

Mechanical Properties of Gellan Gels in Relation to Divalent Cations

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ABSTRACT

Mechanical behavior of gels formed with gellan polymer crosslinked by calcium and magnesium ions was studied to determine the influence of divalent ion type and polymer concentration. Failure strength and deformation were measured in compression and related to concentrations of gellan and bound cations in gel matrices. Insufficient cations formed weak, extensible gels. Maximum gel strength was achieved at 0.5 divalent cations/repeat tetrasaccharide unit, assumed to be the condition for maximal numbers of complete junction zones. At optimum cation levels gels with Ca^{++} were about 1.2 times stronger than gels with Mg^{++} at the same polymer concentration. Excessive cations weakened the gels. Twice as much reduction in gel strength resulted from additional Ca^{++} as compared to the same additional amount of Mg^{++} . Differences between strengths of the gels may be attributable to polymer configurations at junction zones in relation to cation size.

Key Words: gellan, gel, divalent cations, compression, deformation

INTRODUCTION

GELLAN POLYMER is an anionic polysaccharide approved by FDA for use in foods (Anon., 1992). Its monosaccharide units are β -D-glucose, β -D-glucuronic acid and α -L-rhamnose in molar ratios of 2:1:1 (Sanderson, 1990). In water solutions, the polymer forms gels in the presence of cations, possibly through formation of 3-fold double-helical junctions (Upstill et al., 1986; Chandrasekaran et al., 1988a). The native polysaccharide contains $\approx 6\%$ O-acetyl groups which, under suitable conditions, form weak and deformable gels. The O-acetyl groups are lost during normal commercial extraction, and the deacetylated polysaccharide forms much stronger gels (Upstill et al., 1986; Chapman et al., 1987).

Successful application of gellan polymer as a gelling agent to provide desired textural properties in foods depends upon a thorough understanding of relationships between mechanical properties and interactions among gel-forming constituents at the molecular level. It has been found from small displacement dynamic testing that the complex shear and Young's moduli of gellan gels depend upon the concentration and valency of ions (Moritaka et al., 1991). The moduli of gellan gels are larger in certain ranges of ion concentration than others. Both monovalent and divalent cations can yield gellan gels of similar moduli and strength, but much higher concentrations of monovalent cations are required than for divalent cations (Moritaka et al., 1991). This ratio can be as high as 40:1 (Chandrasekaran et al., 1988b).

Effects of polymer and Ca^{++} concentrations on ultimate strength and extensibility of gellan gels have been studied by Tang et al. (1994) using compression, torsion and tension tests. At a given polymer concentration, two distinctive patterns of mechanical response were observed below and above a certain Ca^{++} concentration, referred to as the critical cation level, at which the gel had maximum strength. The gels were soft and deformable below this critical Ca^{++} level, but brittle above. They suggested this critical Ca^{++} level corresponded to the state in

which all anionic sites in potential junction zones along the gellan polymers were occupied by cations so that maximal interactions would take place within the gel network.

Cations have a major effect on mechanical properties of gellan gels and it would be interesting to further investigate their functions in forming three-dimensional polymer networks. Our objectives were: (1) to confirm the findings by Tang et al. (1994) regarding the effects of divalent cations on mechanical response of gellan gels using Mg^{++} in addition to Ca^{++} ; (2) to test the hypothesis that critical cation levels correspond to the state in which all anionic sites in potential junction zones of polymer solutions are occupied by cations; and (3) to correlate mechanical properties of gellan gels to presumed structure of the polymer network at the molecular level.

MATERIALS & METHODS

MECHANICAL PROPERTIES of gellan gels containing different polymer and cation concentrations were determined in large deformation tests under compressive loading. The amount of cations bound to the gellan polymers in forming each gel was derived from the difference between cation concentration in original solutions before gelling and the amount of free cations that could be leached from intact gels. All tests were conducted on gellan gels formed with the divalent cations, Ca^{++} and Mg^{++} .

Sample preparation

Deacetylated gellan polymer (KELCOGEL, Kelco Inc., San Diego, CA) contained small amounts of metals (Table 1). Weighed amounts of gellan powder were dispersed in distilled deionized water at room temperature to form dispersions of desired concentrations. For experiments on gels with calcium, ten polymer concentrations were prepared at equal increments in the range 0.2–2.2%, and for magnesium, six polymer solutions were prepared over the same polymer concentration range. The solutions were gently heated on hot plates from room temperature to 90°C so that the polymers were dissolved and solutions became clear. Predetermined amounts of calcium chloride (CaCl_2) or magnesium chloride (MgCl_2) were added to obtain cation concentrations from 1.5 to 80 mM for calcium solutions and from 1.5 to 60 mM for magnesium solutions. Increments between low cation concentrations were relatively small as compared to higher cation concentrations. This was done in an effort to prepare gels below and above the critical cation levels based on previous experience with calcium-crosslinked gellan gels (Tang et al., 1994). Solutions were allowed to cool to about 70°C and then filled into cylindrical stainless steel molds of 21 mm internal dia and 140 mm length, fitted with rubber stoppers on both ends. Samples were cooled by immersing the sealed molds in running water at 15°C for 15 min and holding them in air at room temperature (22°C) overnight to form consistent gels.

Compression tests

Mechanical properties were determined only by compression tests because previous work has shown failure shear stresses of calcium-gellan gels to be the same when derived from tension, torsion and compression experiments (Tang et al., 1994). Moreover, sample preparation and measurement procedures were far less time consuming for compression testing, so that a larger number of experimental conditions could be explored in a given time.

In preparing specimens for compression tests, six cylindrical sections of 21 mm diam and 20 mm length were cut from each gel when removed from its mold. These specimens were then compressed at a crosshead

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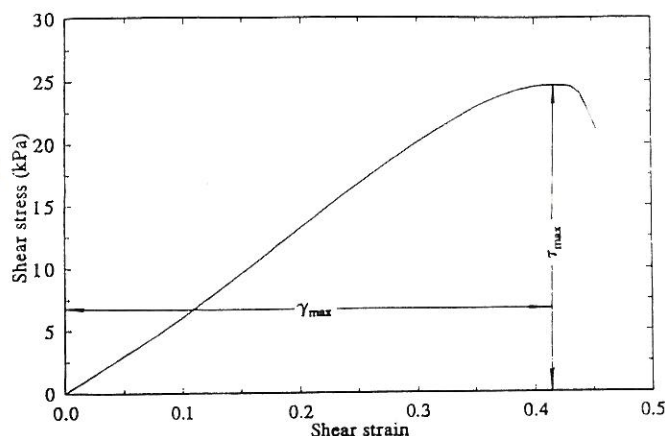


Fig. 1—Typical shear stress-strain relationship in compression. The curve corresponds to a gel sample containing 1% (w/v) gellan polymer and 6 mM added Ca^{++} concentrations.

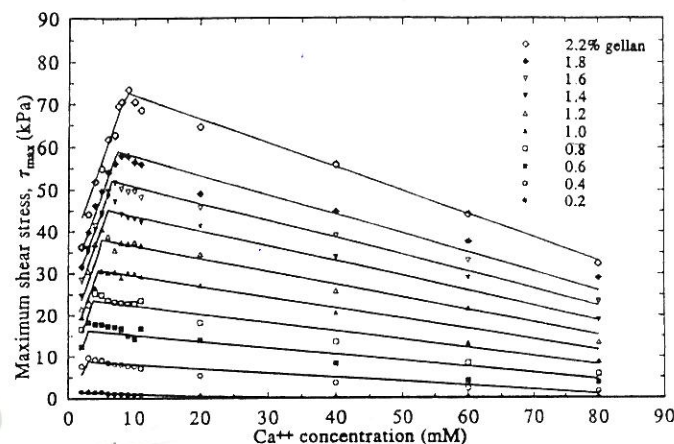


Fig. 2—Maximum shear stress at failure for gellan gels made with different polymer and added Ca^{++} concentrations.

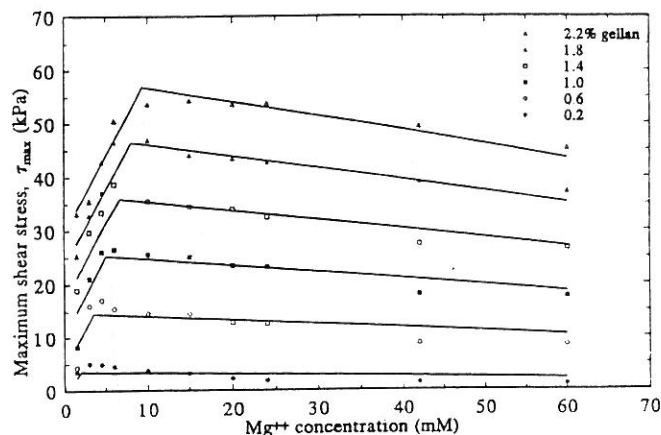


Fig. 3—Maximum shear stress at failure for gellan gels made with different polymer and added Mg^{++} concentrations.

speed of 20 mm/min between lubricated Teflon plates in a mechanical testing machine (Model 1125, Instron Corp., Canton, MA) interfaced with an IBM microcomputer. Contact between the ends of the specimen and compressing surfaces were rendered essentially frictionless by applying a few drops of low viscosity oil to each end. This minimized complications which might otherwise arise due to "barrelling" caused by friction at contact surfaces.

Compression tests were carried out on six specimens from gellan gels at ten polymer concentrations from 0.2 to 2.2% and fourteen Ca^{++} levels from 1.5 to 80 mM. Similar tests were conducted on gellan gels at six

polymer levels (0.2 to 2.2%) and ten Mg^{++} concentrations (1.5–60 mM). From the compression experiments, each gel was characterized by extracting the mean values of force (F_{max}) and deformation (ΔL_{max}) at the moment of failure from the six test specimens.

The maximum true normal strains at failure (ϵ_{max}) in cylindrical specimens were obtained as described by Hamann (1983):

$$\epsilon_{\text{max}} = -\ln \left[1 - \frac{\Delta L_{\text{max}}}{L} \right] \quad (1)$$

and the corresponding failure stresses (σ_{max}) for incompressible gels were derived according to Nussinovitch et al. (1990):

$$\sigma_{\text{max}} = \frac{F_{\text{max}}(L - \Delta L_{\text{max}})}{\pi R^2 L} = \sigma_e (1 - \epsilon_{\text{eng}}) \quad (2)$$

where L and R are the original length and radius of gel specimens, respectively.

Since the mode of failure during compression testing of the cylindrical gels was observed to take place in shear, the shear strains (γ_{max}) and shear stresses (τ_{max}) at failure were obtained from the normal strains and stresses by the following relations:

$$\gamma_{\text{max}} = (1 + \nu) \epsilon_{\text{max}} \quad (3)$$

$$\tau_{\text{max}} = \frac{\sigma_{\text{max}}}{2} \quad (4)$$

Since gellan gels consisted mostly of water and did not contain visible air bubbles, the gels were virtually incompressible under the loading conditions experienced in the tests. Thus, the Poisson's ratio (ν) in Eq. (3) was assumed to be 0.5 (Juvinal, 1967).

Determination of free ion concentration in gels

One hundred grams gels were cut into 2–3 mm pieces and submerged in 150 mL distilled deionized water in beakers. The beakers were covered and left overnight for unbound cations to diffuse into water from gel matrices. The ion concentrations of the solutions were determined with an atomic absorption spectrophotometer (Model 2380, Perkin-Elmer Corp., Norwalk, CT) using an air-acetylene flame. Before testing, standard solutions of 0.2, 1, 3, and 6 mM Ca^{++} and 0.2, 0.6, and 1 mM Mg^{++} were used to produce calibration curves. All sample solutions in three replicates were diluted into these ranges. The wavelength used for Ca^{++} was 422.7 nm, and for Mg^{++} was 285.2 nm. Concentrations of cations in the solutions were related to original weights of gels sampled for extraction. The differences between amounts of metal ion known to be present in the original gel and the amounts extracted, were assumed to be bound with the gellan polymer in forming gel networks.

RESULTS & DISCUSSION

COEFFICIENTS OF VARIATION (C.V.) among replicated tests were 7.8% for shear stresses at failure, 9.1% for shear strains at failure, and 4.9% for metal ion determinations. The means of replicates were used to show data points in some of figures, and in regression analyses using SAS procedures (SAS Institute, Inc., 1985).

Strength and extensibility of gellan gels

A typical shear stress-strain relationship from compression tests is illustrated (Fig. 1). The peak shear stress, τ_{max} , represents the strength of the gel, and the corresponding shear strain, γ_{max} , reflects its extensibility up to the point of failure. The effects of added Ca^{++} and Mg^{++} on gellan gel strength at different polymer concentrations were presented as the data points shown (Fig. 2 and 3). In both cases, the patterns of change in gel strength with increasing cation concentration were similar to those in Tang et al. (1994). In general, strength of gellan gels increased with polymer concentration. At a given polymer concentration, gel strength increased in an approximately linear manner with ion concentration up to a critical cation level. Above that level, the gels gradually decreased in strength with increasing ion concentration. At the critical levels, gellan gels with added Ca^{++} were about 1.2 times stronger than those with added Mg^{++} .

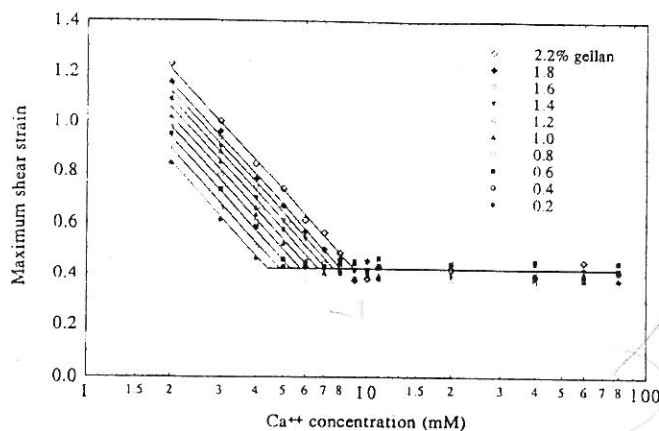


Fig. 4—Maximum shear strain at failure for gellan gels made with different polymer and added Ca^{++} concentrations.

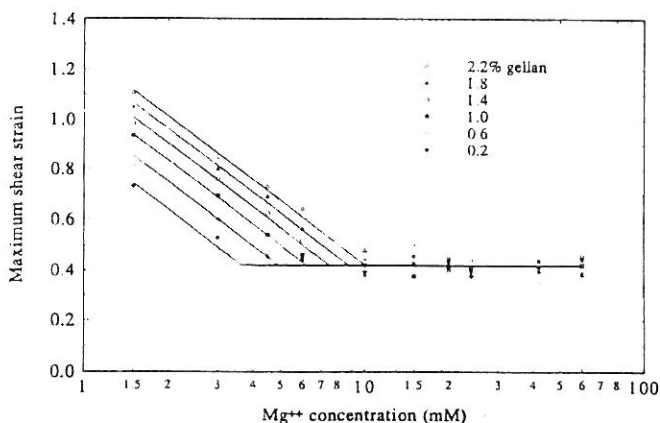


Fig. 5—Maximum shear strain at failure for gellan gels made with different polymer and added Mg^{++} concentrations.

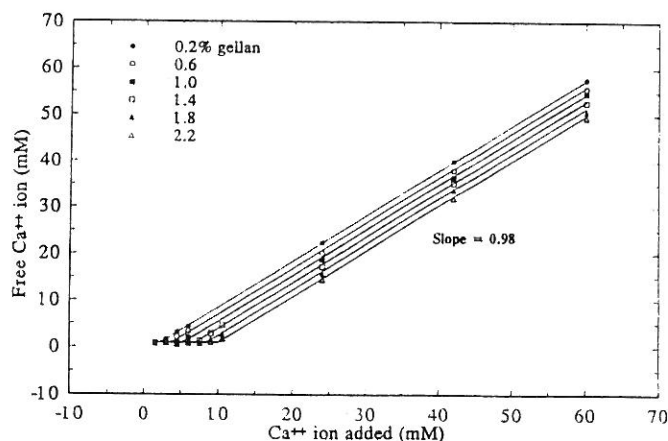


Fig. 6—Relationship between free and added Ca^{++} in gellan gels at different polymer concentrations.

The multiple regression procedure of SAS (SAS Institute Inc., 1985) was used to find the forms of functions which fit the data (Fig. 2 and 3) for shear stresses at failure. The data sets for each polymer concentration were separated into two groups, below and above the cation level x_{cr} that corresponded to maximal failure shear stress. A separate regression equation was developed for each data group. The models drawn as lines (Fig. 2) for calcium-gellan gels are:

$$\tau_{\max} = -8.91 + 19.70 x_g + 4.42 x_c \quad (5)$$

$(x_c \leq x_{cr}; \quad R^2 = 0.976)$

$$\tau_{\max} = -6.23 + 38.12 x_g - 0.25 x_c x_g \quad (6)$$

$(x_c > x_{cr}; \quad R^2 = 0.990)$

and for the gels with Mg^{++} are:

$$\tau_{\max} = -5.18 + 15.78 x_g + 2.89 x_m \quad (7)$$

$(x_m \leq x_{cr}; \quad R^2 = 0.958)$

$$\tau_{\max} = -2.84 + 27.93 x_g - 0.12 x_m x_g \quad (8)$$

$(x_m > x_{cr}; \quad R^2 = 0.995)$

where, τ_{\max} is the maximum shear stress (kPa), x_g stands for the gellan polymer concentration (%), x_c and x_m for the Ca^{++} and Mg^{++} concentrations (mM) added to the gels, respectively. The critical cation concentration (x_{cr} , mM) for each polymer concentration was taken as the intersection of each pair of lines depicted by Eq. (5) and (6) (Fig. 2) and/or by Eq. (7) and (8) (Fig. 3). Based on the coefficient of x_g in Eq. (5) and (7), the effect of polymer concentration on strength of the gels with Ca^{++} was about 1.3 times greater than gels with Mg^{++} below the critical cation levels. The coefficient of the third term in Eq. (6) and (8) indicates that above the critical cation level, the reduction of gel strength with increasing ion concentration for gels with Ca^{++} was about two times more than with Mg^{++} .

The critical ion levels were examined in relation to gellan polymer concentration. The values were expressed as a linear function of polymer concentration in Eq. (9) for Ca^{++} and Eq. (10) for Mg^{++} :

$$x_{cr} = 1.27 + 3.56 x_g \quad r^2 = 0.993 \quad (9)$$

$$x_{cr} = 1.23 + 3.72 x_g \quad r^2 = 0.998 \quad (10)$$

Differences between coefficients of the two equations were small, which suggested that the critical divalent cation levels may be independent of the type of divalent cation.

Deformation of gellan gels at the point of fracture was reflected by the maximum shear strains which were plotted as the data points on semilogarithmic coordinates for added Ca^{++} (Fig. 4) and Mg^{++} (Fig. 5). At each polymer concentration, the maximum strain of both gels decreased in a semilogarithmic with increasing ion concentration up to the critical cation level, and this strain increased with increasing polymer concentration. Above the critical level, the maximum shear strain was independent of both ion and gellan concentrations. The maximum shear strain at failure for the gels below the critical cation levels were related to gellan and ion concentrations using the nonlinear regression procedure (SAS Institute, Inc., 1985) for Ca^{++} :

$$\gamma_{\max} = 0.42 + 1.22 \log \left(\frac{3.96 + 2.27 x_g}{x_c} \right) \quad (11)$$

$(x_c \leq x_{cr}; \quad \text{R.M.S.} = 3.2\%)$

and for Mg^{++} :

$$\gamma_{\max} = 0.42 + 0.85 \log \left(\frac{2.99 + 3.20 x_g}{x_m} \right) \quad (12)$$

$(x_m \leq x_{cr}; \quad \text{R.M.S.} = 3.4\%)$

where R.M.S. indicates Relative Mean Square Error defined as:

$$\text{R.M.S.} = \sqrt{\frac{1}{N} \sum_{i=1}^N \left(\frac{\gamma_{\max,i} - \gamma'_{\max,i}}{\gamma_{\max,i}} \right)^2} \quad (13)$$

in which, $\gamma_{\max,i}$ are the experimental data, $\gamma'_{\max,i}$ are the predicted values from the regression equations and N stands for the number of data points.

Comparing the coefficient of the second term in Eq. (11) and (12) reveals that gellan gels with Ca^{++} were more deformable than gels with Mg^{++} at ion concentrations below the critical ion

levels. Above the critical cation levels, both gels had the same maximum shear strain of about 0.42.

Free divalent cation concentrations

Relationships between concentrations of free (unbound) divalent cations and the total cation concentration added to the solutions prior to gelling were compared for gellan gels with Ca^{++} (Fig. 6) and with Mg^{++} (Fig. 7). When small amounts of cations were added, 0.5 to 0.7 mM concentrations remained as free ions, the rest were bound to gellan polymers. This was true until the added ion concentration exceeded a threshold level marked by a noticeable increase in extracted cations for each polymer concentration. Above this condition, the amount of free cations increased in proportion to added cations.

Stepwise multiple linear regression was used to correlate data of free cations in the gels with gellan polymer concentrations and added cations in the ranges above the threshold cation levels. The regression models are presented as a family of parallel lines represented by Eq. (14) for added Ca^{++} and Eq. (15) for added Mg^{++} :

$$x'_c = -0.34 + 0.98x_c - 3.99x_g \quad R^2 = 0.966 \quad (14)$$

$$x'_m = 0.34 + 0.95x_m - 4.16x_g \quad R^2 = 0.995 \quad (15)$$

where, x'_c and x'_m are the predicted concentrations of the two different free divalent cations (mM). The proportionality factors for x_c and x_m in Eq. (14) and (15) were close to 1, indicating that most of the added cations above the threshold levels remained as free ions in the gels (Figs. 6 and 7). Thus, the threshold cation values mark the state when all the anionic sites along the gellan polymers were occupied by cations. Since the number of anionic sites in a gel increased proportionally with the concentration of polymers, it is expected that larger polymer concentrations would correspond to higher threshold levels. This is demonstrated by shifting of the threshold cation levels and free ion curves to the right with increasing polymer concentrations. The added cation concentration at the threshold levels were related to polymer concentration as follows for Ca^{++} :

$$x_{cr} = 0.93 + 4.07 x_g \quad r^2 = 0.999 \quad (16)$$

and for Mg^{++} :

$$x_{cr} = 0.69 + 4.33x_g \quad r^2 = 0.999 \quad (17)$$

The values of the above cation concentrations varied from 1.7 to 9.9 mM for Ca^{++} and from 1.6 to 10.2 for Mg^{++} in gellan solutions when the polymer concentration increased from 0.2 to 2.2% w/v. Considering the amount of metal ions originally present in the gellan polymer powders (Table 1), these values correspond to 0.4 to 0.7 Ca^{++} , or 0.4 to 0.6 Mg^{++} divalent cations per repeat tetrasaccharide unit in the gellan polymer. The carboxylate group in each repeat unit provides an anionic site for 0.5 divalent cation (Sanderson, 1990). Therefore, the threshold cation concentrations detected by analytical testing appear to correspond to the condition in which the amount of cations were just sufficient to saturate all anionic sites on gellan polymer molecules.

Correlation between compression and analytical test results

The critical cation levels inferred from maximum stresses and strains at failure in the compression tests highly correlated with threshold cation levels from chemical analysis for free Ca^{++} and Mg^{++} (Table 2) over the range of polymer concentrations studied. Correlation coefficients calculated for all possible pairs of critical cation concentrations exceeded 0.81. This supported our hypothesis that the critical cation level detected in the compression tests corresponded to the state of cation saturation on gellan polymers and, therefore, the state of optimal gellan gel structure.

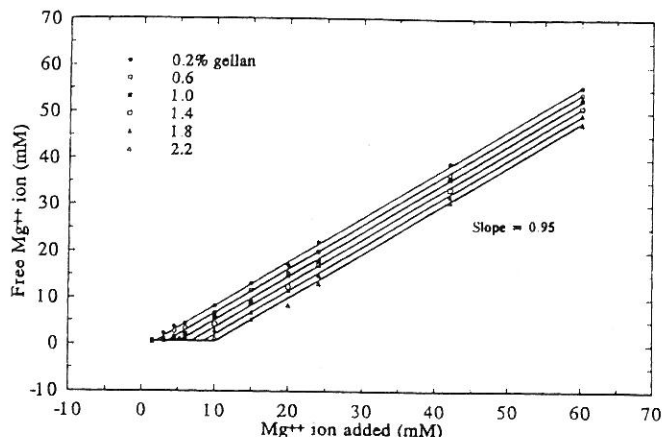


Fig. 7—Relationship between free and added Mg^{++} in gellan gels at different polymer concentrations.

Table 1—Major components in the gellan polymer

Composition ^a	Weight in dry matter (%)
Carbohydrates (dietary fiber)	85
Water	10
Sodium	0.3
Calcium	0.4
Magnesium	0.1
Potassium	4.9
Phosphorus	0.2

^a Provided by the manufacturer, Kelco Inc., San Diego, CA.

Table 2—Critical concentration levels for calcium and magnesium cations derived from mechanical tests of maximum stress and strain at failure and chemical tests to determine bound cations in gellan gels of varying polymer concentrations

Gellan polymer (%)	Calcium (mM)			Magnesium (mM)		
	Stress	Strain	Bound	Stress	Strain	Bound
0.2	2.0	3.3	1.8	2.0	3.5	1.7
0.6	3.8	5.0	3.4	3.5	4.9	3.3
1.0	4.5	6.2	5.0	5.0	6.1	5.0
1.4	6.1	7.1	6.6	6.2	7.4	6.8
1.8	7.6	8.1	8.3	8.0	8.7	8.5
2.2	9.3	9.1	9.9	9.5	10.1	10.2

This appeared to be the case with both divalent cations tested, as indicated by high correlations among the critical ion concentrations for calcium and magnesium.

Gellan gel structure at junction zones

According to Chandrasekaran et al. (1988b), oxygen atoms in the carboxylate groups of adjacent double helices in a crystal gellan structure point toward each other but are separated by about 0.51 nm. Possibly in gellan gels, one divalent cation binds to two carboxylate groups on adjacent polymer molecules to form an interaction site. A few such similar interactions could constitute a junction zone. At cation concentrations below the critical level, anionic sites on the polymers could accommodate additional cations to increase crosslinks at the junction zones. As a result, both the strength and rigidity of the gel would increase. Maximum strength was reached when all possible crosslinks were built and the maximal number of complete junction zones were formed with just sufficient divalent cations. When excessive cations were present during gelling, they might compete for anionic sites without forming junction zones due to repulsive forces. These forces would increase with greater amounts of excessive cations, thereby reducing the number of crosslinks in the junction zones. Excessive cations might also

alter the conformation of the polymers, which might reduce the strength of gel structure, as demonstrated by lower failure shear stresses at cation concentrations above critical levels (Fig. 2 and 3).

The difference between strength of gels with Ca^{++} and Mg^{++} may be related to the different cation sizes. Ca^{++} ions have a diameter of about 0.099 nm which is about 1.5 times that of Mg^{++} ions (Chang, 1991). Below the critical levels, larger Ca^{++} cations may provide stronger binding forces at the junctions and, therefore, a stronger gel than with Mg^{++} . Above the critical level, larger Ca^{++} may provide stronger repulsive forces which reduce the strength of gels at higher rate by excessive Ca^{++} as compared to Mg^{++} .

CONCLUSIONS

FAILURE STRENGTHS OF GELLAN GELS were characterized by critical divalent cation concentrations. For a given polymer concentration, gellan gels were extensible below the critical cation levels and brittle above those levels. Increasing cation concentrations below the critical levels strengthened the gels, but adding cations above the critical levels weakened the gels. The critical cation concentrations appear to correspond to a state in which the anionic sites of the gellan polymers were occupied by a just sufficient amount of divalent cations. The value of the critical divalent cation level was proportional to polymer concentration, but independent of cation type. Gellan gels formed with Ca^{++} were generally stronger than those with Mg^{++} at or close to the critical cation levels. The influence of Ca^{++} on strength of the gels was also stronger than Mg^{++} . The difference between strengths may be attributable to size differences between Ca^{++} and Mg^{++} in relation to potential binding positions at polymer junction zones.

NOMENCLATURE

F	Force (kN)
L	Original compression specimen length (m)
ΔL	Deformation (m)
N	Number of data points
R	Original compression specimen radius (m)
R^2	Coefficient of multiple determination
R.M.S.	Relative Mean Square Error
r^2	Coefficient of determination

x_c	Ca^{++} concentration (mM)
x_{cr}	Critical cation concentrations (mM)
x_c	Gellan gum concentration (%)
x_m	Mg^{++} concentration (mM)
x'_i and x'_m	Concentration of free divalent cation in the gels (mM)
σ_{max}	True normal stress at failure (kPa)
ϵ_{max}	True normal strain at failure
τ_{max}	Shear stress at failure (kPa)
γ_{max}	Shear strain at failure
ν	Poisson's ratio

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