



Gelling temperatures of high acyl gellan as affected by monovalent and divalent cations with dynamic rheological analysis

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Abstract

The effects of Na⁺, K⁺, Ca⁺⁺ and Mg⁺⁺ cations on the sol–gel transitions of high acyl (HA) gellan dispersions were investigated. Gelling temperatures of 1% HA gellan dispersions containing selected cations (monovalent cations, 10–200 mM; divalent cations, 2–80 mM) were determined using dynamic rheological analysis. Gelling temperatures increased with cation concentrations. Divalent cations exhibited greater effects on the gelling temperature than equivalent concentrations of monovalent cations, but the effects between the two different types of divalent cations or the two different monovalent cations were not significantly different. An equation based upon the assumption that the effects of cations on gelling temperatures were additive fitted well with the experimental data ($R^2 = 0.98$, standard error of prediction = 0.58 °C).

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1. Introduction

Gellan gum, a novel polysaccharide food gelling agent secreted by bacteria, provides potential solutions to many gelling agent related problems in the food industry (Sanderson, 1990). One of the major advantages of gellan gum is that both the solution (sol) and solid (gel) states of gellan dispersions can provide a wide range of rheological and functional properties dependent upon the structure of the gellan molecule, concentration of the gellan gum and cations in the dispersion. Commercially available gellan gums can be classified into two categories: high acyl (HA) and low acyl (LA) gellan. Among the two, only HA gellan has glycerate and acetate groups on its glucose residues (Kuo & Mort, 1986). The structural differences between HA and LA gellan results in great disparity in rheological and functional properties between these two products (Huang, Tang, Swanson, & Rasco, 2003; Mao, Tang, & Swanson, 2000).

Physicochemical properties of gellan dispersions were studied with various analytical methods during the last two decades. Gellan in aqueous solutions were characterized with light scattering method (Takahachi, Akutu, Kubota, & Nakamura, 1999), viscometry (Ogawa, 1999), nuclear magnetic resonance (NMR) (Matsukawa, Tang, & Watanabe, 1995) and rheometry (Takigawa, Nakajima, & Masuda, 1999). Sol–gel transitions of gellan dispersions were assayed with differential scanning calorimetry (Lzumi, Saito, & Soma, 1999; Miyoshi & Nishinari, 1999a,b), rheometry (Miyoshi & Nishinari, 1999a,b; Tang, Tung, & Zeng, 1997a,b), electron spin resonance (Tsutsumi, Ozawa, Okada, Hiraoki, & Ishii, 1999), NMR (Annaka, Honda, Nakahira, Seki, & Tokita, 1999) and dielectric methods (Shinyashiki, Sakai, Yamada, & Yagihara, 1999). The textural properties of gellan gels were studied extensively using texture analyzer and rheometry (Mao et al., 2000, 2001; Tang, Tung, & Zeng, 1995, 1996). Studies of gellan gum reported the effects of cations, sugars and pH on the physicochemical properties of gellan dispersions as well as the mechanisms of interactions of gellan gum with other gelling agents. However, most of these studies focused on

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LA gellan, while few studies were reported on the basic properties of HA gellan. Investigations on the properties of HA gellan are necessary to promote utilization of this bacterial polysaccharide in food and pharmaceutical industry.

Gellan random coils form double helices and subsequently aggregate to form three dimensional networks in an appropriate aqueous environment. Both monovalent and divalent cations stabilize the networks through cross-linking gellan double helices via carboxylate groups of gellan molecules. However, monovalent and divalent cations follow different mechanisms on gellan gelation. Divalent cations (M^{++}) cross-link double helices directly (double helix- M^{++} -double helix), while monovalent cations (M^+) cross-link double helices indirectly (double helix- M^+ -water- M^+ -double helix) (Chandrasekaran, Radha, & Thailambal, 1992; Chandrasekaran & Thailambal, 1990). As a result of different gelation mechanisms, divalent cations are more effective on gel formation than monovalent cations (Sanderson, 1990).

The objectives of the study were to investigate the effects of Na^+ , K^+ , Ca^{++} and Mg^{++} cations on the sol–gel transitions of HA gellan dispersions, and to determine the gelling temperatures of HA gellan dispersions containing selected cations.

2. Materials and methods

2.1. Gellan solution preparation

Commercial HA gellan powder (Kelcogel LT100), provided by CP Kelco US, Inc. (Wilmington, DE, USA),

Table 1
Compositions of commercial and clarified HA gellan gums and their cation concentrations in 1% gellan dispersions

	Commercial gellan		Clarified gellan	
	(%) (w/w) ^a	mM of 1% gellan ^b	(%) (w/w) ^a	mM of 1% gellan
Solid	92.3	N/A	93.8	N/A
Ash	5.8	N/A	7.5	N/A
Mg^{++}	0.141	0.588	0.038	0.158
Ca^{++}	0.321	0.803	0.232	0.580
Na^+	0.649	2.820	1.671	7.265
K^+	2.029	5.203	1.908	4.892
Monovalent ^c	N/A	8.023	N/A	12.157
Divalent ^d	N/A	1.391	N/A	0.738
Ionic strength ^e	N/A	0.012	N/A	0.014

^a Data provided by CP Kelco US, Inc.

^b Cation concentrations in 1% gellan dispersions prepared by dispersing 1 gram gellan powder in 100 ml deionized water.

^c Sum of concentrations of K^+ and Na^+ .

^d Sum of concentrations of Ca^{++} and Mg^{++} .

^e Ionic strength calculated as half the sum of each ion concentration times the square of the charge.

was used in the study. Table 1 presents the initial cation compositions in gellan powder and cation molar concentrations in 1% gellan–water dispersions (see section 2.3 for information about clarified gellan). To prepare 1% gellan solutions, one gram of gellan powder was dispersed into 100 ml of deionized water in a 250 ml beaker and heated to the boiling point in 15 min with constant stirring. To compensate for the loss of water during heating, weight loss of gellan dispersion was recorded and additional water was added. Predetermined cations were added to prepare gel dispersions with Ca^{++} or Mg^{++} concentrations (not including the initial cation concentrations in the gellan) ranging from 2 to 80 mM (2, 4, 6, 8, 15, 30, 60, 80 mM) or with K^+ or Na^+ concentrations ranging from 10 to 200 mM (10, 20, 30, 50, 100, 150, 200 mM). The salts (KCl , $NaCl$, $CaCl_2$ and $MgCl_2$) used as cation additives were ACS reagents (Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA). Gellan dispersions were maintained at a temperature slightly below the boiling point and stirred for two min to dissolve the salt and then used for rheological tests.

2.2. Rheological tests

Dynamic rheological tests were conducted with an AR2000 rheometer (TA Instruments, New Castle, Delaware, USA) using a standard cup-and-bob geometry system (DIN 12620). The end of the bob was conical. The dimensions of the geometry system were as following: stator inner radius, 15.00 mm; rotor outer radius, 14.00 mm; cylinder immersed height, 42.00 mm and gap 5.92 mm.

A series of strain sweep tests were performed to estimate the linear viscoelastic range (LVE) of 1% gellan dispersions at selected temperatures (30, 55, 70, 85, 98 °C). During the strain sweep tests, change in storage (G') and loss moduli (G'') with change in controlled strain was recorded for each selected temperature starting at 98 °C and gradually decreasing to 30 °C. The applied strain ranged from 0.25 to 25%, and the angular frequency was 10 rad/s. Results from the strain sweep tests were used to determine the controlled strain applied to the temperature ramp tests to ensure the latter conducting in the LVE region.

G' describes the elastic energy storage and G'' describes the viscous energy loss of viscoelastic materials during shearing of dynamic rheological tests. In other words, G' represents the elastic solid-like behavior and G'' represents the viscous fluid-like behavior of viscoelastic materials. A strain sweep test is normally used to examine the dependence of G' and G'' on the shear strain, and thus determine the limit of LVE range. For shear strain less than a critical value, both G' and G'' exhibit a constant value with an increase in shear strain. The LVE shear strain range is exceeded when G' or G'' start to decrease significantly, which may be determined visually or with special analysis software program (Mezger, 2002).

Temperature ramp tests were used to determine the change in rheological properties (e.g. G' and G'') with

change in temperature from 98 to 50 °C for 1% gellan dispersions with selected concentrations of Na^+ , K^+ , Mg^{++} or Ca^{++} . Tests were conducted at an angular frequency of 10 rad/s and 5% controlled strain. The cooling rate of gellan dispersions was 0.6 °C/min. The gelling temperature of each gellan dispersion was identified as the intersection of the extrapolation of the rapidly increasing G' and the temperature axis (Hsieh, Regenstein, & Rao, 1993; Tang, Tung, & Zeng, 1997b). Duplicate experiments were conducted for each gellan dispersion with selected cation concentration.

2.3. Commercial HA gellan gum vs. clarified HA gellan gum

Commercial HA gellan (Kelcogel LT100) is not purified and contains residues (e.g. protein) from the fermentation process, which decreases the clarity of gellan dispersions. To determine the possible effects of these residues on rheological properties of gellan, temperature ramp tests were conducted for representative treatments of clarified HA gellan (Kelcogel EX-8593) dispersions and results were compared with those of commercial HA gellan. Clarified HA gellan provided by CP Kelco was purified using ion exchange and contains a greater sodium concentration than commercial HA gellan (Table 1). One percent clarified HA gellan, 0.984% clarified HA gellan with cation adjustment (adjusted clarified gellan), and 1% commercial HA gellan with or without cation adjustment (adjusted commercial gellan) were prepared for the temperature ramp tests. Adjusted clarified gellan and adjusted commercial gellan dispersions were prepared at the same levels of solid and cations (solid 0.0092%, Na^+ 7.15 mM, K^+ 5.20 mM, Mg^{++} 0.59 mM and Ca^{++} 0.80 mM) by adding a pre-determined amount of Na^+ , K^+ , Mg^{++} and/or Ca^{++} . All samples used in the study were commercial HA gellan unless specified otherwise.

3. Results and discussion

Fig. 1 presents results from the strain sweep tests. Gellan dispersions exhibited sol character ($G' < G''$) at

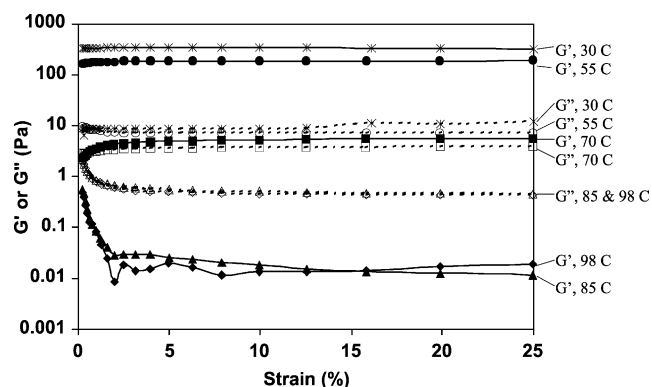


Fig. 1. G' and G'' of 1% gellan dispersion at selected temperatures with strain sweep test (0.25–25% strain, 10 rad/s).

temperatures greater than 70 °C and gel character ($G' > G''$) at temperatures equal to or smaller than 70 °C. At temperatures lower than 70 °C (i.e. 30, 55 °C), both G' and G'' exhibited a constant value and there was no abrupt change in G' or G'' with change in oscillation strain. This indicates that 0.25–25% controlled strain for 1% gellan dispersions was within the LVE range at the specified test condition (10 rad/s, ≤ 55 °C). At higher temperatures (85 and 98 °C), both G' and G'' of gellan dispersions decreased with increase in oscillation strain at low oscillation strains ($< 2.0\%$) and then reached a constant value. Although a decrease of G' and G'' with increasing oscillation strain (from 0.25 to ca. 2%) was observed, this might not necessarily indicate that the limit of LVE range for oscillation strain was exceeded. The initial decrease of G' and G'' may result from instrumental and geometry inertia and/or sensitivity of the instrument. The oscillation strains or oscillation stress used to calculate the G' and G'' at low controlled strains ($< 2\%$) may be too small for precise control or determination. Similar explanation can be given for the slight increase in G' and G'' with increasing oscillation strain from 0.25% to about 1.5% for gellan dispersions at 70 °C. Based upon the strain sweep test, an oscillation strain less than 25% is believed to be in the LVE range and a 5% controlled strain with 0.5% initial tolerance was selected for temperature ramp experiments.

Rheological properties and gelling temperatures for 1% commercial and clarified HA gellan dispersions were close (Fig. 2), in spite of the two products having different concentrations of solids and cations and a disparity in the amount of fermentation residues. The G' or G'' vs. temperatures curves for the two products were very similar (Fig. 2) and the difference between the curves of two products was similar to that of replicated experiments of the same treatment of commercial or clarified gellan dispersion. At temperatures higher than about 75 °C, G' and G'' values were almost constant and the gellan dispersion had sol

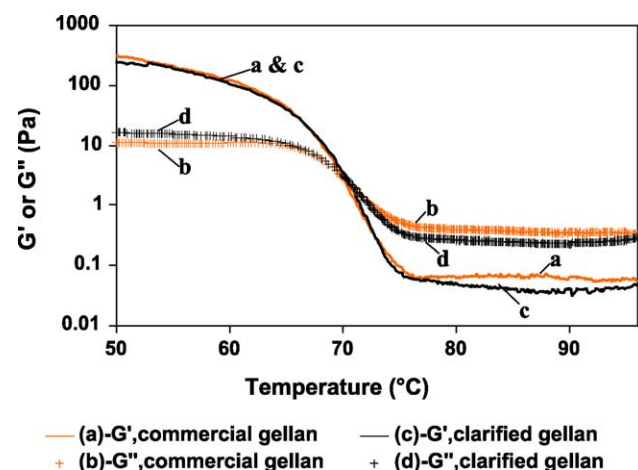


Fig. 2. G' and G'' of 1% commercial and clarified gellan dispersion obtained by cooling from 98 to 50 °C.

character ($G'' > G'$). As temperature decreased to about 75 °C, G' and G'' for both products increased and the value of G' increased more rapidly than G'' . The onset of the steep increase of G' and G'' at about 75 °C may be attributed to the coil-helix transition of gellan molecules (Miyoshi & Nishinari, 1999a). The temperature where G' is equal to G'' (cross-over point of G' and G'') is normally defined as the sol-gel transition temperature, also called gel point or gelling temperature. The cross-over gelling temperature for 1% commercial gellan was 69.9 °C and for the clarified gellan was 70.1 °C. The gelling temperature determined by the method of G' extrapolation was 67.5 °C for both 1% commercial gellan and clarified gellan. Because of the difficulties in obtaining reliable G' values at temperature higher than gel point for the dispersions with high cation concentrations, the G' and G'' cross-over point method was not selected as the final method for gelling temperature determination in this study. As temperature was continuously decreased, G' and G'' reached constant values and the gellan dispersions displayed rigid solid behavior.

Commercial gellan powder contains less solid (dry weight, 92.3 vs. 93.8%) and monovalent cations than the clarified gellan powder. This should have caused a relatively lower gelling temperature for commercial gellan compared to that of clarified gellan (Tang, Tung, & Zeng, 1997a,b; Takigawa, Nakajima, & Masuda, 1999). However, commercial gellan powder has larger concentrations of divalent cations than the clarified gellan powder, which tends to increase the gelling temperature of the former. The divalent cations have much larger effects on rheological properties of gellan (Tang, Tung, & Zeng, 1997a) than the monovalent cations because of the different gelation mechanisms between divalent cations and monovalent cations. Divalent cations cross-link double helices directly while the monovalent cations cross-link double helices indirectly (Chandrasekaran & Thailambal, 1990; Chandrasekaran, Radha, & Thailambal, 1992). Therefore, although the difference in total divalent cations (1.391 vs. 0.738) is much less than the difference in total monovalent cations (8.023 vs. 12.157 mM) for these two products, the former may have compensated for the effects of the latter and solid content. The ionic strength of the clarified gellan was about 17% higher than the commercial gellan indicating that the gelling temperature of gellan may not be directly related to the ionic strength Table 1.

The similarity in change of G' and G'' values with temperatures between 1% commercial and clarified gellan dispersions implies that the fermentation residue may only have minor effect on the rheological properties of gellan dispersions. This was confirmed through the comparison of the experimental results for adjusted commercial and adjusted clarified gellan dispersions with equivalent concentrations of solid and cations. The changes in G' and G'' with temperatures for adjusted commercial and adjusted clarified gellan dispersions were very similar (figures were not shown here). In addition, the cross-over gelling

temperature for both the adjusted commercial and clarified gellan was 71.4 °C (69.5 and 69.0 °C, respectively using extrapolated gelling temperature). The adjusted gellan dispersions had higher concentrations of cations resulting in greater gelling temperatures than gellan dispersions without cation adjustments as previously reported (e.g. 69.9 and 70.1 °C for commercial and clarified gellan respectively).

Fig. 3a exhibits the effect of Ca^{++} on the G' of gellan dispersions. The addition of Ca^{++} increased gelling temperature of gellan dispersions (Table 2 and Fig. 3a). In the range of 50–70 °C, G' increased with increase in Ca^{++} concentration from 0 to 15 mM, but then decreased with further addition of Ca^{++} from 30 to 80 mM. This may imply that gellan dispersions with higher Ca^{++} concentrations (≥ 30 mM) exhibit different gelation mechanisms from gellan dispersions with relatively lower Ca^{++} concentrations (≤ 15 mM). The effects of change in Ca^{++} concentration on G'' exhibited a similar trend (Fig. 3b). The temperature determined through the intersection of the extrapolation of the rapidly increasing G'' and the temperature axis increased with the increase in Ca^{++} concentration. The G'' for high Ca^{++} gellan dispersions

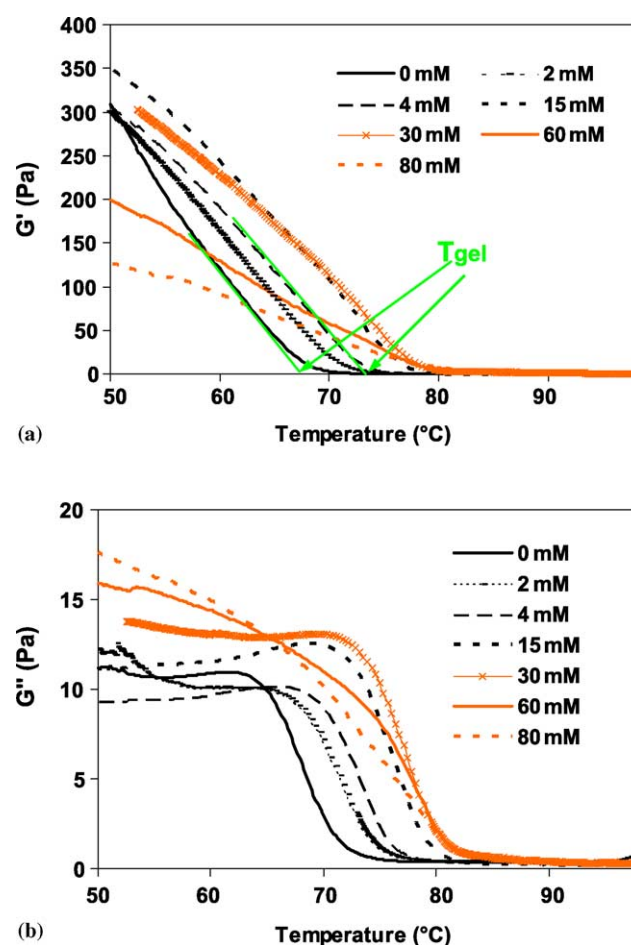


Fig. 3. (a) G' , and (b) G'' of 1% commercial gellan dispersions containing selected calcium concentrations (0–80 mM) cooling from 98 to 50 °C.

Table 2
Mean gelling temperature ($n = 2$) of gellan dispersions with selected concentrations of Ca^{++} , Mg^{++} , Na^+ and K^+

Cation added (mM)		2	4	6	8	15	30	60	80
Divalent	$T_{\text{gel}} (\text{Ca}^{++})^a$	71.2	73.3	74.7	75.1	76.6	78.2	80.8	80.5
	$T_{\text{gel}} (\text{Mg}^{++})$	71.4	73.1	74.7	75.1	76.8	78.5	80.1	80.8
Cation added (mM)		10	20	30	50	100	150	200	
Monovalent	$T_{\text{gel}} (\text{K}^+)$	71.0	72.6	74.3	75.2	78.5	80.3	81.6	
	$T_{\text{gel}} (\text{Na}^+)$	71.1	72.8	73.7	75.2	78.0	79.5	80.8	

^a Standard deviations for gelling temperatures were between 0 and 0.42 °C.

(≥ 30 mM) also followed different trend from low Ca^{++} gellan dispersions (≤ 15 mM).

Single factor ANOVA (Microsoft Excel 2000; Microsoft Co., Redmond, WA, USA) ($P < 0.05$) demonstrated that addition of equivalent concentration of Ca^{++} and Mg^{++} resulted in no significant differences in gellan gelling temperatures. In Fig. 4a, G' vs. temperature curves overlapped for samples with the same level of Ca^{++} and Mg^{++} (≤ 15 mM), which means that small concentrations of Ca^{++} and Mg^{++} (≤ 15 mM) had the same effects on G' and G'' for gellan dispersions. However, there was a disparity between the effects of a large concentration (≥ 30 mM) of Ca^{++} and Mg^{++} on G' or G'' of gellan dispersions, which may indicate that the molecular size of divalent cations affects the rheological properties (Fig. 4b).

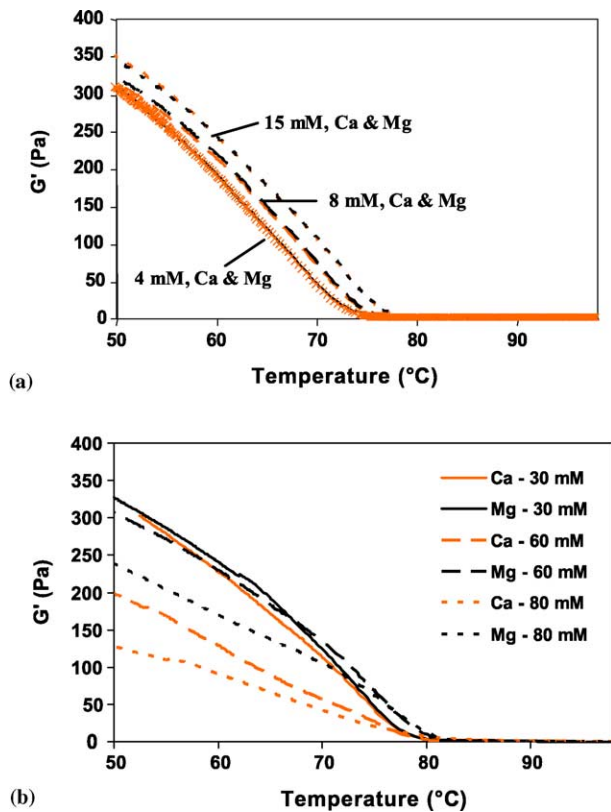


Fig. 4. G' of 1% gellan dispersions containing equivalent concentration of calcium and magnesium at (a) 2–15 mM and (b) 30–80 mM cooling from 98 to 50 °C.

Na^+ and K^+ exhibited equivalent or very similar effect on the gelling temperature, G' and G'' of gellan dispersions (Fig. 5). The G' or G'' vs. temperature curves overlapped, and ANOVA showed no significant difference in gellan gelling temperatures for equivalent concentrations of Na^+ and K^+ over the tested range between 10 and 200 mM.

Tang, Tung, and Zeng (1997a,b) developed a model to predict the gelling temperatures of LA gellan dispersions based on composition:

$$\frac{1}{T_{\text{gel}}} = A \ln[X_g] + B \ln[X_i] + C \quad (1)$$

where A , B and C are constants, $[X_g]$ is the gellan concentration, and $[X_i]$ is the cation concentration. For gellan dispersions with more than one type of cations, the expression was derived as

$$\frac{1}{T_{\text{gel}}} = A \ln[X_g] + B \ln\left(\sum_{i=1}^n (a_i[X_i])\right) + C \quad (2)$$

where n is the number of cation types, $[X_i]$ is the concentration of cation i and a_i is a constant value corresponding to $[X_i]$. Eq. (2) was based upon the assumption that different types of cations contributed independently to the gelation mechanisms and the effects of these cations is additive.

Our study indicated a linear relationship between $1/T_{\text{gel}}$ and $\ln[\text{divalent}]$ or $\ln[\text{monovalent}]$, which implies that Eq. (2) for LA gellan may also be applicable to the gelling

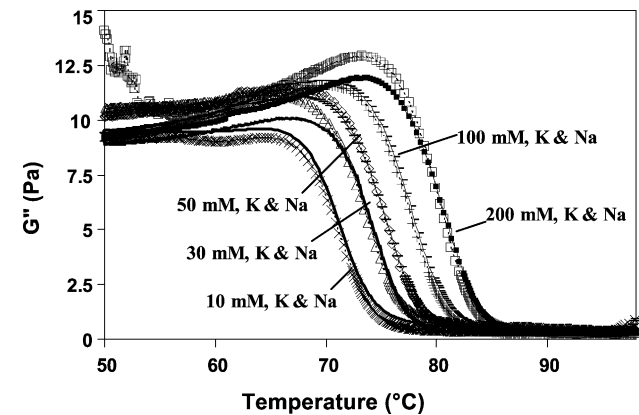
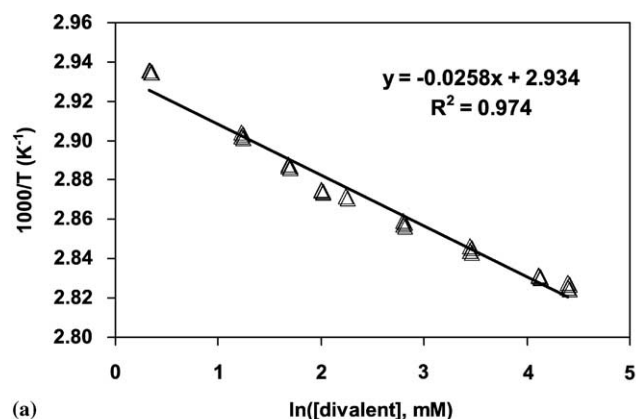
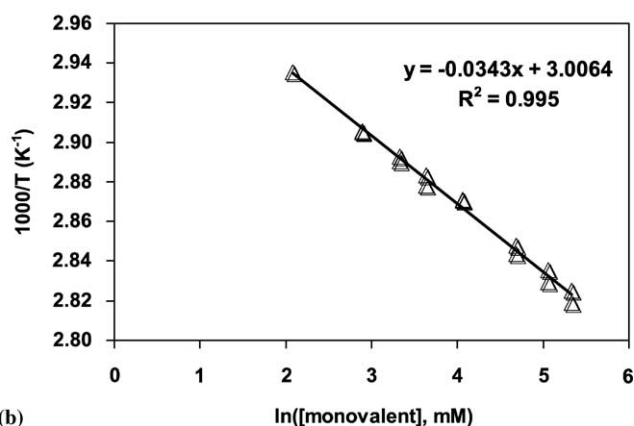


Fig. 5. G'' of 1% commercial gellan dispersions containing selected sodium and potassium concentrations (10–200 mM) cooling from 98 to 50 °C.



(a)



(b)

Fig. 6. Relationship of 1% HA gellan gelling temperatures with concentrations of (a) divalent cations, and (b) monovalent cations.

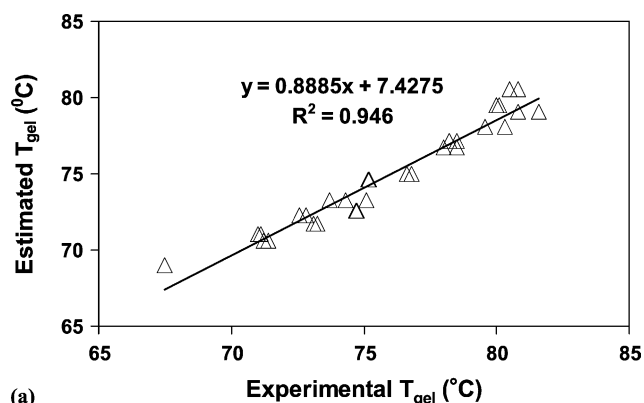
temperatures of HA gellan (Fig. 6). Eq. (3) deriving from Eq. (2) and their slightly modified Eq. (4) were proposed for determining gelling temperatures of 1% HA gellan dispersions:

$$\frac{1}{T_{\text{gel}}} = A + B \ln([X_d] + C[X_m]) \quad (3)$$

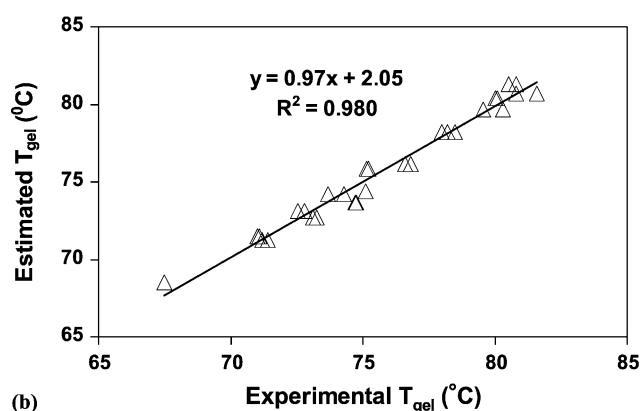
$$\frac{1}{T_{\text{gel}}} = A + B \ln[X_d] + C \ln[X_m] \quad (4)$$

where A , B , C are constants, $[X_d]$ is the concentration of divalent cations and $[X_m]$ is the concentration of monovalent cations.

A multiple linear regression method (Microsoft Excel 2000; Microsoft Co., Redmond, WA, USA) was used to fit the data into Eq. (4) and a nonlinear regression method (SAS 8.1; SAS institute, Inc, Cary, NC, USA) was used to fit the data into Eq. (3). A , B , and C constants were determined as 0.00296 , -3×10^{-5} , 0.2641 for Eq. (3) and 0.003 , -2.6×10^{-5} , -3.1×10^{-5} for Eq. (4). The measured gelling temperature and the gelling temperature predicted by the Eq. (3) and Eq. (4) are presented in Fig. 7. The standard error of gelling temperatures estimated by Eq. (3) was 1.39°C and by Eq. (4) was 0.58°C . The R^2 of estimated temperatures vs. measured gelling temperatures was 0.946 and 0.980 for Eqs. (3) and (4), respectively. Both Eqs. (3) and (4) fitted the data well, but the Eq. (4) performed better



(a)



(b)

Fig. 7. Experimental gelling temperature (T_{gel}) against estimated gelling temperature of 1% HA gellan dispersions from (a) Eq. (3), and from (b) Eq. (4).

than Eq. (3) based upon the standard error and R^2 of estimated temperatures vs. measured gelling temperatures.

4. Conclusions

Rheological properties for 1% commercial and clarified HA gellan dispersions without added cations were similar. The similarity between the 1% commercial and clarified gellan dispersions implies that the fermentation residues may have negligible effect on rheological properties of gellan. Gelling temperature of gellan dispersions increased with cation concentrations. There were no significant differences on the effects of two divalent cations, Ca^{++} and Mg^{++} , or of two monovalent cations, K^+ and Na^+ on gelling temperatures. Equations based upon the assumption that the effects of cation concentrations were additive fitted well with the experimental data ($R^2 = 0.98$, standard error of prediction = 0.58).

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