

Gelling Temperatures of Gellan Solutions as Affected by Citrate Buffers

R. Mao, J. Tang and B.G. Swanson

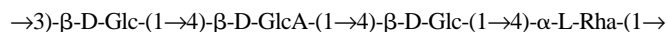
ABSTRACT

Effects of citrate buffers at pH 3.5 and 5.0 on gelling temperatures of gellan solutions with 0.4–1.8% gellan and 1.5–60 mM Ca^{2+} were studied. Partial dissociation of the carboxyl groups in gellan polymer in pH 3.5 solutions resulted in weakened gels. The pH 3.5 buffer exhibited weak chelating ability for Ca^{2+} . The gelling temperature of gellan solutions at pH 3.5 was quantitatively related to polymer and cation concentrations using a similar model to that for gellan water solutions. The pH 5.0 buffer exhibited strong chelating ability. Gelling temperatures at pH 5.0 were generally lower than those at pH 3.5, except at low calcium concentrations.

Key Words: gellan, gelling temperature, citrate buffer, cation concentration

INTRODUCTION

GELLAN GUM IS AN EXCELLENT POLYSACCHARIDE GELLING AGENT approved by the FDA for food use (Pszczola, 1993). Gellan is an anionic polysaccharide with repeating unit:



When gellan solutions are cooled from elevated temperatures, the carboxylate group of the glucuronic acid (GlcA) reacts with cations to form cross-links between the linear gellan polymer chains, resulting in three dimensional gel networks.

Gelling temperatures (T_{gel}) of gellan solutions between 20°C and 75°C were reported at selected polymer concentrations and cation concentrations (Sanderson, 1990; Moritaka et al., 1992; Nakamura et al., 1993; Tanaka et al., 1993). Tang et al. (1997a) developed a quantitative model to relate T_{gel} of gellan solutions to polymer and cation concentrations:

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

where, T_{gel} is absolute temperature (K), $[X_p]$ is polymer concentration (g/mL), $[X_i]$ is cation concentration (mM), and

$$A = [(n - l)R]/\Delta H \quad (2)$$

$$B = mR/\Delta H \quad (3)$$

where R is the gas constant, and ΔH is the enthalpy change of gelation reaction:

$$nX_l + mX_i = lJ \quad (4)$$

where J , X_i and X_l represent molar concentrations of cross-links, cation, and free cross-linking loci on the polymer chains, respectively. With regard to cross-linking mechanism, Morris (1979) pointed out

Authors Mao and Tang are with the Dept. of Biological Systems Engineering, Washington State Univ., Pullman, WA 99164-6120. Author Swanson is with the Dept. of Food Science & Human Nutrition, Washington State Univ., Pullman, WA 99164-6376. Address inquiries to Dr. Juming Tang.

that the traditional concept of polysaccharide gel network involving 'point cross-linking' of disordered chains should be superseded by a 'junction-zone' model. Therefore, J in Eq (4) represents molar concentration of cross-linking regions. The relationship expressed in Eq (1) describes well the gelling temperature of gellan solutions prepared in distilled water without the addition of acid and buffer systems (Tang et al., 1997a, b).

Research on the gelling temperatures of gellan solutions has mainly focused on solutions at neutral or "as is" pH, i.e., the deionized distilled water was used as solvent without pH adjustment. Many food gels are, however, prepared at acid pH. Matsuhashi (1990) reported that reducing the pH decreased the gelling ability of agents such as agar. Information on the effects of pH on the gelling temperature of gellan solutions is scarce. Moritaka et al. (1995) reported that the exothermic enthalpy of gellan solutions during gelation decreased and the gelling temperature decreased as the pH was reduced from neutral to 4.0. Camelin et al. (1993) reported that the gellan gel was weakened at pH 4.0 compared to neutral pH. In those studies, the pH was adjusted with a strong acid (HCl) or base (NaOH).

Citric acid is a natural constituent and common metabolite of plants and animals (Bouchard et al., 1979). It is an organic acid widely used in the food industry accounting for more than 60% of acidulants consumed (Dziezak, 1993). Citric acid is often added to soft drinks and desserts to complement fruit flavors. It also contributes to tartness, chelates metal ions, acts as a preservative or controls pH so that a desired sweetness can be achieved (Dziezak, 1993). Data from experiments in which citrate buffer controls pH of food gels instead of HCl or NaOH should be more applicable to the food industry. Chelating ability of the citrate group for divalent cations would, however, complicate the effects with citrate buffer.

The objectives of this study were to investigate the effects of citrate buffers on gelling temperature of gellan solutions and to develop quantitative relationships between gelling temperatures with polymer and cation concentrations at low pH in order to provide information for preparing low pH gellan gel based desserts. The pH of many fruit juices is buffered near 3.5 to provide a balanced condition for protection against contaminating microorganisms, for stability of the sweetener aspartame, and to afford some dental protection against very low pH (<2.0) erosion effects (Matthews, 1993; Batchelor, 1993). The "as is" pH of a typical gellan solution containing a commonly used calcium ion concentration (1.5–60 mM) is about 5.0. Therefore, in this research pH 3.5 and pH 5.0 citrate buffers were selected for studying the effects of pH on gelling temperatures of gellan solutions.

MATERIALS & METHODS

Preparation of 0.05M citrate buffers

A pH 3.5 buffer was prepared by mixing 370 mL 0.1 M citric acid with 130 mL 0.1 M sodium citrate and 500 mL water. The solution was adjusted to pH 3.50 with a small amount of 0.1 M sodium citrate and the pH was measured with an AP5 pH Meter (Fisher Scientific, Pittsburgh, PA). This buffer contained 0.05 M total citrate groups and 39 mM sodium ions. A pH 5.0 buffer was similarly prepared by adding 170 mL of 0.1 M citric acid and 330 mL of 0.1 M sodium citrate to 500 mL water. This buffer had 0.05 M total citrate groups, and a sodium ion concentration of 99 mM.

Gelling temperature

Deacylated gellan gum powder (KELCOGEL F) was provided by NutraSweet Kelco Company (San Diego, CA). Gellan powder at five selected gum concentrations (0.4–1.8% w/v) was dispersed in citrate buffers in a 400 mL beaker with a magnetic stirrer. The mixtures were heated to 96–98°C, and the temperature was maintained for 1 min to produce a clear solution. Calcium chloride was added to the hot gellan solutions to prepare dispersions containing selected calcium concentrations between 1.5 mM and 60 mM. In measuring gelling temperatures, a pre-calibrated fine gauge type T thermocouple was inserted into a hot gellan solution, and the temperature was read with a Digi-Sense Thermocouple Thermometer (Model 91100-20, Cole-Parmer Instrument Co., Vernon Hills, IL). The solution was cooled to room temperature with constant stirring. When close to gelation, a small piece of gel was observed on the thermocouple wire just above the solution surface. At that time, the stirring speed was reduced to maintain a flat liquid surface. The sol-gel transition was indicated by a sharp increase in turbidity in the bulk liquid. The liquid quickly turned into a gel. The measured solution temperature at the start of the sol-gel transition was noted as gelling temperature. The measurement was repeated three times.

The reliability of the above method was checked against more elaborate methods reported by Tang et al. (1997a), using 1% gellan solutions containing 8 Ca²⁺ concentrations and prepared in deionized distilled water (without buffer). The measured gelling temperatures compared well with reported results (Table 1). There was no difference between gelling temperatures measured by the different methods ($P < 0.05$).

Preparation of gellan gels for compression test

Gellan solutions were prepared following the described procedures. After adding calcium chloride into the boiling gellan solutions, they were continuously stirred for 2 to 3 min and poured into metal tubes (i.d. 21 mm). These tubes were preheated to 80°C to prevent partial gelling on the metal tube wall during pouring. The gels were set by submerging the tubes in running tap water at 15°C for 15 min. The gels were held for 24 h at room temperature (2°C) before being removed from the tubes and sliced to make specimens 21 mm long for compression tests.

Compression test

Cylindrical test specimens of 21 mm dia and 21 mm l were compressed at air conditioned room temperature (22°C) between lubricated flat metal surfaces on The TA.XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY/Stable Micro Systems, Godalming, Surrey, UK). The texture analyzer was interfaced with Texture Expert software running in Windows 95. Tests were replicated six times. The pre-test speed was set at 2.0 mm/s. The gels were deformed at a constant crosshead speed of 0.3 mm/s until failure. True failure shear stress (Tang et al. 1994), which takes into account enlarged cross-sectional areas of the gels during compression, was used to represent the gel strength.

Statistical analyses

The ANOVA procedure of Microsoft Excel (Microsoft Corp., 1995) was used to determine the difference between gelling temperatures measured by different methods (Table 1), or affected by different pH buffers. The 3D-plane regression procedure of SigmaPlot 4.0 (SPSS Inc., 1997) was used to fit the gelling temperature to Eq (1) and to calculate the standard errors. A significance level of $P < 0.05$ was chosen for all statistical analyses.

RESULTS & DISCUSSION

Gelling properties in pH 3.5 citrate buffer

The mean gelling temperatures of gellan in pH 3.5 buffer systems increased with calcium and polymer concentrations (Table 2). The quantitative relationship of gelling temperatures of gellan solutions in

Table 1—Gelling temperatures of 1% gellan solutions, prepared with deionized distilled water (without buffer) with added calcium measured by three methods

Test methods	Added Ca ²⁺ (mM)							
	2	4	6	8	14	20	30	40
This work	35.3	39.8	43.6	46.6	52.8	57.2	62.8	64.0
DRT ^a	35.0	40.0	44.0	46.5	53.5	58.0	64.0	65.5
DVO ^b	35.0	39.5	43.0	46.0	52.0	56.0	62.0	63.0

^aDynamic rheological testing, data extracted from Tang et al., 1997a.

^bDirect visual observation, data extracted from Tang et al., 1997a.

Table 2—Mean gelling temperatures(°C) of gellan solutions prepared with pH 3.5 citric buffer system^a

Gellan (%w/v)	Added Ca ²⁺ (mM)						
	1.5	3.0	6.0	10.0	24.0	42.0	60.0
0.4	29.5 (0.7)	32.3 (0.4)	34.9 (0.5)	38.9 (0.7)	46.7 (0.7)	51.8 (0.5)	54.8 (0.6)
0.6	33.0 (0.2)	35.1 (1.0)	39.6 (0.7)	45.5 (0.5)	51.5 (1.6)	56.4 (0.8)	59.9 (0.4)
1.0	38.0 (0.5)	40.3 (1.5)	45.5 (1.1)	49.4 (0.4)	57.4 (1.9)	62.1 (1.4)	66.2 (1.2)
1.4	42.0 (0.4)	45.0 (0.2)	48.2 (0.3)	54.0 (0.9)	59.5 (1.3)	65.6 (0.8)	70.5 (1.6)
1.8	44.8 (0.5)	48.6 (0.3)	54.8 (0.6)	59.6 (0.4)	66.1 (1.2)	70.5 (2.3)	72.6 (2.4)

^a() Standard deviation.

the pH 3.5 buffer system with calcium and polymer concentrations was determined by fitting Eq (1) to the experimental data.

For a mixture of cations present in a gellan solution, Tang et al. (1997b) reported that in terms of the effect on gelling temperature, a mole of Mg²⁺ was equivalent to a mole of Ca²⁺, while a mole of Na⁺, or of K⁺, was equivalent to 0.0721 mole or 0.111 mole of Ca²⁺, respectively. According to manufacturer data, the gellan powder contained 85% polysaccharide, 0.4% Ca, 0.1% Mg, 4.9% K, and 0.3% Na. The pH 3.5 citrate buffer contained 39 mM Na⁺. All cations were converted to yield a total equivalent Ca²⁺ concentration $[X_i]$. $1/T_{gel}$ was plotted against $\ln[X_i]$, and a linear relationship was obtained for each gellan concentration (Fig. 1). Eq (1) was fitted to the experimental data to obtain the following coefficients:

$$\begin{aligned} A &= -1.03 \pm 0.04 \times 10^{-4} \text{ K}^{-1} \\ B &= -(1.06 \pm 0.02) \times 10^{-4} \text{ K}^{-1} \\ C &= (2.89 \pm 0.02) \times 10^{-3} \text{ K}^{-1} \end{aligned} \quad (5)$$

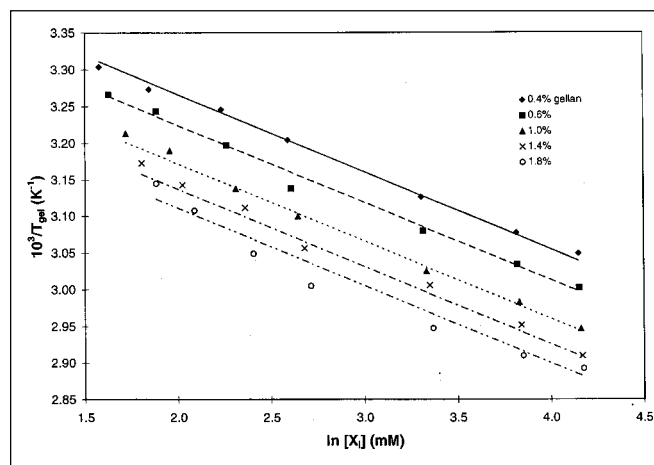


Fig. 1—Relationship between gelling temperatures of gellan solutions, prepared with pH 3.5 citrate buffer, and ion concentration. The scattered data are means calculated from the measured temperatures, and continuous curves were obtained from Eq (6).

Thus with 0.05 M pH 3.5 citrate buffer, the following relationship was obtained:

$$1/T_{\text{gel}} = -1.03 \times 10^{-4} \ln[X_p] - 1.06 \times 10^{-4} \ln[X_i] + 2.89 \times 10^{-3} \quad (6)$$

The R^2 for this equation was 0.99 (Fig. 2).

Tang et al. (1997a) obtained the values for A, B, and C in Eq (1) for gellan solutions prepared with deionized distilled water:

$$\begin{aligned} A &= -5.82 \times 10^{-5} \text{ K}^{-1} \\ B &= -1.01 \times 10^{-4} \text{ K}^{-1} \\ C &= 3.33 \times 10^{-3} \text{ K}^{-1} \end{aligned} \quad (7)$$

The value of B was very similar for both gels [Eq (5) and (7)], while the value of A for gellan solutions in a pH 3.5 citrate buffer system was about 2× the value of A for gellan solutions without addition of acid or buffer solutions. Chandrasekaran et al. (1988) and Chandrasekaran and Thailambal (1990) indicated that cross-links in gellan gels were formed as associations of two pairs of double stranded polymer chains. Thus, the stoichiometric coefficients l and n in reaction Eq (4) were 1 and 4, respectively. Based on Eq (2), the ΔH for reaction (4) could be calculated as,

$$\begin{aligned} \Delta H &= [(n - l)R]/A = [(4-1) \times 8.314]/(-1.03 \times 10^{-4}) \\ &= -2.42 \times 10^{-5} (\text{J/mol}) = -242 (\text{kJ/mol}) \end{aligned} \quad (8)$$

Comparing it with the $\Delta H = -426.8 \text{ kJ/mol}$ reported by Tang et al. (1997a) for gels prepared without adjustment of pH, the gelling ener-

gy in pH 3.5 citrate buffer was about half that without buffer. The gels formed in pH 3.5 citrate buffer were, therefore, weaker than those without buffer. This was supported by the results of compression tests. The effect of calcium and gellan concentrations on the strength of gels was compared (Fig. 3) as represented by failure shear stress. Comparing with a similar plot of gellan gels formed in water (Fig. 4, from Tang et al., 1994), failure shear stresses of the buffered gels were lower than those without buffer except at very low calcium concentrations. At those levels, the sodium ions provided by the buffer might have strengthened the gel. Observations of lower failure shear stress for the buffered gels were consistent with their lower gelling energy than gels formed in water.

The reason for reduced gelling ability and weakened gel formation by buffered gellan systems at pH 3.5 could be explained by the reduced percent of disassociated carboxyl groups of gellan polymers. The pK_a of glucuronic acid in gellan polymer chains is ≈ 3.5 (NutraSweet Kelco Company, 1996). The mole percent of the protonated (COOH) and ionized (COO⁻) species of glucuronic acid would change with pH (Fig. 5) according to the following relation:

$$pH = pK_a + \log ([\text{COO}^-]/[\text{COOH}]) \quad (9)$$

It is possible that at pH of 7.0, almost all glucuronic acid was ionized. When the pH was reduced to 5.0, 97% of the glucuronic acid was ionized. At pH 3.5, however, only 50% of the glucuronic acid was ionized, and the other 50% was in the protonated state. Only the ionized species (COO⁻) in the gellan chains formed ionic bonds with cations, which might account for the reduced gelling energy (ΔH) compared with that at "as is" pH, i.e. the gels prepared without buffer.

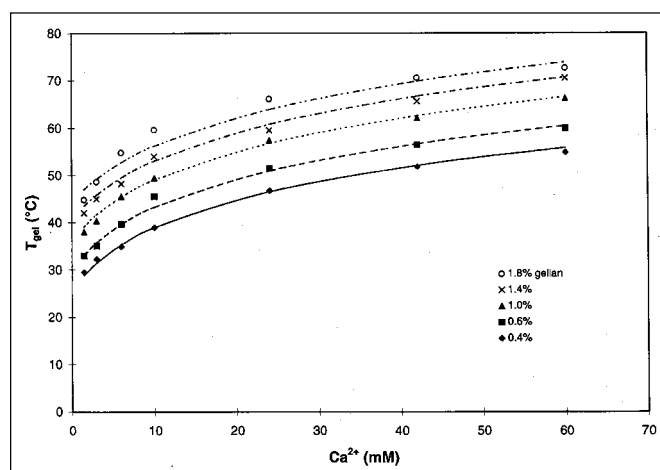


Fig. 2—Gelling temperatures of gellan solutions prepared in pH 3.5 citrate buffer as described by Eq (6) (curves), compared with experimental data (scattered points).

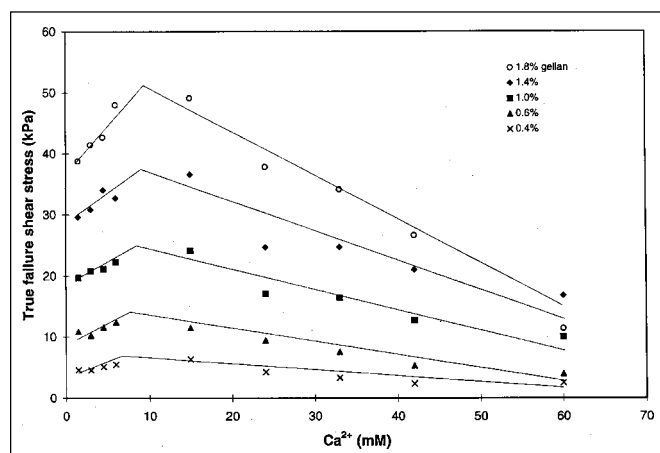


Fig. 3—True failure shear stress of gellan gels made from pH 3.5 citrate buffer as affected by polymer and calcium concentrations.

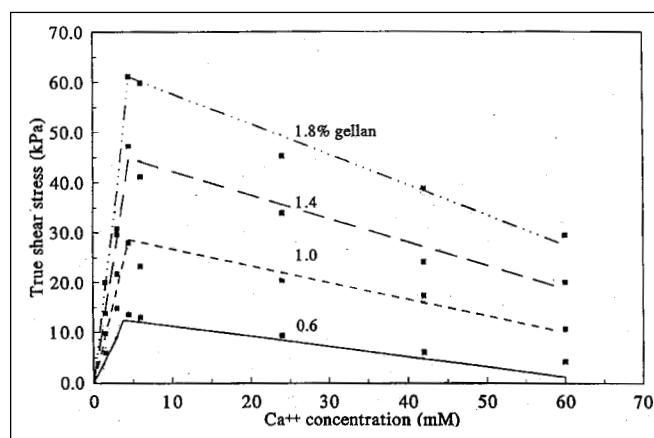


Fig. 4—True failure shear stress of gellan gels made from deionized distilled water in compression tests deformed at a constant cross-head speed of 20 mm/min. (Reproduced from Tang et al., 1994).

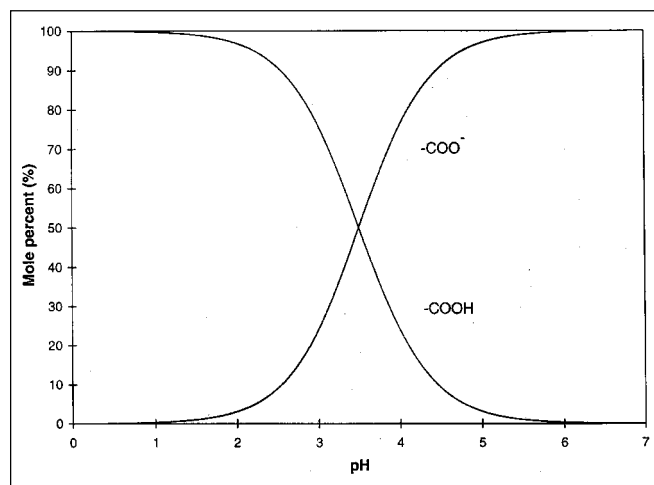


Fig. 5—Mole percent of -COO⁻ and -COOH in gellan chain at different pH as calculated by Eq. (9).

Based on Eq (2) and (3), the stoichiometric coefficient m in reaction Eq (4) was calculated as,

$$m = [B(n-1)]/A = [-1.06 \times 10^{-4} \times (4-1)]/(-1.03 \times 10^{-4}) \approx 3 \quad (10)$$

That is, 3 Ca^{2+} ions were used with four strands of gellan polymer chains to form one cross-linking region at the gelling point. This result could be used in conjunction with observations from earlier x-ray studies to estimate the size of the cross-linking region at the gelling point. According to the x-ray results of Chandrasekaran and Thailambal (1990), gellan polymer chains form a left-handed, three-fold double helix with a 5.63 nm pitch. A gellan chain within a pitch has three repeating tetrasaccharide units and, therefore, three carboxyl groups. Four strands of gellan chains in one pitch would have a total of 12 carboxyl groups. As mentioned, only six carboxyl groups were, however, in the ionized state at pH 3.5 to contribute six negative charges. Three Ca^{2+} would be needed to neutralize those six carboxylate groups in each pitch. Based on our calculation in Eq (10), three divalent cations were used for each cross-linking region. We may, therefore, conclude that, at the gelling point, the length of the cross-linking region along the strand of calcium gellan was exactly the length of one pitch (5.63 nm).

An average gellan consists of about 800 tetrasaccharide repeat units. At the gelling point, only a few of these units would be required for forming cross-links in a continuous three-dimensional network. As gelation continues, cross-link density may increase and, most likely, the cross-linking regions would propagate to form more rigid gels as indicated by a sharp increase in G' and light absorbance (Tang et al., 1997a). The crosslinking region may grow from one pitch to much longer length. Nakajima et al. (1996) reported, by scanning tunneling microscopy, that the average strand length of calcium gellan gel after full gelation was 70 nm.

Gelling properties in pH 5.0 citrate buffer

The pH of gellan solutions prepared in deionized distilled water is around 5. To study the effect of the buffer system on gelling temperatures of gellan solutions, the gelling temperatures of gellan solutions prepared at various polymer and calcium concentrations in pH 5.0 citrate buffer were measured (Table 3). Comparing with other results (Table 2) showed that in the low range of $[\text{Ca}^{2+}]$, the T_{gel} at pH 5.0 was higher than that at pH 3.5, but in the high range of $[\text{Ca}^{2+}]$, the T_{gel} at pH 5.0 was lower than that at pH 3.5. Therefore, the effect of calcium ion on T_{gel} at pH 5.0 was less than at pH 3.5. A plot of $1/T_{\text{gel}}$ vs. $\ln[X_i]$ for experimental data at pH 5.0 was developed, but no clear linear relationship was observed. Instead, a linear relationship was observed in a plot of T_{gel} vs $[\text{Ca}^{2+}]$ for each polymer concentration (Fig. 6).

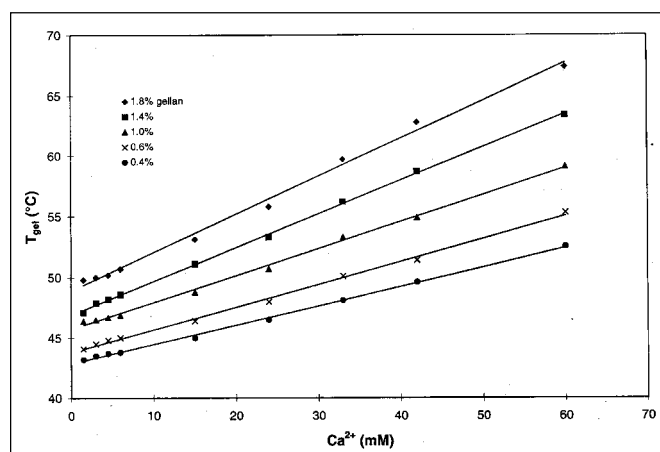


Fig. 6—Gelling temperature of gellan in pH 5.0 citrate buffer as affected by polymer and calcium concentrations.

Table 3—Mean gelling temperatures ($^{\circ}\text{C}$) of gellan solutions prepared with pH 5.0 buffer system^a

Gellan (%w/v)	Added Ca^{2+} (mM)								
	1.5	3.0	4.5	6.0	15.0	24.0	33.0	42.0	60.0
0.4	43.2 (0.1)	43.5 (0.1)	43.7 (0.2)	43.8 (0.1)	45.0 (0.4)	46.5 (0.2)	48.1 (0.5)	49.6 (0.7)	52.5 (1.1)
0.6	44.1 (0.1)	44.5 (0.3)	44.8 (0.2)	45.0 (0.1)	46.4 (0.7)	48.0 (0.6)	50.1 (0.6)	51.4 (0.6)	55.3 (0.9)
1.0	46.4 (0.1)	46.5 (0.1)	46.7 (0.1)	46.9 (0.1)	48.8 (0.3)	50.7 (0.3)	53.3 (1.1)	54.9 (0.5)	59.1 (1.1)
1.4	47.1 (0.2)	47.9 (0.3)	48.2 (0.3)	48.6 (0.3)	51.1 (0.4)	53.3 (1.4)	56.2 (0.6)	58.7 (0.8)	63.4 (1.6)
1.8	49.8 (0.2)	50.0 (0.2)	50.2 (0.3)	50.7 (0.3)	53.1 (0.5)	55.8 (0.7)	59.7 (1.2)	62.6 (1.3)	67.4 (1.4)

^a() Standard deviation.

Chelating ability of citrate buffers at pH 3.5 and 5.0

Citrate compounds chelate dissolved calcium ions in aqueous gellan solutions (NutraSweet Kelco Company, 1996). The chelating reactions require the fully deprotonated species (Cit^{3-}) or the mono-protonated species (HCit^{2-}) as ligands. The mole percent of each species of a polybasic acid at any pH can be calculated from pK_a values. For citric acid, the $\text{pK}_1=3.128$, $\text{pK}_2=4.761$, and $\text{pK}_3=6.396$ (Bouchard and Merritt, 1979). Based on those values, the mole percent of various protonated species of citrate as functions of pH were calculated and plotted (Fig. 7). At pH 5.0, > 60% of the total citrate groups were present as HCit^{2-} or Cit^{3-} capable of chelating cations (Fig. 7). At pH 3.5, however, > 95% of the total citrate groups were H_3Cit or H_2Cit^- with little chelating effect. This might have resulted in the observed different behaviors of T_{gel} as affected by $[\text{Ca}^{2+}]$ at pH 3.5 and 5.0. Since the pH 5.0 buffer had a very limited chelating effect, the $T_{\text{gel}}-[\text{Ca}^{2+}]$ relationship for gellan solutions was similar to that without buffer (Tang et al., 1997a) and could be described by Eq (1). The pH 5.0 buffer exhibited a strong chelating ability that removed calcium ions from the solution. Thus, with pH 5.0 citrate buffer, the $T_{\text{gel}}-[\text{Ca}^{2+}]$ relationship was quite different from results without buffer and the T_{gel} in the buffered systems were usually lower. Since the pH 5.0 citrate buffer contained much higher $[\text{Na}^+]$ (99mM) than the pH 3.5 buffer (39mM), we hypothesized that at low $[\text{Ca}^{2+}]$ range the T_{gel} at pH 5.0 would be higher than at pH 3.5.

CONCLUSIONS

CITRATE BUFFER GENERALLY DECREASED THE GELLING ABILITY of gellan solutions and lowered gelling temperature. Citrate buffer at pH 3.5 also weakened the gellan gel strength. The mechanism for reducing gelling ability might be different between the pH 3.5 and 5.0 buffer systems. At pH 3.5, the chelating ability of citrate ion was not important. But only half of the carboxyl groups in gellan polymer

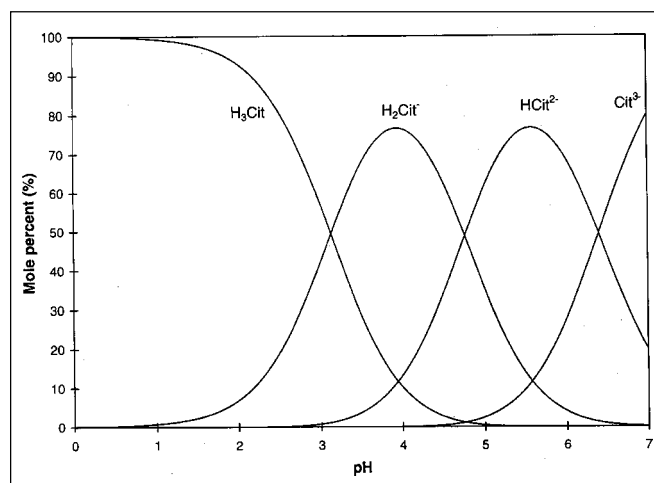


Fig. 7—Mole percent of different ionized species of the citrate group.

chains were in the ionized state at that pH to form fewer ionic bonds with divalent cations. At pH 5.0, the main factor in reducing the gelling temperature of gellan solutions was the chelating ability of the citrate ions. Because of the insignificant effects of chelating ability of citrate at pH 3.5, changes in gelling temperature as affected by polymer and cation concentrations followed a pattern similar to gellan solutions prepared without addition of buffer or acid. This pattern was described by a model in our earlier study. Coefficients of the model were used to predict gelling temperatures and to estimate gelling energy and size of the cross-linking region at the beginning of the gelling process.

REFERENCES

- Batchelor, V.J. 1993. Soft drinks, microbiology. In *Encyclopaedia of Food Science Food Technology and Nutrition*, R. Macrae, R.K. Robinson, and M.J. Sadler (Ed.), Vol.6, p.4200-4206. Academic Press, London.
- Bouchard, E.F. and Merritt, E.G. 1979. Citric acid. In *Encyclopedia of Chemical Technology*, 3rd ed., H.F. Mark, M. Grayson, and D. Eckroth (Ed.), Vol.6, p.150-179. John Wiley & Sons, New York.
- Camelin, I., Lacroix, C., Paquin, C., Prevost, H., Cachon, R., and Divies, C. 1993. Effect of chelators on gellan gel rheological properties and setting temperature for immobilization of living bifidobacteria. *Biotechnol. Prog.* 9: 291-297.
- Chandrasekaran, R. and Thailambal, V.G. 1990. The influence of calcium ions, acetate and L-glycerate groups on the gellan double helix. *Carbohydr. Polym.* 12: 431-442.
- Chandrasekaran, R., Puigjaner, L.C., Joyce, K.L., and Arnott, S. 1988. Cation interactions in gellan: an X-ray study of the potassium salt. *Carbohydrate Research* 181: 23-40.
- Dziezak, J.D. 1993. Acids, natural acids and acidulant. In *Encyclopaedia of Food Science Food Technology and Nutrition*, R. Macrae, R.K. Robinson, and M.J. Sadler (Ed.), Vol.1, p.10-16. Academic Press, London.
- Matsushashi, T. 1990. Agar. In *Food Gels*, P. Harries (Ed.) p.1-52. Elsevier, New York.
- Matthews, C. 1993. Soft drinks, chemical composition. In *Encyclopaedia of Food Science Food Technology and Nutrition*, R. Macrae, R.K. Robinson, and M.J. Sadler (Ed.), Vol.6, p.4189-4194. Academic Press, London.
- Microsoft Corporation 1995. Microsoft Excel for Windows 95, Version 7.0a.
- Morris, E.R. 1979. Polysaccharide structure and conformation in solutions and gels. In *Polysaccharides in Food*, J.M.V. Blanshard and J.R. Mitchell (Ed.), p.15-31. Butterworths, London.
- Moritaka, H., Nishinari, K., Nakahama, N., and Fukuba, H. 1992. Effect of potassium chloride and sodium chloride on the thermal properties of gellan gum gels. *Biosci. Biotechnol. Biochem.* 56(4): 595-599.
- Nakajima, K., Ikehara, T., and Nishi, T. 1996. Observation of gellan gum by scanning tunneling microscopy. *Carbohydr. Polym.* 30: 77-81.
- Nakamura, K., Harada, K., and Tanaka, Y. 1993. Viscoelastic properties of aqueous gellan solutions: effect of concentration on gelation. *Food Hydrocolloids* 7(5): 435-447.
- NutraSweet Kelco Company 1996. *Gellan Gum*, 3rd ed. NutraSweet Kelco Company, San Diego, CA.
- Pszczola, D.E. 1993. Gellan gum wins IFT's food technology industrial achievement award. *Food Technol.* 47(9): 94-96.
- Sanderson, G.R. 1990. Gellan Gum. In *Food Gels*, P. Harries (Ed.), p.201-232. Elsevier, New York.
- SPSS Inc., 1997. *Transforms & Regressions Reference Manual (SigmaPlot 4.0 for Windows 95, NT & 3.1)*. SPSS Inc., Chicago, IL.
- Tanaka, Y., Sakurai, M., and Nakamura, K. 1993. Ultrasonic velocities in aqueous solutions. *Food Hydrocolloids* 7(5): 407-415.
- Tang, J., Lelievre, J., Tung, M.A., and Zeng, Y. 1994. Polymer and ion concentration effects on gellan gel strength and strain. *J. Food Sci.* 59(1): 216-220.
- Tang, J., Tung, M.A., and Zeng, Y. 1997a. Gelling temperature of gellan solutions containing calcium ions. *J. Food. Sci.* 62(2): 276-280.
- Tang, J., Tung, M.A., and Zeng, Y. 1997b. Gelling properties of gellan solutions containing monovalent and divalent cations. *J. Food. Sci.* 62(4): 688-692.

Ms received 10/13/98; revised 1/26/99; accepted 3/10/99.

These studies were supported by Grant 97-35503-4304 from the USDA NRI Competitive Grant Program. We thank NutraSweet Kelco Company for providing the gellan gum.