

Rheological Properties of Gellan Gum in Aqueous Media^{†,††}

Masakuni TAKO, Ayano SAKAE and Sanehisa NAKAMURA

Laboratory for Chemistry of Sugar Technology, College of Agriculture,
University of the Ryukyus, Nishihara,
Okinawa 903-01, Japan

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The flow behavior and dynamic viscoelasticity of deacetylated gellan gum solutions were measured with a rheogoniometer. The gellan gum showed Newtonian behavior with a range of concentration below 0.9%, but plastic flow behavior above 1.0% at 25°C. A gelation occurred in polysaccharide concentrations over 0.8% upon cooling, and a melting temperature was observed at 15 and 20°C at 0.8 and 1.0% concentrations, respectively. In the presence of CaCl₂ (6.8 mM), the dynamic modulus of gellan gum showed a very high value at low temperature at the concentration of 0.2%, and increased with increasing temperature up to 80°C, which was estimated to be a melting temperature, then it decreased. However, the gellan showed a little increase of dynamic modulus even at the concentration of 0.4% in the presence of NaCl (16.6 mM) or KCl (13.5 mM). Gelation occurred for gellan gum in acid and alkaline range after reaching a pH 2.6 and 11.2 by addition of 100 mM HCl and Ca(OH)₂. The rheological characteristics of the gellan gum molecules might be essentially attributed to intra- and inter-molecular associations, possible structures of which have been proposed.

Gellan gum produced by *Pseudomonas elodea* has potential industrial application as a gelling agent alternate to agar and carrageenans.^{1~6)} The primary structure of the polysaccharide consists of tetrasaccharide repeating units, 3)-β-D-Glcp-(1→4)-β-D-GlcpA-(1→4)-β-D-Glcp-(1→4)-α-L-Rhap-(1.^{7,8)} The polysaccharide contains 6% of O-acetyl groups at C-6 of -3)-β-D-Glcp-(1⁹⁾; under suitable conditions, the polymer forms weak elastic gels, however, it forms stiff, brittle gels on deacetylation as in agarose. A tertiary structure of the gellan gum may consist of two identical left-handed, 3-fold double helices in the solid state,^{10,11)} similar to that of agarose.¹²⁾

We have discussed the molecular origin for rheological characteristics of κ-^{13,14)} and ι-carrageenan,¹⁵⁾ and agarose¹⁶⁾ in aqueous solutions. We concluded that the rheological characteristics of these polysaccharide molecules might be essentially attributed to intra-

and inter-molecular associations, respectively. The structure of gellan gum is similar to that of carrageenans and agarose in the view point of α-1,3-linkage.

We report herein the flow behavior and dynamic viscoelasticity of deacetylated gellan gum, and its rheological properties are analysed with respect to its association characteristics, in comparison with those of carrageenans^{13~15)} and agarose,¹⁶⁾ to propose possible structures of intra- and inter-molecular associations in aqueous solution.

Materials and Methods

Materials. Gellan gum was supplied by Sanei Kagaku Co., Ltd. and was dissolved in hot water (95°C) as a 0.2% solution which was filtered through Celite 545 (which had been treated in boiling 3M HCl for 30 min, and washed with distilled water until the pH was 6.5) with a keeping temperature at 70°C. KCl was added to a concentration of 0.05%, and then ethanol (2 vols.) was added. The precipitate was dried *in vacuo*. Purified gellan gum was redissolved in hot water (90°C) and the solution deionized,

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with keeping at 65°C, by passage through a column of Amberlite 120 (H⁺), and made neutral with 50 mM KOH. The solution was filtered through Celite 545 again. Ethanol (2 vols.) was added to the filtrate in the presence of 0.05% KCl, and the precipitate was dried *in vacuo*. To prepare solutions, the gellan gum was dissolved in hot distilled water (90°C) for 20 min, and for salt-containing solutions, it was dissolved in hot water, then a solution of KCl, NaCl, CaCl₂, or MgCl₂ which was dissolved in minimum volume of water, was added to the hot polymer solution.

Specific rotation. Specific rotation was measured at 589 nm with an automatic digital polarimeter DIP 180 (Japan Spectroscopic Co., Ltd.) for a solution of 0.5% in water at various temperatures with cooling.

Infrared spectroscopy. Infrared spectroscopy was done with an IR spectrophotometer IR 440 (Shimadzu Seisakusho Co., Ltd.) for samples dispersed in KBr discs.

Atomic absorption spectroscopy. For atomic absorption spectroscopy, the cation concentration was measured with an atomic absorption spectrophotometer (508, Hitachi Seisakusho Co., Ltd.).

Viscosity and dynamic viscoelasticity measurements. Viscosity at various shear rates (1.19~95.03 s⁻¹) and dynamic viscoelasticity at a steady frequency (3.77 rad/sec) were measured with a rheogoniometer consisting of a coaxial cylinder (1.8 cm diam.) and a rotating outer cylinder (2.2 cm diam.), 6.0 cm long (IR 103, Iwamoto Seisakusho Co., Ltd.). The temperature of the sample was controlled by circulating oil from a Thermo-cool (LCH-130F, Toyo Co., Ltd.) over a temperature ranging from 0 to 90°C, raised at a rate of 1°C/min. by steps. Shear rate (*D*), shear stress (*S*), and apparent viscosity (*η*), were calculated with the equation of Margules.¹⁷⁾ Dynamic viscosity (*η'*) and elasticity (*G'*) were calculated by a modification of Markovitz's equation.¹⁸⁾ The loss tangent was calculated from the relationship $\tan \delta = G''/G'$, where *G''* is the loss modulus ($\omega\eta''$) and ω is the frequency of the outer cylinder.

Results

The identity of the polysaccharide as a deacetylated gellan gum was established by IR spectroscopy, by the absence of a band at 1730 cm⁻¹. The potassium concentration of gellan gum was 2.6% (w/w).

The flow curves, at 25°C, of gellan gum with a range of concentration below 0.9% approximated to Newtonian behavior, and plastic behavior at 1.0%, the yield value of which was

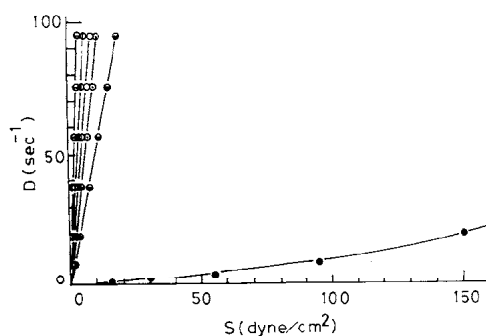


Fig. 1. Flow Curves of Gellan Gum at Various Concentrations and 25°C.

Concentrations: ⊖, 0.2%; ⊕, 0.4%; ○, 0.6%; ⊙, 0.8%; ⊚, 0.9%; ●, 1.0%.

estimated to be 10 dyn/cm² as shown in Fig. 1. The curves at concentrations below 0.9%, where there was a shift to a very low shear-stress, were in contrast to that of 1.0% solution. This may be caused by the breakdown of an intermolecular association, where micro gel-particles may exist in part. Though the flow curve of 1.0% solution increased linearly with increasing shear-rate up to 57.0 sec⁻¹ it decreased with further increases of shear-rate. This indicates that the intermolecular association of gellan gum molecules is only stable under low shear-rates <57.0 sec⁻¹ even at a high concentration (1.0%). The measurement of the flow behavior was not done because of gel-formation at a concentration >1.2%. In the presence of KCl (not reported in the figure), the curve of gellan gum (0.2%) was shifted over to a higher shear-stress than that of polymer alone, and approximated to Newtonian behavior for salt concentration <13.5 mM, pseudoplastic = 30.0 mM, and plastic behavior >40.5 mM, in which the flow curve increased linearly with increasing shear-rate until 19.0 sec⁻¹ but it decreased with further increases of shear-rate. This indicates that the intermolecular association of the gellan gum molecules is broken down even in the presence of salt as in polymer alone by shear-force. A solution of gellan gum (0.2%), however, gelled in the presence of divalent cations, CaCl₂ (6.8 mM) or MgCl₂ (5.3 mM), even

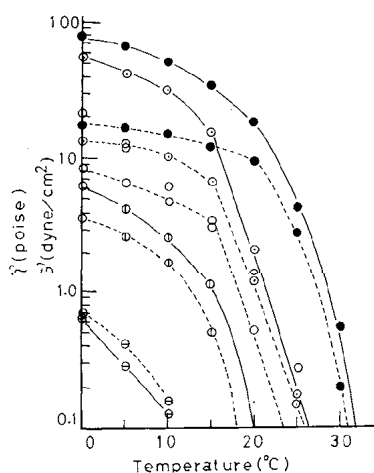


Fig. 2. Effects of Temperature on the Dynamic Viscoelasticity at 3.77 rad/sec at Various Concentrations. \ominus , 0.2%; \oplus , 0.4%; \circ , 0.6%; \odot , 0.8%; \bullet , 1.0%. -----, dynamic viscosity; —, dynamic modulus.

at room temperature (25°C).

Gelation occurred for the gellan gum at concentrations $>0.8\%$, on cooling. As shown in Fig. 2, the dynamic modulus of gellan gum was very large at 0.8 and 1.0%, and decreased gradually with increases in temperature. On the other hand, the dynamic viscosity was also high, and kept constant until 15 and 20°C, then it decreased rapidly with the increase in the temperature as in the dynamic modulus. The $\tan \delta$ of the solution at various concentrations was high about 0.85~2.6 (not reported in the figure) even at a low temperature (0°C). This indicates that the intermolecular association of gellan gum molecules is not so strong as that of ι -carrageenan¹⁵⁾ or agarose¹⁶⁾ molecules, and broken down rapidly with increasing temperature up to 25~30°C even at high concentrations (0.8 and 1.0%). The temperature, 15 and 20°C, at a concentration of 0.8 and 1.0%, was estimated to be the melting temperature of gellan gum. Such melting temperature was also observed in an aqueous solution of κ -carrageenan,¹³⁾ ι -carrageenan,¹⁵⁾ and agarose.¹⁶⁾ This suggests that the gellan gum molecule involves an intramolecular association, as in the above polysaccharides, in addition to an intermolecular

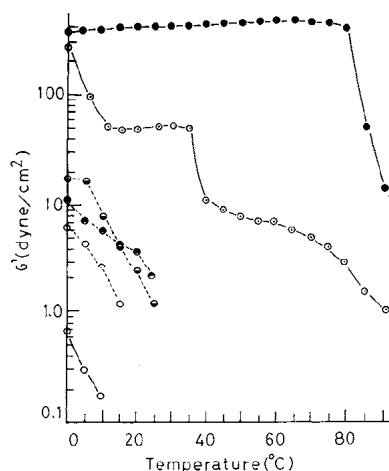


Fig. 3. Effects of Temperature on the Dynamic Modulus. at 3.77 rad/sec, for Aqueous Solution of Gellan Gum after Addition of Salts.

\circ , gellan gum alone; \ominus , with addition of KCl (13.5 mM); \bullet , NaCl (16.6 mM); \odot , CaCl₂ (6.8 mM), \oplus , MgCl₂ (6.8 mM). —, 0.2% gellan gum solution; -----, 0.4% solution.

association.

As shown in Fig. 3, a very large dynamic modulus was observed on addition of CaCl₂ (6.8 mM) to a 0.2% solution of gellan gum, and increased with the increase of temperature until 80°C, which was estimated to be the melting temperature, then it decreased rapidly. This indicates that the gellan gum molecules associate tightly with Ca²⁺ where D-glucuronic acid residues may contribute to cation-bridges on the different molecules with ionic force.¹⁵⁾ The dynamic modulus, showing increases with the increase of temperature in the presence of CaCl₂, suggests that an intramolecular association may also be involved in addition to the Ca²⁺ bridges.^{13,15,16,19~21)} The dynamic modulus also showed a large value on addition of MgCl₂ (6.8 mM) at low temperatures, but it decreased rapidly with increasing temperature to 15°C and kept constant until 35°C, then it decreased rapidly again. Such profile may be caused by the breakdown of intermolecular Mg²⁺ bridges under steady frequency (3.77 rad/sec). This indicates that the intermolecular Mg²⁺ bridges on gellan gum molecules are not as strong as

those of Ca^{2+} bridges, may be due to the difference in cation structure.²²⁾ A smaller increase of the dynamic modulus was observed on addition of NaCl (16.6 mM) or KCl (13.5 mM) even at 0.4% solution of gellan gum. This indicates that the gellan gum molecules associate more with divalent cations than that with univalent cations.

A very large dynamic modulus for gellan gum (0.4%) was observed in acid and alkaline range after reaching a pH 2.6 and 11.2 by addition of 100 mM HCl and $\text{Ca}(\text{OH})_2$ at 0°C, a behavior that parallels that of κ -¹³⁾ and *t*-carrageenan,¹⁵⁾ and agarose,¹⁶⁾ as shown in Fig. 4. The dynamic modulus of the gellan gum solution was increased by the addition of KOH (100 mM), but it decreased after reaching pH 11.7. On the other hand, the dynamic

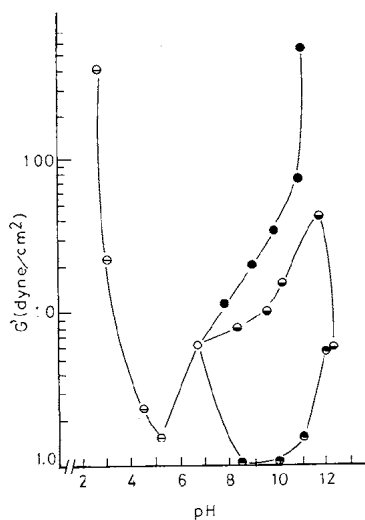


Fig. 4. Effects of pH on the Dynamic Modulus at 3.77 rad/sec, for Aqueous Solution of 0.4% at 0°C.

The pH value was adjusted with 100 mM KOH (●), NaOH (●), $\text{Ca}(\text{OH})_2$ (●), or HCl (○); ○, gellan gum alone.

modulus of the gellan gum solution was decreased by the addition of NaOH (100 mM) until pH 10.0, then it increased a little. The dynamic viscoelasticity was lower than that of gellan gum alone upon the addition of urea (4.0 M), however, it showed large values when in co-existence with CaCl_2 (6.8 mM) at low temperatures (not reported in the figure).

The specific rotation, at 0.5%, of gellan gum at various temperatures is summarized in Table I. After dissolving the sample at 90°C, measurement was done by cooling from 80 to 10°C. The specific rotation increased a little with decreasing temperature until 50°C, then it kept constant, showing a value of -52° ; after reaching 20°C, it increased gradually again with further decrease of temperature. This indicates that there are two-step mechanisms for the conformational transition at the temperatures of 50 and 20°C, as in agarose solution,¹⁶⁾ in aqueous solution.

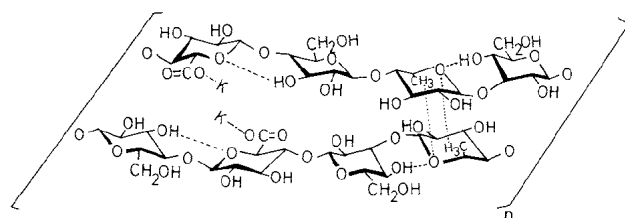
Discussion

It has been suggested that the deacetylated gellan gum molecules are involved in both intra- and inter-molecular association. A very large dynamic modulus was observed on the addition of 6.8 mM CaCl_2 to a 0.2% solution of gellan gum at low temperature and it increased gradually with increase of the temperature until 80°C. The result, showing large dynamic modulus even at high temperature (80°C), suggests that the methyl group of the L-rhamnosyl residues may contribute to an intermolecular association as in xanthan, on which the pyruvate methyl groups^{19~21,23)} and in methylcellulose, on which the methylether, may contribute.²⁴⁾ This bonding, to which the

Table I. SPECIFIC ROTATION OF GELLAN GUM AT 589 nm^a

	Temperature (°C)								
	80	70	60	50	40	30	20	10	5
Gellan gum	-58.5	-56.6	-54.2	-52.0	-52.0	-52.0	-52.0	-43.3	-38.7

^a For a solution in water, *c* 0.5%.



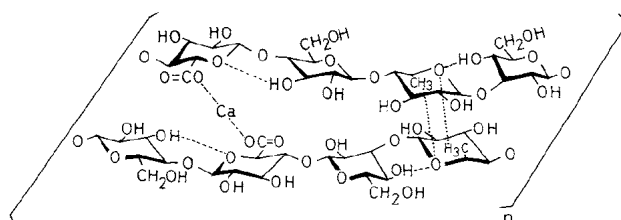
Scheme 1. Possible Mode of Intra- and Inter-molecular Associations of Gellan Gum in Aqueous Solution.
Associations: ----, hydrogen force; ·····, van der Waals' force of attraction; ·-·-, ionic force.

methyl groups contribute, may co-operate with the intermolecular Ca^{2+} bridges in keeping an intramolecular association even at high temperature (80°C) to preserve gelation. The tendency of the curve, increasing the dynamic modulus for an increase in temperature, is in agreement with that of κ -¹³⁾ and ι -¹⁵⁾ carrageenan, and agarose.¹⁶⁾ Furthermore, the change of the dynamic viscoelasticity of gellan gum solution is in agreement with the specific rotation, suggests an intramolecular hydrogen bonding as agarose.¹⁶⁾

Thus, we propose a mode of intramolecular hydrogen bonding between the OH-4 of the D-glucosyl residue and the adjacent hemiacetal oxygen atom of the L-rhamnosyl residue, as illustrated in Scheme 1. This bonding is likely owing to the flexibility of the α -L-(1 \rightarrow 3)-linkage between L-rhamnosyl and D-glucosyl residues, and it may take place below the transition temperature (50°C), as in agarose molecule.¹⁶⁾ The L-rhamnosyl residues may adopt a ${}^4\text{C}_1$ pyranose-ring conformation²⁵⁾ at high temperature (50°C) caused by large kinetic energy and Brownian motion of the polymer and water molecules, on which the methyl group has an axial orientation.

Thus, an intermolecular association may be proposed between the methyl group and the hemiacetal oxygen atom of the L-rhamnosyl residues on different molecules, as illustrated in Scheme 1. As the methyl groups of the L-rhamnosyl residues are oriented in the axial conformation, their peripheries are surrounded by hydrophobic carbon and hydrogen atoms, and may attract each other on different molecules by a cage effect.^{16,26)} Consequently, the intermolecular association may take place

with a hydrophobic van der Waals' force of attraction.²⁷⁾ The intermolecular van der Waals' force may be stable below the melting temperature (20°C). The result of the specific rotation determination suggests that the molecules of gellan gum may adopt a random coil at a temperature above 50°C , but it may keep a rod-like conformation²⁾ at a temperature ranging from 50 to 20°C owing to the formation of intramolecular hydrogen bonding. The intermolecular van der Waals' force may take place due to the decrease of the kinetic energy and brownian motion of the polymer and water molecules at a temperature $< 20^\circ\text{C}$, at which there could be an intramolecular hydrogen bonding between OH-3 of the D-glucosyl and adjacent hemiacetal oxygen atom of the D-glucuronosyl residues.²⁸⁾ The formation of the intermolecular association below 20°C agrees with the breakdown at 25°C under shearing-force even at high concentration (0.9%; Fig. 1). The intra- and inter-molecular associations result in insolubility in cold water, because, as the polymer molecules bind, their periphery is surrounded by hydrophobic carbon and hydrogen atoms. The gellan gum molecules are hydrated at a high temperature $> 90^\circ\text{C}$, where dissociation occurs owing to an increase of kinetic energy and the Brownian motion of polymer and water molecules. This model corresponds to a double-stranded helix. A tertiary structure of gellan gum molecules may consist of two identical, left-handed, 3-fold helices in aqueous solution as in the solid state.¹¹⁾ Consequently, we suggest that the cavity in the double-stranded helix is occupied by water molecules, the arrangement of which are very nearly tetrahedral, in a manner similar to that



Scheme 2. Possible Mode of an Intermolecular Ca^{2+} Bridge of Gellan gum in Aqueous solution.
Associations: ----, hydrogen force; ·····, van der Waals' force of attraction; —·—, ionic force.

of the L-rhamnosyl residues in aqueous solution.

In the presence of CaCl_2 , an intermolecular Ca^{2+} bridge, as in *ι*-carrageenan,¹⁵⁾ may take place between carboxyl oxygen atoms of the D-glucuronosyl residues on different molecules with ionic force, as illustrated in Scheme 2. The intermolecular Ca^{2+} bridges, including the intra- and inter-molecular hydrogen bonding and van der Waals' force, result in gelation up to a high temperature (80°C), as the polymer molecules bind tightly. This model also corresponds to a double-stranded helix.

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