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

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The effects of pH on DSC curves and the breaking stress of gellan gum gels with and without NaCl or KCl were investigated. The breaking stress of all gels increased with decreasing pH down to pH 4. The difference between the breaking stress of nonheated gels and that of gels reheated for 2 h at 90 °C became larger with increasing pH. The exothermic enthalpy (ΔH) of all samples became minimum at pH 4. The DSC cooling and heating curves for all samples did not show any peak at pH 2. Repeated observations of DSC curves were carried out from 99.9 to 1.0 °C. Exothermic peaks of all gels at pH 4 and 6 shifted to lower temperatures, and the peaks of DSC heating curves of gels without salt at pH 8 shifted to higher temperatures with increasing numbers of heating and cooling cycles.

Keywords: *Gellan gum; gel; breaking stress; enthalpy; pH; salt*

INTRODUCTION

Gellan gum is a linear anionic heteropolysaccharide and contains L-rhamnose, D-glucose, and D-glucuronic acid (Jansson et al., 1983; O'Neill et al., 1983). The polymer has one carboxyl side group and one *O*-acetyl substituent per chemical repeat unit. Deacetylation of gellan gum has been done by a heat treatment at alkaline pH (Kang et al., 1982). The polymer is an extremely good gelling agent (Nakamura et al., 1993; Shimazaki et al., 1993). Progressive deacetylation results in increasing brittleness of the gels. The carboxyl side groups in glucuronosyl residues induce electrostatic repulsion in gellan solution and inhibit gelation, but the introduction of monovalent and divalent cations promotes gelation (Carroll et al., 1982, 1983; Pettitt et al., 1986; Brownsey et al., 1984; Attwool et al., 1986; Chandrasekaran et al., 1988; Milas et al., 1990; Moritaka et al., 1991; Tsutsumi et al., 1993; Watase and Nishinari, 1993; Miyoshi et al., 1994a,b). It is well-known that the traditional gelling agents such as agarose and carrageenan decrease the gelling ability at low pH. Research and development of gelling agents that form a gel at low pH is important to the production of, e.g., dessert jellies containing fruit juices. Gellan gum is used as a gelling agent with salt in the food industry. From this point of view, the effects of pH, NaCl, or KCl on differential scanning calorimetry (DSC) curves and stress–strain curves of gellan gum were investigated by thermal and rheological measurements.

MATERIALS AND METHODS

Gellan gum was supplied by San-Ei Gen F.F.I Corp. (Osaka, Japan). The metal content in the sample is shown in Table 1. The acetyl side group was not detected with an enzymatic method using Mannheim-Boehringer acetic acid UV method (Bergmeyer and Mollerlng, 1974) and by using NMR (A-600, JEOL, Japan) at 70 °C (Y. Goda, private communication, 1995). The sample was swollen for 30 min at room temperature and dissolved at 90 °C for 20 min. The concentration of gellan gum gels examined in the present work ranged from 0.5 to 1.0% (w/w). A solution of KCl or NaCl was added to

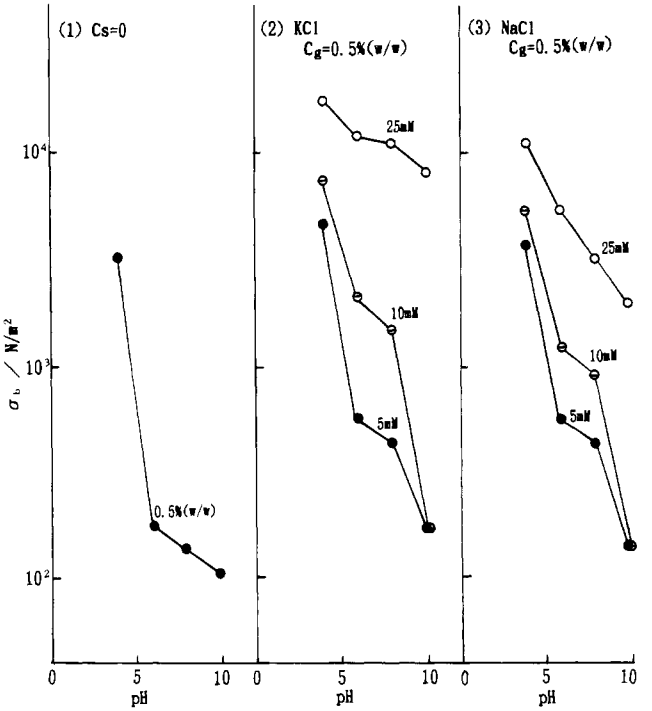

Figure 1. Breaking stress σ_b of gellan gels as a function of pH at 20 °C: (1) without salt (figures beside each curve represent the concentration of gellan gum); (2, 3) concentration of gellan gum 0.5% (w/w) with alkali metal salt (figures beside each curve represent the concentration of the salt).

Table 1. Metal Content in the Gellan Sample

metal	wt in dry matter	metal	wt in dry matter
sodium	0.20%	heavy metals	<10 μg/g
potassium	4.00%		
calcium	0.50%		
magnesium	0.20%		

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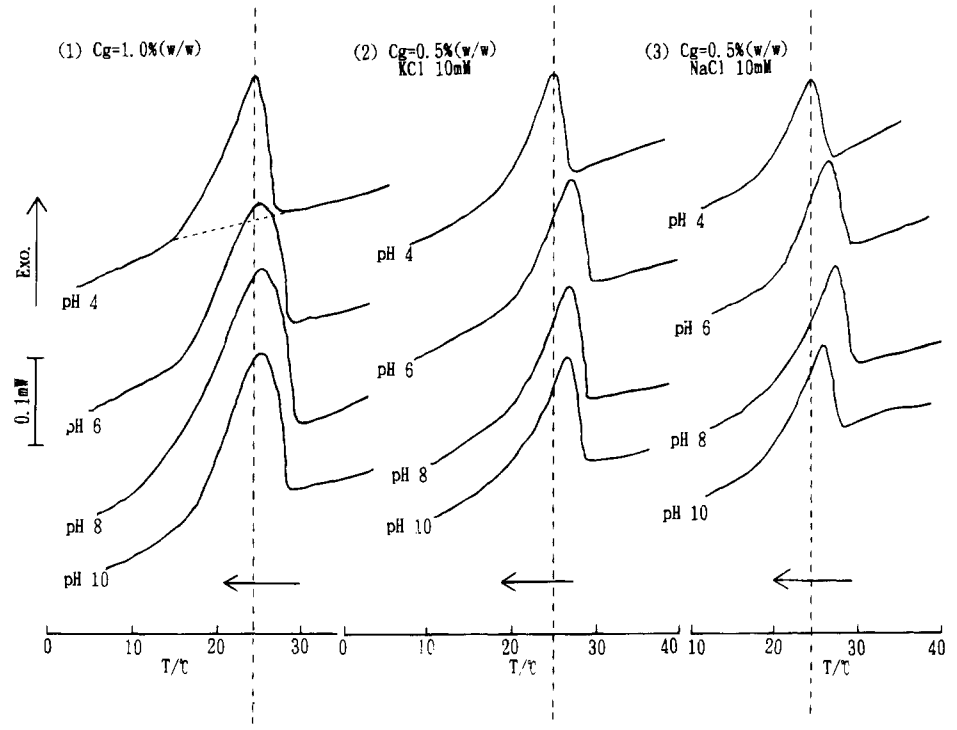

Figure 2. Cooling DSC curves of 1.0% (w/w) gellan gum solutions and 0.5% (w/w) gellan gum solutions containing 10 mM KCl or 10 mM NaCl at various pH values. The cooling rate was 1.0 °C/min. Figures beside each curve represent the pH of the gellan gum solution.

the hot 0.5% (w/w) gellan solution. The pH was adjusted using HCl or NaOH.

DSC Measurements. Differential scanning calorimetry (DSC) measurements were done with a Setaram micro DSC calorimeter (Setaram, Caluire, France). Approximately 950 mg of the sample was hermetically sealed into the DSC pan, and the reference pan was filled with exactly the same amount of distilled water (±0.5 mg). The two pans were then placed inside the calorimeter and kept at 99.9 °C for 1 h. The temperature was lowered from 99.9 to 1.0 °C at 1.0 °C/min and kept for 10 min and then raised again at the same rate up to 99.9 °C (Moritaka et al., 1992).

Breaking Stress. The breaking stress was measured by uniaxial compression using a Rheolometer (Iio Denki., Ltd, Tokyo, Japan). The sample solution (18 mL) prepared at 90 °C was poured into a sample case, 55 mm in diameter, and was kept at 10 °C for 120 min. The diameter of the plunger used was 40 mm. The breaking stress of the gel was taken as the maximum of the stress in the first "bite". Details of the experimental procedures were given previously (Moritaka et al., 1991).

RESULTS AND DISCUSSION

Breaking Stress. Figure 1 shows the breaking stress of 0.5% (w/w) gellan gels with and without NaCl or KCl at various pH values. The breaking stress of all samples increased with decreasing pH. The breaking stress of gellan gum gels containing KCl or NaCl increased with increasing salt concentration. Potassium and sodium ions promote aggregation and gelation of gellan gum (Tako et al., 1989; Moritaka et al., 1991, 1992; Watase and Nishinari, 1993; Miyoshi et al., 1994a,b). At pH 2, all samples became turbid and showed a phase separation. Carboxyl side groups present in glucuronosyl residues of gellan gum molecules repel each other and then inhibit the aggregation of single or double helices of gellan gum molecules. Hydrogen ions compensate negative charges of carboxyl groups and shield the electrostatic repulsion of gellan gum molecules. Therefore, the increase in hydrogen ions may enhance the number of junction zones in gellan gum gels. On the contrary, hydroxide ions may accelerate electrostatic repulsion of gellan gum molecules.

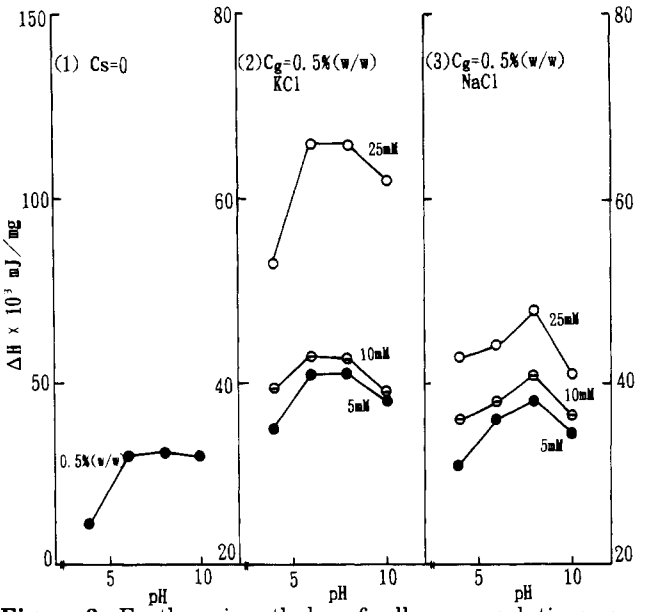

Figure 3. Exothermic enthalpy of gellan gum solutions as a function of pH: (1) without salt (figures beside each curve represent the concentration of gellan gum); (2, 3) concentration of gellan gum 0.5% (w/w) with alkali metal salt (figures beside each curve represent the concentration of the salt). The cooling rate was 1.0 °C/min.

Cooling DSC. Figure 2 shows DSC cooling curves for gellan gum gels, with [Figure 2(2),(3)] and without [Figure 2(1)] KCl or NaCl, at various pH values. Only one exothermic peak was observed for gellan gum gels at various pH values. The DSC cooling curves for gellan gels with and without salt did not show any peak at pH 2. The exothermic peaks of gellan gum gels with

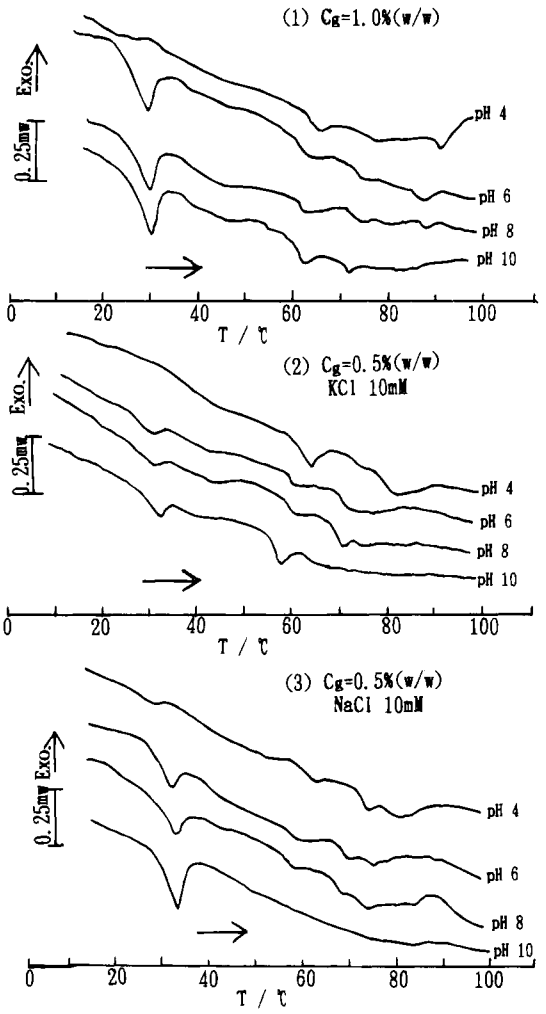

Figure 4. Heating DSC curves of 1.0% (w/w) gellan gum solutions and 0.5% (w/w) gellan gum solutions containing 10 mM KCl or 10 mM NaCl at various pH values. The heating rate was 1.0 °C/min. Figures beside each curve represent the pH of the gellan gum solution.

and without salt at pH 4 were observed at lower temperatures than the exothermic peaks at the other pH values. The exothermic peaks of gellan gels with and without salt over the pH range 6–10 were observed at approximately the same temperature. Gellan gum molecules might be hydrolyzed at pH 4 during heating DSC measurement. The enthalpy (ΔH), calculated from the area enclosed by the DSC peak and the base line, is shown as a function of pH in Figure 3. The exothermic enthalpy ΔH increased with increasing concentration of gellan gum and alkali metal salt. The value of ΔH decreased with decreasing pH. The reason for the shift of the endothermic peak to a lower temperature and for the decreased endothermic enthalpy with decreasing pH is attributed to the acid hydrolysis at lower pH values during DSC measurements.

After adjustment of the pH, the sample for differential scanning calorimetry was heated during measurement for a long time. On the other hand, the sample for breaking stress measurement was not heated after adjustment of the pH. So, in the differential scanning calorimetry, junction zones in gellan gum gels may be disintegrated by acid, at low pH values. On the contrary, junction zones in gellan gum gels for breaking stress measurement may not be destroyed by acid, and the gelation of gellan gum molecules may be accelerated by hydrogen ions. The value of ΔH for gellan gum gels

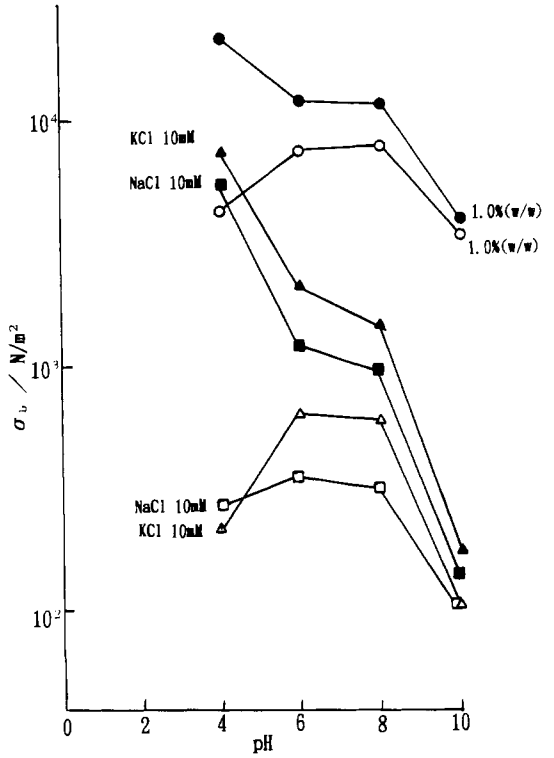

Figure 5. Breaking stree σ_b of gellan gels heated at 90 °C for 120 min and of nonheated gellan gels as a function of pH. Heated sample: (○) 1.0% (w/w) gellan gum; (△) 0.5% (w/w) gellan gum containing 10 mM KCl; (□) 0.5% (w/w) gellan gum containing 10 mM NaCl. Nonheated sample: (●) 1.0% (w/w) gellan gum; (▲) 0.5% (w/w) gellan gum containing 10 mM KCl; (■) 0.5% (w/w) gellan gum containing 10 mM NaCl.

with and without NaCl became maximum at pH 8. Gelation of gellan gum may be expedited at pH 8, probably by deacetylation during cooling and heating DSC measurement. Deacetylation of gellan gum can be achieved by heating at alkaline pH (Kang et al., 1982). The condition of DSC measurement may coincide with that of the deacetylation process. Acetyl substituents affect gelation by hindering crystallization of segments of neighboring polymer chain (Robinson et al., 1991). Deacetylation promotes the aggregation process, giving rise to stronger gels. A small amount of acetyl side groups, which cannot be detected through the enzymatic method and NMR measurement, may be removed during heating and cooling DSC measurement.

The enthalpies of gellan gum gels containing KCl at pH 6 and 8 were not so different. The enthalpy of a gellan gum gel containing KCl was larger than that of a gellan gum gel 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: potassium ions are more effective than sodium ions in enhancing gelling ability (Moritaka et al., 1991). Uedaira and Ohsaka (1993) 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., they may strengthen the structure of junction zones more effectively than do sodium ions.

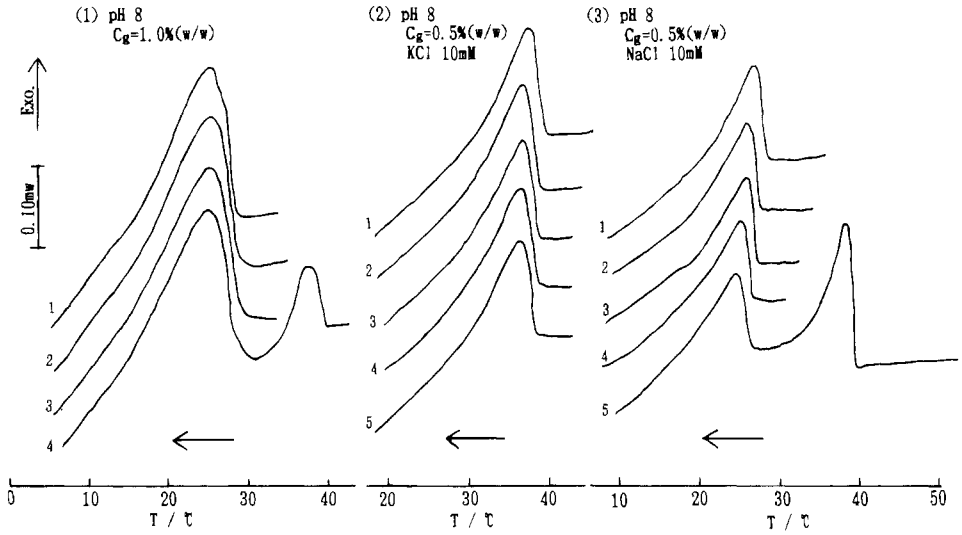

Figure 6. Cooling DSC curves of 1.0% (w/w) gellan gum solutions and 0.5% (w/w) gellan gum solutions containing 10 mM KCl or 10 mM NaCl at pH 8. The cooling rate was 1.0 °C/min. Figures beside each curve represent the number of repeated cycles of heating and cooling.

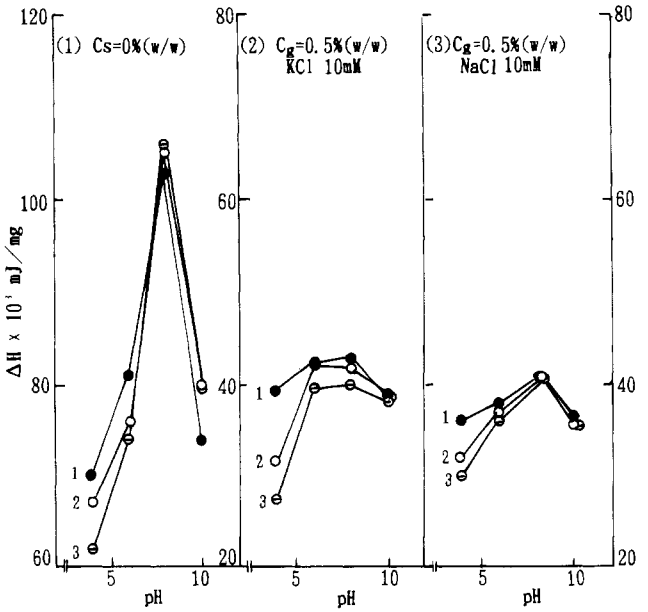

Figure 7. Exothermic enthalpy of gellan gum solutions as a function of pH: (1) without salt; (2, 3) concentration of gellan gum 0.5% (w/w) with alkali metal salt. The cooling rate was 1.0 °C/min. Figures beside each curve represent the number of repeated cycles of heating and cooling.

Heating DSC. Figure 4(1) shows the DSC heating curves of 1.0% (w/w) gellan gum gels at various pH values. The lowest temperature endothermic peak observed at around 30 °C at pH 4 was very small, but the peak became larger and shifted to higher temperatures with increasing pH. Many peaks were observed for all samples at various pH values. Robinson et al. (1991), however, reported only one endothermic peak in a heating DSC curve for Na^+ gellan [1.0% (w/w)]. The endothermic peak temperature for a 1.0% (w/w) gellan gum gel containing 25 mM NaCl was reported to be about 30 °C (Robinson et al., 1991). In the present measurement, in addition to the lowest temperature endothermic peak at around 30 °C, many other peaks were observed for 1.0% (w/w) gellan gum gel without salt at higher temperatures. The gellan sample used in the present study is a potassium type; however, the content of calcium, magnesium, and sodium ions is not negligible, as shown in Table 1. Various endothermic peaks at different temperatures suggest the presence of various junction zones with different thermal stabilities which are formed in the presence of various metal ions. Figure 4(2),(3) shows the DSC heating curves of 0.5% (w/w) gellan gum gels containing 10 mM KCl or NaCl. The lowest temperature endothermic peak at around 30 °C shifted to higher temperatures and became sharper with increasing pH. The endothermic peak of all samples around 90 °C became broader at pH 10 than at other pH values. The endothermic peak at around 30 °C is attributed to the disintegration of main junction zones formed by the aggregation of single or double helices of gellan gum molecules. These junction zones are unstable at lower pH values. Removal of a very small amount of acetyl group may cause the endothermic peak at around 90 °C, and this removal is expedited with increasing pH.

Breaking Stress for Reheated Samples. The rheological properties of gellan gum gels with and without salt are quite consistent with the thermal properties described above, as for the effects of gellan gum and salt concentration; however, as for the effect of pH, the rheological results for gellan gum gels with and without salt seem to contradict the thermal results. The difference between rheological and thermal properties may be caused by heating of samples during DSC measurement. Thereupon, gellan gum gels with and without salt were kept at 10 °C overnight and then reheated for 2 h at 90 °C. The pH dependence of breaking stress for reheated gellan gum gels shown in Figure 5 was similar to the pH dependence of ΔH for nonheated gellan gum gels (Figure 3). The breaking stress of reheated gellan gum gels was small in comparison to that of nonheated gels. In the case of Figure 1, samples were not heated after adjustment of pH, but in the case of Figure 5, samples were kept at 10 °C overnight and then reheated for 2 h at 90 °C. The difference between the breaking stress of nonheated gels and that of reheated gels became larger with decreasing pH. At lower pH, gellan gum molecules may be hydrolyzed by acid, and tight binding of helices and association of helices may be inhibited. The breaking stress of reheated 1.0% (w/w) gellan gum gel at pH 8 was smaller than that of nonheated gel. This phenomenon was different from the thermal result. The difference between rheological and thermal properties may be caused by the difference in the heating condition.

Repeated Cooling DSC Curves. The sample was cooled from 99.9 to 1.0 °C at a scan rate of 1.0 °C/min,

kept at 10 °C for 10 min, and then heated again at the same rate up to 99.9 °C. This procedure was repeated several times. Exothermic peaks of gellan gum gels at pH 4 with and without KCl or NaCl became smaller and shifted to lower temperatures with increasing numbers of repeated cycles. An exothermic peak of gellan gum gels without salt at pH 8 splits into two peaks at the fourth cycle [Figure 6(1)]. An exothermic peak of gels containing NaCl at pH 8 splits into two peaks at the fifth cycle [Figure 6(3)]. These findings suggest that the gel formation of gellan gum occurs in two steps. The split of an exothermic peak into two peaks indicates that gellan gum forms the junction zones with two different thermal stabilities. The detailed mechanism of this phenomenon, however, has not yet been clarified. Gels containing KCl at pH 8 showed only one peak at higher temperatures than gels without salt [Figure 6(1)] and with NaCl [Figure 6(3)] at the fourth or fifth cycle [Figure 6(2)]. This result indicates that the junction zones of gellan gum containing KCl at pH 8 are stable to heat.

Figure 7 shows the pH dependence of endothermic enthalpy ΔH for DSC heating curves of gellan gum gels without salt [Figure 7(1)], with 10 mM KCl [Figure 7(2)], and with 10 mM NaCl [Figure 7(3)]. The enthalpy of all samples at lower pH values of 4 and 6 decreased with increasing numbers of cycles (Figure 7). The gellan gum may be hydrolyzed by acid during DSC measurement. The enthalpy of 1.0% (w/w) gellan gum gels without salt at higher pH values of 8 and 10 increased, and the midpoint transition temperature in a cooling DSC curve shifted to higher temperature with increasing numbers of cycles. The gellan gum molecules may have acetyl side groups that cannot be detected through an enthymatic method and NMR measurement. The gellan solution may be deacetylated at higher pH values. This should be explored in the future. The enthalpy of gellan gum gels containing 10 mM KCl at pH 4–8 decreased with increasing numbers of cycles [Figure 7(2)]. The enthalpy of gellan gum gels containing 10 mM NaCl at pH 8 has not been changed by repeating the heating and cooling up to the third cycle.

Conclusion. Gellan gum gels at pH 2 became turbid and had no practical use. Gellan gum at pH 4 formed a colorless and transparent gel and gelation was promoted, but resistance to heat was definitely superior to that of gels prepared at other pH values. Gellan gum over the pH range 6–10 would be an excellent gelling agent for processed foods that need to be reheated. In the present work, the pH of gellan gum gels was adjusted with either NaOH or HCl. As the gelation of gellan gum gels was greatly influenced by anions or cations, the effects of pH on the gelation of gellan gum may be changed by the addition of salts.

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