Effects of Potassium Chloride and Sodium Chloride on the Thermal Properties of Gellan Gum Gels

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The exothermic and endothermic peaks in cooling and heating curves of differential scanning calorimetry (DSC) for gellan gum gels without and with potassium chloride and sodium chloride were analyzed. The gelling and melting temperatures shifted to higher temperatures with increasing gellan and salt concentration in the concentration range of gellan from 0.3 to 2.0% (w/w). The exothermic and endothermic enthalpy increased with increasing gellan and salt concentrations. Cooling DSC curves showed one exothermic peak for samples with salts and at low gellan concentration. Heating DSC curves showed many peaks for all samples except 0.3% (w/w) gellan gum gels. The sol–gel transition of samples was examined numerically by using a zipper model approach. The introduction of cations increases the number of junction zones or zippers and decreases the rotational freedom of parallel links. This makes the structure of junction zones more heat resistant, and increases the elastic modulus of the gel.

Gellan gum is a microbial polysaccharide derived from Pseudomonas elodea. The polymer contains L-rhamnose, D-glucose, and D-glucuronic acid in the molar ratios 1:2:1 and it has a carboxyl side group.1,2) Gellan gum is an extremely good gelling agent. Gelation is sensitive to the type, valency, and concentration of cations present in salts added to the dispersion before gelation. Physical-chemical studies suggest that the junction zones of the gels arise due to the association and possibly crystallisation of sections of the polymer chain. Acetyl groups inhibit and cations promote such intermolecular association.3–6)

Differential scanning calorimetry (DSC) has been used to study the gel–sol transition for thermo-reversible gels. Endothermic peaks arising from the gel-to-sol transition as well as exothermic peaks arising from the sol-to-gel transition have been observed in heating and cooling DSC curves respectively by many investigators.7–14) Since these peaks are equivalent to the maximum of heat capacity, a zipper model approach was proposed to explain the gel–sol transition of thermo-reversible gels.15) In this treatment, the gel-to-sol transition is governed by a number of zippers, \( N \), the number of parallel links constituting a zipper, the rotational freedom \( G \) of parallel links, and the energy \( \varepsilon \) required to open a parallel link. Recently, it was shown that the addition of sucrose to agarose or gelatin gels increased \( N \) and decreased \( N \) and \( G \) from DSC and rheological measurements based on the zipper model approach and a rubber elasticity theory of gels.16) Application of the zipper model approach is expected to shed some light on molecular mechanism of gel–sol transitions of gellan gels.

In this work, heating and cooling DSC measurements were done to clarify the mechanism of the gel–sol transition of gellan solutions without and with NaCl or KCl.

Materials and Methods

Gellan was supplied by San-ei Co., Ltd. As will be shown later, endothermic and exothermic peaks of gellan are influenced by the presence of metal ions. Therefore, it is necessary to know the metal content in the sample. The metals contained in the sample are shown in Table I. The powdered sample was mixed with water to swell for 30 min at room temperature to form a gellan solution of the concentration range from 0.3 to 2.0% (w/w). The container was fitted with a reflux condenser. The sample was dissolved at 90°C for 20 min. For salt-containing samples, the salt was dissolved in hot water, then a solution of KCl or NaCl dissolved in a minimum volume of water was added to the hot gellan solution. The concentration of gellan solutions containing KCl or NaCl was chosen as 0.3, 0.5, and 0.7% (w/w). The sample was cooled at room temperature for 1 h and then kept at 10°C overnight.

Differential scanning calorimetry (DSC) measurements were done with a Seicarom micro DSC calorimeter. Approximately 950 mg of the sample was sealed into the DSC pan hermetically, and the reference pan was filled with exactly the same amount (± 0.5 mg) of distilled water. The two pans were then placed inside the calorimeter and kept at 99.9°C for 1 hr. Then the temperature was lowered to 1.0°C at 1.0°C/min and then raised again at the same rate up to 99.9°C.

Results and Discussion

Cooling DSC

Figure 1 shows cooling DSC curves for gellan gels of various gellan gum polymer concentrations (Cg). Only one exothermic peak was observed for gellan gels of low concentrations, but the peak splits into multiple peaks for gellan gels of higher concentrations than 1.3% (w/w). The splitting of a single peak to multiple peaks is especially clearly seen at a lower scanning rate (Fig. 2). Multiple endothermic peaks are often observed in the melting of polymorphic crystals.17) In such a case, the heating DSC

<table>
<thead>
<tr>
<th>Metal</th>
<th>Weight in dry matter</th>
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<tbody>
<tr>
<td>Sodium</td>
<td>0.20%</td>
</tr>
<tr>
<td>Potassium</td>
<td>4.00%</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.50%</td>
</tr>
<tr>
<td>Magnesium</td>
<td>0.20%</td>
</tr>
<tr>
<td>Heavy metals</td>
<td>&lt;10 μg/g</td>
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</tbody>
</table>
The curve at a higher heating rate shows only one peak instead of multiple endothermic peaks as reported for a seaweed polysaccharide gel.\(^{18}\) The mid-point transition temperature in a cooling DSC curve will be called the setting temperature, \(T_s\) hereafter. Figure 3 shows the \(T_s\) and the incipient temperature at which gelation begins, \(T_i\). Both \(T_s\) and \(T_i\) shifted to higher temperatures with increasing gellan polymer concentration (\(C_g\)). The difference between \(T_s\) and \(T_i\) became larger with increasing \(C_g\); the gelation curve of concentrated gellan solution became broader than that of dilute solution. The exothermic enthalpy of setting, \(\Delta H_s\), calculated from the area enclosed by the DSC peak and the base line, are shown as a function of gellan polymer concentration \(C_g\) in Fig. 4. Enthalpy (\(\Delta H_s\)) increased with increasing \(C_g\) due to the stabilization of the gellan network structure in gellan gels. The gelation of gellan solution is promoted by the aggregation of double or single helices of the molecules.\(^{19}\) Gels consist of junction zones and flexible chains that connect the junction zones. Both ends of a flexible chain are bound to two of the junction zones by weak secondary interactions such as hydrogen bonds. The number of junction zones and flexible chains may increase with increasing \(C_g\). Figure 5 shows

Fig. 1. Cooling DSC Curves of Gellan Gum Solutions of Various Concentrations. Figures beside each curve represent the concentration of gellan gum. Cooling rate, 1.0 °C/min.

Fig. 2. Cooling DSC Curves of 1.5% (w/w) Gellan Gum Solutions. Cooling rate, 0.2 °C/min.

Fig. 3. Gelling and Incipient Temperatures of Gellan Gum Gels of Various Concentrations as a Function of Gellan Polymer Concentration. Cooling rate, 1.0 °C/min. (○) gelling temperature; (△) incipient temperature.

Fig. 4. Exothermic Enthalpy of Gellan Gum Solutions of Various Concentrations without Salt as a Function of Polymer Concentration.

Fig. 5. Cooling DSC Curves of 0.5% (w/w) Gellan Gum Solution Containing KCl or NaCl of Various Concentrations. Figures beside each curve represent the concentration of KCl or NaCl. Cooling rate, 1.0 °C/min.
cooling DSC curves of 0.5% (w/w) gellan gum gels containing KCl or NaCl of various concentrations. Figure 6 shows the $T_g$ of gellan gum gels containing KCl or NaCl as a function of salt concentration $C_s$. At the same concentration of KCl or NaCl, $T_g$ shifted to higher temperatures with increasing $C_s$. The setting temperature ($T_s$) shifted to higher temperatures with increasing salt concentration ($C_s$) for gels of the same gellan polymer concentration ($C_g$). These results suggest that cations promote the tight binding of helical polymers, and then strengthen the junction zones, because cations shield the electrostatic repulsion of the carboxyl groups in gellan molecules. The gellan solution containing KCl showed a higher $T_g$ than the gellan solution containing NaCl of the same concentration. We found a similar tendency for the sol-to-gel transition temperature of gellan solutions containing NaCl or KCl from the plot of dynamic shear modulus against the temperature.\(^{20}\) Potassium ions are more effective in enhancing the gelling ability. Ueda\(^{21}\) reported that a potassium ion is a structure-breaking ion, while a sodium ion is a structure-making ion. Therefore, the water molecules surrounding potassium ions are more mobile than those surrounding sodium ions, and potassium ions can obtain access to carboxyl groups. There are water molecules between sodium ions and carboxyl groups. Therefore, potassium ions may promote the helix formation and association of helices, i.e., strengthen the structure of junction zones more effectively than do sodium ions. The difference between $T_s$ and $T_g$ became almost zero at all salt concentrations. In the presence of monovalent cations, the gelation will speed up more than in the case of gellan without salt. The exothermic enthalpy of the sample containing salt increased with increasing $C_g$ (Fig. 7).

**Heating DSC**

Figure 8 shows the heating DSC curves of gellan gum gels of various concentrations. The lowest endothermic peak temperature around 30°C will be called melting temperature, $T_{m}$ hereafter. The $T_{m}$ for a 0.3% (w/w) gellan gum gel is 25.1°C and for a 2.0% (w/w) gellan gum gel is 32.4°C. The $T_{m}$ shifted to higher temperatures and the endothermic enthalpy of melting increased with increasing $C_g$. Only one endothermic peak was observed for a 0.3% (w/w) gel, but many peaks were observed for gels of higher concentrations. We reported the temperature dependence of Young's modulus $E'$ of gellan gels with and without salt.\(^{20}\) Young's modulus $E'$ decreased monotonically with increasing temperature. Figure 9 shows heating DSC curves of gellan gels containing KCl (Fig. 9-1) or NaCl (Fig. 9-2). The $T_{m}$ for 0.5% (w/w) gellan gum without salt was 26.5°C, while the $T_{m}$ for 0.5% (w/w) gellan gum containing 25mM KCl was 45.1°C and that containing 25mM NaCl was 36.0°C. The number of endothermic peaks increased and shape of these endotherms became complicated with increasing $C_g$. Although the shape of endotherms is very complicated, it was reproducible. We reported that the storage Young's modulus of...
0.4% (w/w) gellan gels containing KCl or NaCl was less temperature-dependent than that of more concentrated gellan gels (0.99% (w/w)) without salt. Williams et al. reported that the exothermic peak of 1.2% xanthan shifted from 50°C to 85°C in the presence of 0.04 mol/dm³ NaCl. The secondary bond of network structure in the gellan gels containing salt was rendered more stable to heat. The thermal rupturing of cross-links was inhibited with comparison to gels without salt. Potassium is far more effective than sodium in shifting the Tm to higher temperatures; this corresponds well with the increase in the gelation temperature.

**Heat of reaction of cross-links**

Eldridge and Ferry proposed the following equation for the melting temperature of gelatin gels.

\[
\log C = \frac{-\Delta H}{2.303 \cdot R \cdot T_m} + \text{const.} \tag{1}
\]

where C is the concentration of gelatin, Tm is the melting temperature, \(\Delta H\) is the heat of reaction in kJ/mol of cross-links and R is the gas constant. Figure 10 shows the Eldridge–Ferry plot of 0.3—1.0% (w/w) gellan gum. Since the relationship between the logarithm of gelatin concentration and the reciprocal of the setting temperature was found to be linear, equation 1 might be used to calculate the heat of reaction. The heat of reaction (\(\Delta H\)) for crystallite formation of gellan gels was about 98 kJ/mol. We reported that the heat value of gellan gels obtained from melting temperature was 38 kJ/mol. The difference of results might be caused by the measuring method and gellan concentration. Figure 10 showed the Eldridge–Ferry plot for gellan gels containing KCl or NaCl. The plot gave a straight line in each case, and the slope of the line is proportional to the heat released on forming 1 mol of junction zones. The slope increased with increasing salt concentration. The gellan gum gel containing KCl had a much larger \(\Delta H\) than that containing NaCl of the same concentration as in the case of the exothermic enthalpy. Potassium ions were more effective in promoting the molecular association in gellan solution than sodium ions. This difference resulted from the difference of the electrostatic shield of repulsion between carboxyl groups in gellan molecules. Tan et al. examined the melting temperature of atactic polystyrene gels with various molecular weights and found that \(\Delta H\) increased with increasing molecular weight of atactic polystyrene. Watase and Nishinari reported that the \(\Delta H\) of iota-carrageenan gels was smaller than that of kappa-carrageenan gels. Iota-carrageenan gels are known to have more sulfate than kappa-carrageenan. In this work, the gelling ability was enhanced with increasing salt concentration and then \(\Delta H\) increased.

**Application of a zipper model**

It has been widely accepted that most thermo-reversible gels consist of somewhat crystalline regions, called junction zones, and somewhat amorphous regions. One of us and his coworkers proposed a method to analyze DSC endotherms or exotherms by using a zipper model that has been used for explaining the helix-coil transition and the melting of DNA. The gel-to-sol transition for thermo-
Thermal Properties of Gellan Gum with Monovalent Cations

Compared to sodium ions, potassium ions expedited gelation. The increase in the number of zippers \( N \) by the addition of salt is consistent with rheological results reported previously. The elastic modulus of gellan gels increased with increasing concentrations of added salt. This was explained by the electrostatic shield of carboxyl groups by cations, and the tight binding of gellan helices to form junction zones. Thus, the introduction of cations increases the number of junction zones or zippers \( N \) and decreases the rotational freedom \( G \) of parallel links. This makes the structure of junction zones more heat resistant, and increases the elastic modulus of the gel.

Acknowledgment. We wish to thank Mr. Kaneko of Rigaku Corporation for his cooperation in DSC experiments.

References


Fig. 11. The Solid Curve Represents the Observed Cooling DSC Curves of 0.5% (w/w) Gellan Gums Containing KCl of Various Concentrations. The dotted curves were obtained by using Eqs. (2) and (3) with \( N = 100, k = 2000k \):

\[
G^o = \frac{0.5x}{(kT)}
\]

where \( k \) is Boltzmann’s constant. Figure 11 shows cooling DSC curves of 0.5% (w/w) gellan gum gels containing KCl of various concentrations. Solid curves represent the observed cooling DSC curves and dotted curves are obtained by curve fitting, using Eqs. (2) and (3). Calculated curves fitted observed curves fairly well. When \( c = 2000/k \) and \( N = 100 \), the number of zippers \( N \) increased and the rotational freedom of the constituting links \( G \) decreased with increasing salt concentration. The sample containing NaCl showed the same tendency. With gellan solutions without salt, the number of zippers increased and the rotational freedom of the constituting links decreased with increasing gellan concentration. These results suggest that the molecular association in gellan solutions was promoted by cations due to the electrostatic shield of repulsion between carboxyl groups in gellan molecules.