helices by the inter-chain bridging (Kanesaka, Watanabe, & Matsukawa, 2004; Nishinari & Miyoshi, 1999).

Rheology method is a convenient way to monitor the gelation process of a viscoelastic fluid because its viscoelasticity changes dramatically at the gel point. The rheology results revealed that the gelation temperature of gellan solution increased with increasing cation concentration and the order of effectiveness for monovalent cations promoting the gelation followed the Hofmeister sequence (Miyoshi, Takaya, & Nishinari, 1996; Morris, Richardson, & Whittaker, 1999; Nishinari & Miyoshi, 1999). Circular dichroism (CD) spectrum was used to detect the conformation change of gellan chains from single coil to double helix during the gelation: a peak of the molar ellipticity appeared at about 201 nm ($[\theta]_{201}$)

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and the conformational transition temperature became higher with increasing cation concentration (Kanesaka et al., 2004; Matsukawa, Huang, & Watanabe, 1999; Ogawa, Matsuzawa, & Iwahashi, 2002). An exothermic peak in differential scanning calorimetry (DSC) at the cooling curve and an endothermic peak at the heating curve were observed for the transition of coil to helix and helix to coil, respectively. The DSC results indicated that the gelation characteristics of gellan gum depended on the nature of the added cations (Miyoshi et al., 1996). The spin-lattice relaxation time T_1 and the spin-spin relaxation time T_2 of nuclear magnetic resonance (NMR) were adopted to monitor the change in gellan chain mobility during the sol–gel transition in the presence of monovalent and divalent cations to reveal the hydrogen bonding and conformation change of the gellan molecules (Matsukawa, Huang, et al., 1999; Matsukawa, Tang, & Watanabe, 1999).

Up to now, only a few studies concern the critical behavior of the sol–gel transition in the gellan gum aqueous solutions with cations. The gel point becomes a very important reference state in discussing the structure and properties of different gellan gum gels, and the critical exponents of the sol–gel transition are significant for quantitative description of the hydrogel structure at the critical state. The problem is, however, that making "critical gel" measurement on physically gelling systems is intrinsically very difficult.

Winter and Chambon (Winter & Chambon, 1986; Chambon & Winter, 1987) found experimentally that the storage and loss moduli G' and G'' exhibited a power law on angular frequency ω as $G' \sim G'' \sim \omega^n$ at the gel point. Thus, the relaxation modulus $G(t)$ became $G(t) = St^{-n}$, where S was the gel strength and n the relaxation critical exponent. With the Kramers–Krönig relation, tangent of the loss angle δ was read

$$\tan\delta = \tan\left(\frac{n\pi}{2}\right) \qquad (1)$$

This relation suggested an interpolation method to determine the gel point accurately, i.e., ω independence of $\tan\delta$ provides the gel point and relaxation critical exponent n.

In this research, the effect of added salts KCl or $CaCl_2$ on the sol–gel transition of a deacetylated K-type gellan gum (Gelrite®), which is the most popular product of gellan gum used in food and biomedical industries, was investigated and the gel point was determined by rheology methods. Moreover, the critical exponents of the sol–gel transition and the critical gel structure were discussed for further understanding the gellan gum gel.

2. Experimental

2.1. Gellan gum sample

Deacetylated potassium-type gellan gum (Gelrite®) was purchased from Sigma and purified as follows: the aqueous gellan gum solution of ~2.5 wt% was first dialyzed against distilled water at 70 °C using a cellulose tubular membrane until conductivity of the water outside became constant before and after refreshing. Then, the solution was filtered and freeze-dried to produce purified dry sample. Metal cation content in the purified gellan sample was determined by atomic absorption spectrum as K^+: 0.22 wt%, Na^+: 0.063 wt%, Ca^{2+}: 0.029 wt%, and Mg^{2+}: 0.0025 wt%. The aqueous gellan gum solution was prepared in the same way as that described by Matsukawa et al (Matsukawa, Tang, et al., 1999). The powdered gellan gum was mixed with deionized water and stirred at room temperature overnight to homogenize the suspension. This homogeneous suspension was heated to 70 °C stirred for 2 h, and then heated to 90 °C for 30 min till complete dissolution. Subsequently, KCl or $CaCl_2$ solution of appropriate concentration was added to desired concentration of K^+ or Ca^{2+} at 90 °C under vigorous stirring for another 30 min. Finally, completely homogenized solution was directly put into a preheated incubator to achieve equilibrium at the specified temperature. Gellan gum concentration C_g in this work was kept at 1.0 wt%. The amount of added cations was several ten times higher than that originally present in the sample. Thus, the cation effect on the gelation predominantly came from the added salts.

2.2. Rheology measurements

Rheology measurements were conducted to determine the gel point with a strain-controlled ARES-RFS rheometer and a stress-controlled AR-G2 rheometer using different fixtures, including parallel plates of diameter of 25 and 50 mm, a cone-plate of 60 mm diameter with the cone angle of 0.03 rad. Temperature was controlled by a Peltier plate with an accuracy of ±0.1 °C. Frequency sweep from 10^{-2} to 10^2 rad/s at a given temperature was carried out within the linear viscoelasticity region to determine the gel point. For the pregel solutions, viscosity η was measured at different shear rates and desired temperatures to determine the zero-shearing viscosity η_0.

3. Results and discussion

3.1. Observation of sol–gel transition

In this work, we determined the gelation temperature (gel point) for the sol–gel transition in aqueous gellan gum solutions with different salt concentrations using rheology methods. It is well-known that gelation of a gellan solution with added salts should be incubated at a specified temperature for equilibrium. We tested this incubating time by the frequency sweep at a given temperature. Fig. 1 demonstrates the dynamic moduli of a gellan gum solution incubated at 30 °C for different incubating times. Because the dynamic moduli after 48 h incubation agreed well with those after 72 h incubation, we took 48 h as the incubation time and carried out the rheology measurement after the incubation.

Fig. 2 shows the angular frequency ω dependence of the dynamic moduli for 1.0 wt% gellan gum solutions containing KCl of 25 mM (A) and $CaCl_2$ of 2.0 mM (B) at indicated temperatures for instances. Commonly, at high temperature, G' and G'' are proportional to $\omega^{1.44-1.79}$ and $\omega^{0.92-0.97}$, respectively, and G'' is always higher than G' without any plateau in the curve of G' vs ω in the low frequency

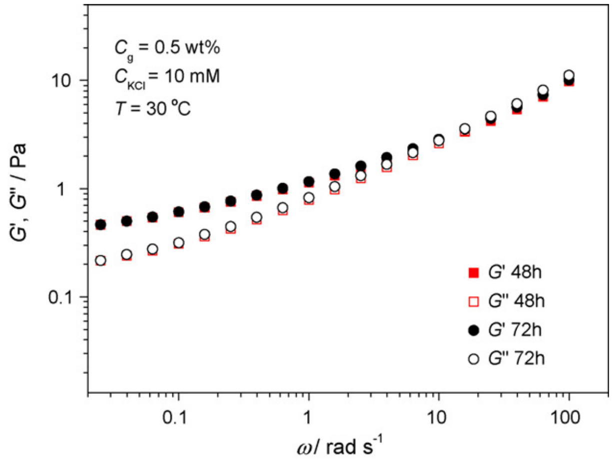

Fig. 1. Angular frequency ω dependence of storage modulus G' (solid symbol) and loss modulus G'' (open symbol) for the gellan gum solution of $C_g = 0.5$ wt% with KCl of 10 mM after incubated at 30 °C for 48 and 72 h, respectively.

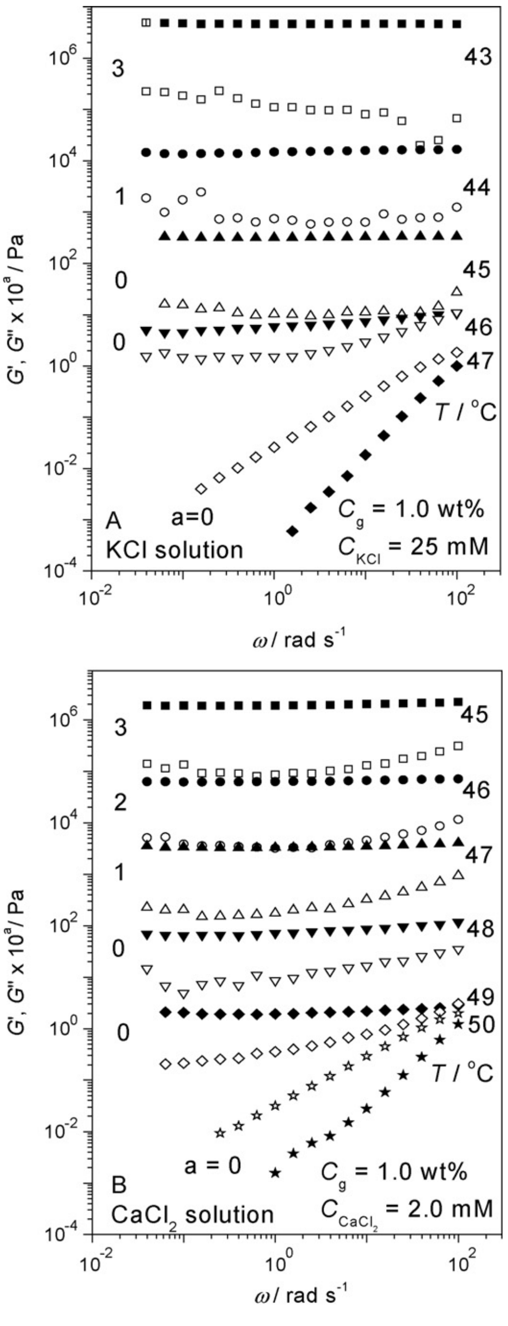

Fig. 2. Angular frequency ω dependence of storage modulus G' (solid symbol) and loss modulus G'' (open symbol) for 1.0 wt% gellan gum solutions with $C_{KCl} = 25$ mM (A) and $C_{CaCl_2} = 2.0$ mM (B) at indicated temperatures. The data were vertically shifted by a factor of 10^a with given a value to avoid overlapping.

range. These are the typical characteristic of a viscoelastic polymer fluid without network structure (Ferry, 1980). As temperature is decreased, the storage modulus G' becomes higher than the loss modulus G'' and a plateau appears in the curve of G' vs ω, indicating the network formation. Therefore, sol–gel transition occurs on cooling in the gellan gum solutions containing either K$^+$ or Ca^{2+} cations.

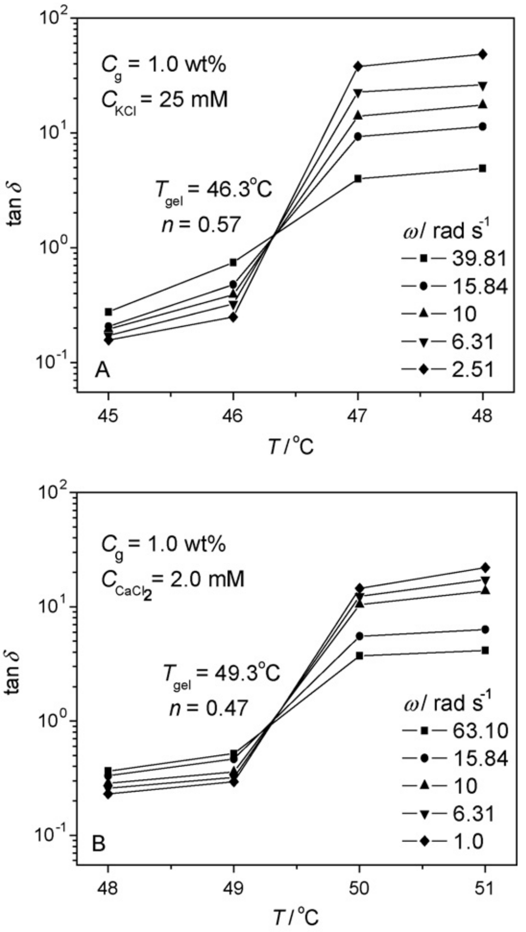

Fig. 3. tan δ at indicated ω plotted against T for 1.0 wt% gellan gum solutions with 25 mM KCl (A) and 2.0 mM CaCl$_2$ (B) to determine the gel point T_{gel} and critical exponent n according to the Winter's criterion.

The gel point T_{gel} was determined according to the Winter's criterion of $\tan\delta = \tan(n\pi/2)$ using the interpolation method, where $\tan\delta$ was independent of frequency. Fig. 3 presents this interpolation with the data from Fig. 2 as examples. T_{gel} and critical exponent n determined in this way are summarized in Table 1. The uncertainty in the n value comes from determination of the intersecting point from the $\tan\delta$ vs T curve. Consequently, the Winter's criterion is available for determination of the points in the gellan gum solutions with salts.

The ω dependence curves of G' and G'' just at the gel point, i.e., the power law of $G' \sim G'' \propto \omega^n$, were not obtained. This is due to the high temperature sensitivity of the gelation in the gellan gum solutions with added salts as seen from Fig. 2, which leads to difficulty to adjust the test temperature just at the gel point. Nishinari and Miyoshi observed the power law of $G' \sim G'' \propto \omega^n$ with $n = 0.5$ for the Na-type gellan gum solutions of 2.5 and 3.0 wt% at the gel point. But for the gellan gum solution of 3.5 wt%, the power law failed (Nishinari & Miyoshi, 1999). For the calcium cation induced gelation in aqueous alginate solutions, we observed power law behavior at a specified Ca^{2+} concentration owing to the insensitivity of temperature for that gelation (Lu, Liu, Dai, & Tong, 2005).

3.2. Salt concentration dependence of critical exponents

We can see from the data in Table 1 that T_{gel} increases but n decreases with increasing the salt concentration of KCl or CaCl$_2$. This means that there is no universal n for the gelation in these solutions even at such a low concentration of 1.0 wt%. The gelation process of the gellan gum in aqueous solutions involves the coil–double helix transition and aggregation of the double helices, the latter acts as the junction of cross-linkers. Thus, this gelation process is probably hierarchical, depending on the helix length of the gellan gum chains and the spatial distribution of the double helix already existing in the solution. This dependence will destroy

Table 1
Critical exponents for gelation in 1.0 wt% of gellan gum aqueous solutions with KCl or $CaCl_2$.

Salt	C_{salt} (mM)	T_{gel} (°C)	n^a	z	k	n^b	d_f
KCl	10	40.4	0.72 ± 0.01	3.53 ± 0.11	1.84 ± 0.28	0.66 ± 0.04	1.71 ± 0.02
	15	43.2	0.69 ± 0.02	3.38 ± 0.12	2.84 ± 0.50	0.54 ± 0.05	1.75 ± 0.02
	20	45.1	0.67 ± 0.01	2.86 ± 0.08	2.87 ± 0.17	0.50 ± 0.02	1.78 ± 0.01
	25	46.3	0.57 ± 0.01	2.90 ± 0.06	3.27 ± 0.12	0.47 ± 0.02	1.92 ± 0.01
$CaCl_2$	1.0	42.3	0.63 ± 0.01	2.78 ± 0.21	4.44 ± 0.10	0.38 ± 0.02	1.84 ± 0.01
	1.5	45.4	0.58 ± 0.01	2.52 ± 0.18	4.16 ± 0.42	0.37 ± 0.04	1.90 ± 0.01
	2.0	49.3	0.47 ± 0.01	2.49 ± 0.08	3.19 ± 0.44	0.43 ± 0.05	2.03 ± 0.01
	2.5	53.3	0.37 ± 0.04	2.11 ± 0.10	1.42 ± 0.13	0.59 ± 0.03	2.15 ± 0.04

[a] The critical exponent according to the Winter's criterion.
[b] The critical exponent calculated from $n = z/(z + k)$.

the randomness of the cross-linking occurrence, resulting in no universal n for the sol–gel transition.

Whether the critical exponent n is universal or dependent on the structure details of the system is still a problem, which is significant for describing and predicting this kind of phase transition theoretically. The gelation induced by chemical cross-linkers seems to follow the universal prediction fairly well (Adam, Lairez, Karpasas, & Gottlieb, 1997; Adolf, Martin, & Wilcoxon, 1990; Donald & Eric, 1990; Takahashi, Yokoyama, & Masuda, 1994). As for the physical gelation, the universality is sometimes broken owing to the transient nature of the physical cross-linking formed by intermolecular interactions. In the vicinity of the gel point, the zero shear viscosity η_0 and the equilibrated shear modulus G_e follow the power laws of $\eta_0 \sim \varepsilon^{-k}$ and $G_e \sim \varepsilon^{z}$, where ε is the relative distance from the critical point $\varepsilon = |p - p_c|/p_c$ (Stauffer, Coniglio, & Mireille, 1982). Here, k and z are the critical exponents for viscosity and modulus, respectively, p is the actual cross-linking probability and p_c is the value of p at the critical gel point. If the gelation occurs in a completely random way, the critical exponent n, k, and z are expected to be universal and independent of the system details (Adolf et al., 1990).

The shear viscosity η for the gellan gum solutions at temperatures slightly above the gel point was measured through shear rate sweep to evaluate the zero-shear viscosity η_0 and η_0 was plotted in Fig. 4 against the relative distance to the critical gel point ε, where $\varepsilon = |T - T_{gel}|/T_{gel}$ with T replacing p as suggested by literature (Takenaka, Kobayashi, Hashimoto, & Takahashi, 2002). The η value at the lowest shear rate ($10^{-3}\ s^{-1}$) was taken as η_0 for the gellan gum solutions containing C_{KCl} or C_{CaCl_2}, which approaches to 10^3 Pa s. The equilibrium modulus G_e was estimated from the plateau value of storage modulus G' at temperatures slightly below T_{gel} and plotted against ε in Fig. 5. The G_e values in Fig. 5 are very small, even approaching to 10^{-2} Pa at low ε, reflecting the initial network over the whole specimen. Ross-Murphy argued that the critical exponents depended on the ε range and suggested a suitable interval of $0.01 < \varepsilon < 0.1$ (Ross-Murphy, 2007). The ε in the present work was restricted in the range of $0.01 < \varepsilon < 0.1$ as seen from Figs. 4 and 5. The exponent k and z evaluated from the slope are summarized in Table 1.

The critical exponent n can be calculated from the exponents z and k as $n = z/(z + k)$ (Winter & Mours, 1997) and the calculated n values are also listed in Table 1. The n evaluated in this way is smaller than the corresponding n determined with the Winter's criterion (except for the systems containing 2.0 and 2.5 mM $CaCl_2$). This discrepancy may be due to the error in determining η_0 at such a low shear rate. All of the critical exponent n, z, and k in Table 1 vary with salt concentration and no universal value can be found for the sol–gel transition in the gellan gum solutions containing KCl or $CaCl_2$. There are several theoretical models proposed to describe the critical exponents at the gel point. The Rouse limit without hydrodynamic interaction gives $n = 0.66$, $z = 2.7$ and $k = 1.35$, the Zimm limit with hydrodynamic interaction gives $n = 1$, $z = 2.7$, and $k = 0$, and the percolation analogue to the electrical network predicts $n = 0.71$, $z = 1.94$, and $k = 0.75$ (Winter & Mours, 1997). These models are based on the assumption of random cross-linking and universal exponent. A lot of experiments have been performed on various gelation systems and typically observed value is 0.7–1.7 for k and 1.8–3.0 for z (Adam, Delsanti, Durand, Hild, & Munch, 1981; Adam et al., 1997; Fujii, Yano, Kumagai, & Miyawaki, 2000; Martin, Adolf, & Wilcoxon, 1988; Mortimer, Ryan, & Stanford, 2001). For the physical gelation, n varies from 0.11 to 0.8 (Horst & Winter, 2000; Izuka, Winter, & Hashimoto, 1997; Richtering, Gagnon, Lenz, Fuller, & Winter, 1992). The salt concentration dependence of the critical exponent n in Table 1 cannot be described with any available models because all of them lead to a constant critical exponent.

3.3. Fractal dimension for critical gels

In order to reveal further the structure of the critical gel, we related the critical exponent to the fractal dimension. Based on the

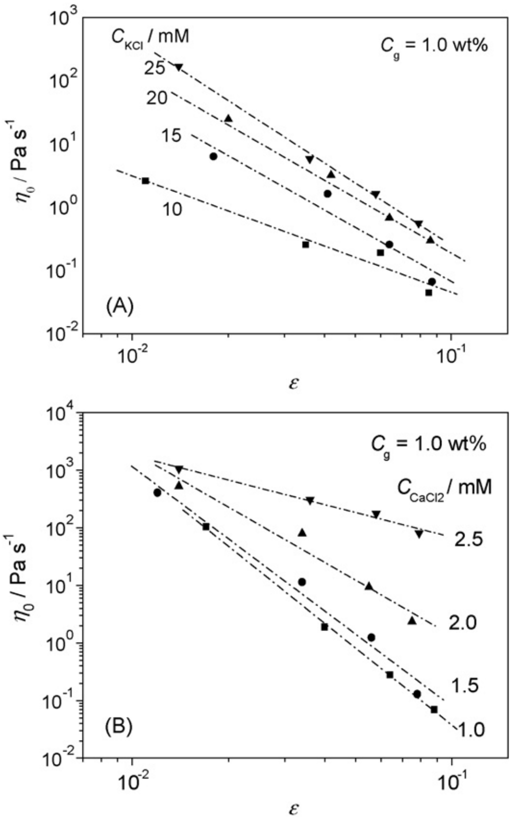

Fig. 4. Zero-shear viscosity η_0 plotted against ε, the relative distance from the gel point, for 1.0 wt% gellan gum solutions with indicated C_{KCl} (A) and C_{CaCl_2} (B).

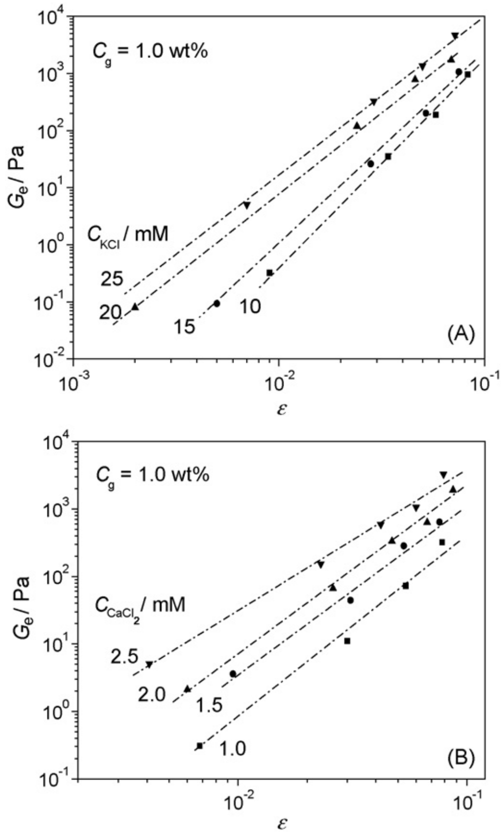

Fig. 5. Equilibrium modulus G_e plotted against ε, the relative distance from the gel point, for 1.0 wt% gellan gum samples with indicated C_{KCl} (A) and C_{CaCl_2} (B).

fractal analysis of the critical gel, several relations between the critical exponent n and the fractal dimension d_f have been proposed. Muthukumar derived a theoretical expression for the frequency dependence of the complex viscosity in terms of the arbitrary fractal dimension of the molecular cluster (Muthukuma, 1985). Then, Muthukumar and Winter proposed a relation between n and d_f with complete screen of the excluded volume (Hess, Vilgis, & Winter, 1988; Muthukuma & Winter, 1986). Muthukumar further investigated the cross-linking process for polydisperse systems using a hyperscaling relation from the percolation theory (Muthukuma, 1989). When hydrodynamic interaction is completely screened out and the excluded volume effect remains in the cluster, we have

$$n = \frac{d}{d_f + 2} \tag{2}$$

If the hydrodynamic interaction as well as the excluded volume effect is screened out completely, n is expressed by

$$n = \frac{d}{\bar{d}_f + 2} = \frac{d(d + 2 - 2d_f)}{2(d + 2 - d_f)} \tag{3}$$

where $\bar{d}_f$ is the fractal dimension of the gel network where the excluded volume effect is fully screened and d is the space dimension. If only partial screening exists, the fractal dimension takes a value in between d_f and $\bar{d}_f$. According to this theory, a looser filling in the space will lead to a lower d_f value.

We have calculated d_f from n for the present critical gels using Eqs. (2) and (3). The unreasonable value of d_f higher than 3 was obtained from Eq. (2) (data not shown) for the critical gel of gellan gum, because d_f cannot be higher than d when $d \le 3$. The double helix of gellan molecules behaves as a rigid chain at the critical gel state and the excluded volume may be ignored (Takahashi, Akutu, Kubota, & Nakamura, 1999). Consequently, we relied our further discussion on the d_f values evaluated from Eq. (3) and plotted in Fig. 6 as a function of salt concentration.

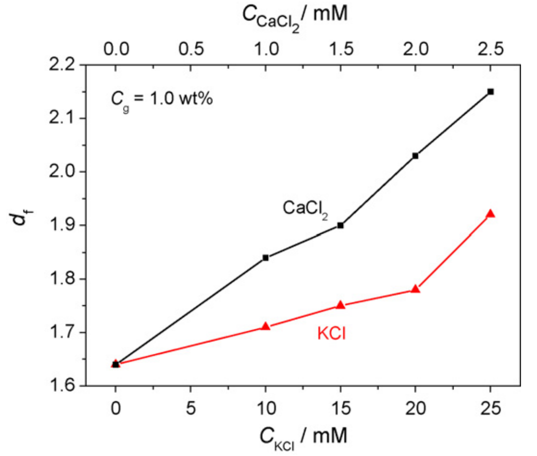

Fig. 6. Salt concentration dependence of the fractal dimension d_f calculated from n using Eq. (3) for the critical gellan gum gels of 1.0 wt%.

At constant gellan gum concentration, the d_f value for the critical gel increases with increasing salt concentration, indicating that the cluster of the critical gellan gel is filled more densely when adding salts. With decreasing temperature, the gellan chain undergoes the coil-double helix transition and the aggregation of double helices finally induces the cross-linked network (Nijenhuis, 1997). According to their SAXS results, Yoguchi et al. reported that the gellan molecules existed as a single coil at 60 °C but a double helix at 10 °C at the concentration of 1.5 wt% without added salts. When 50 mM of KCl was added into the above system, 18% of gellan molecules were in the single chain state and 72% in the double helix state at 60 °C; while at 10 °C, 17% were in the double helix state, 17% in the 2-double helix state, and the remaining 66% in the 4-double helix state (Yoguchi, Urakawa, Kitamura, Wataoka, & Kajiwara, 1999). Hence, the addition of KCl enhances the aggregation of the gellan double-helices, leading to an increase in d_f with increased space filling density of the critical gel.

The d_f value for the critical gellan gels with CaCl$_2$ is higher than that with KCl (Fig. 6). Because ionic bonds of Ca^{2+} with the carboxyl groups of different double-helices bridge different gellan chains, causing a denser filling structure than that in the critical gel with K$^+$.

4. Conclusions

The sol–gel transition in aqueous systems of gellan gum with adding K$^+$ or Ca^{2+} was investigated using rheology methods. The gel point T_{gel} and relaxation critical exponent n were determined using the Winter's criterion With an increase in the salt concentration, T_{gel} increased but n decreased. This means that the concept of universal critical exponent fails for this sol–gel transition because this gelation belongs to the cross-linking of existing macromolecules, deviating from the original hypothesis of random connection. At fixed gellan concentration, the fractal dimension d_f increased with increasing salt concentration when hydrodynamic interaction and excluded volume were ignored. Addition of Ca^{2+} showed a much stronger promotion to the gelation in the aqueous gellan gum solutions and induced a denser filling structure in the critical gel than addition of K$^+$ did.

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