EFFECT OF pH BUFFERS ON MECHANICAL PROPERTIES OF GELLAN GELS

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ABSTRACT

The strength and deformability of calcium cross-linked gellan gels as affected by pH 3.5 and 5.0 citrate and acetate buffers were measured by large compressive deformation test until failure. The trend of dependence of gel strength on polymer and calcium concentrations was similar to gels formed in distilled water without pH adjustment. A critical calcium concentration was observed for each gellan concentration. Gels formed at the critical calcium concentration exhibited the maximum strength. The chelating effect of pH 5.0 citrate buffer greatly increased the critical calcium concentration. The failure strain, representing the deformability, of gellan gels formed in buffers behaved differently from gels formed in distilled water. In the pH 3.5 buffer systems, gellan gels were brittle regardless of gellan and calcium concentrations. In the pH 5.0 buffer systems, gellan gels were brittle at high calcium concentrations and ductile at calcium concentrations less than 24 mM in citric buffer and less than 6 mM in acetate buffer.

INTRODUCTION

Gellan gum is a gel forming polysaccharide produced by the microbe *Sphingomonas elodea* (formerly *Pseudomonas elodea*) (Kang *et al*. 1982) and approved by the U.S. Food and Drug Administration in 1992 for food use

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(Pszczola 1993). The monosaccharide units are β-D-glucose, β-D-glucuronic acid and α-L-rhamnose in molar ratios of 2:1:1 (Sanderson 1990). The carboxyl groups of glucuronic acid react with cations in solutions to form crosslinks between the linear gellan polymer chains, resulting in three dimensional networks.

Mechanical properties of hydrogels are of practical interest because of the widespread use of gels in the food industry. Important mechanical properties include failure stress (related to gel strength) and failure strain (related to the deformability) of gel systems (Hamann 1983; Foegeding 1992; Tang et al. 1994). Large failure stress indicates a strong gel and high failure strain indicates a very deformable gel. Wood (1979) pointed out that it was the failure property, rather than the apparent modulus measured at small deformation, that correlated with the gel strength assessed in the mouth.

Tang et al. (1994) observed that the true failure shear stresses measured in compression, torsion and tension test modes were the same and the true failure shear strain by tensile testing was about half that measured by the other two modes. In all three modes, a unique pattern of mechanical responses was observed for gellan gels. That is, at a critical calcium concentration the gel had the maximum strength and below or above the critical concentration the gel strength decreased linearly with calcium concentration for each polymer concentration. The gels were soft and deformable below this critical calcium concentration, but brittle above it. This behavior was also observed in magnesium crosslinked gellan gels (Tang et al. 1995).

The published literature on the mechanical properties of gellan gels is mainly focused on gels formed at neutral or "as is" pH, i.e., the deionized distilled water was used as solvent and no pH adjustment of gelling solutions (Moritaka et al. 1991; Tang et al. 1994, 1995, 1996). Limited information on the effect of pH on the mechanical properties of gellan gels is reported in the literature. Moritaka et al. (1995) reported that when the pH of gellan solutions was reduced from neutral to 4.0, the gel strength decreased for reheated gels (gels kept at 10C overnight then reheated at 90C for 2 h), but increased for nonreheated gels. At pH 4.0, gellan gels were less rigid compared to neutral gels (Camelin et al. 1993). Sanderson (1990) reported that gellan gels were slightly weaker and more brittle at pH 4.0 than "as is" pH. In those studies, Sanderson (1990) used citric acid to control the pH, while others used a strong acid (HCl) or base (NaOH) to adjust the pH, and only a few gellan-cation combinations were examined.

Citric acid is a natural constituent and common metabolite of plants and animals. It is the most widely used organic acid in foods and pharmaceuticals and also used in applications to sequester ions and act as a buffer (Bouchard and Merritt 1979). Citric acid accounts for more than 60% of acidulants consumed in the food industry (Dziezak 1993). It is, therefore, desirable to study the effect

of a citrate buffer system on gel properties. Introducing citrate buffers into a gelling system, however, will inevitably make the system more complicated because of chelating ability of citrate groups. Preliminary tests in our laboratory indicated that in some gellan calcium combinations, reducing pH with citrate buffer could strengthen the gels, while with other gellan calcium combinations, weakened gels were observed. Thus, a detailed systematic study was required to investigate the comprehensive effect of citrate buffer.

Our objective was to investigate the effect of pH and chelation of citrate buffers on the failure properties of gellan gels to control the texture of gellan gels in acid food systems such as fruit juices. The pH of many fruit juices is buffered near 3.5 to provide balanced conditions for protection against contaminating microorganisms, for maximum stability of the intense 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 calcium ion concentration from 1.5 mM to 60 mM, commonly studied in the literature, is around 5.0. Therefore, pH 3.5 and pH 5.0 citrate buffers were considered in this study. To separate the effect of pH from possible chelating effects of the buffer system, citrate and acetate buffers with same pHs were used as the medium to make gels. Citrate buffer and acetate buffer represent two typical pH buffers widely used in the food industry, with the former able to chelate cations while the latter cannot.

MATERIALS AND METHODS

Preparation of pH Buffers

Two citrate and acetate buffers were prepared: 0.05M citrate buffers and 0.1M acetate buffers at pH 3.5 and 5.0 (Table 1). The concentrations of citrate group, acetate group and sodium ion in the buffers were also shown in Table 1.

TABLE 1.
PREPARATION OF pH BUFFERS AND THEIR COMPONENTS

Buffers	Prepared from			Buffer containing	
	acid[a] (vol%)	salt[b] (vol%)	water (vol%)	citrate or acetate(mM)	[Na$^+$] (mM)
Citrate 0.05M pH 3.5	37	13	50	50	39
0.05M pH 5.0	17	33	50	50	99
Acetate 0.1M pH 3.5	47	3	50	100	6
0.1M pH 5.0	15	35	50	100	70

[a] 0.1M citric acid for citrate buffers, or 0.2M acetic acid for acetate buffers.
[b] 0.1M sodium citrate for citrate buffers, or 0.2M sodium acetate for acetate buffers.

Preparation of Gellan Gels

Low acyl gellan gum powder (KELCOGEL F) was provided by NutraSweet Kelco Company, San Diego, CA who provided the following composition data: carbohydrate (dietary fiber), 85%; water, 10%; Na, 0.3%, Ca, 0.4%; Mg, 0.1%; K, 4.9%; and P, 0.2%. Gellan powder at selected polymer concentrations (0.4-1.8% w/v) was dispersed in buffered solutions in a 400 mL beaker with a magnetic stirrer. The mixtures were heated to 96-98C and held at the same temperature for 1 min to give clear solutions. Calcium chloride at eight or nine selected concentrations between 1.5 and 60 mM was added to the hot gellan solutions. The solutions were stirred for 2 to 3 min and poured into stainless steel tubes (inner diameter 21 mm) preheated to 80C. The gels were set by cooling the tubes in running tap water at 15C for 15 to 30 min, held for about 24 h at room temperature (22C) before being removed from the tubes for compression tests.

Compression Tests

Cylindrical test specimens 21 mm in diameter and 21 mm long were compressed between lubricated flat metal surfaces fitted to The TA.XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY/Stable Micro Systems, Godalming, Surrey, UK) with a 5 kg load cell and interfaced with Texture Expert software. The gels were deformed at a constant crosshead speed of 0.3 mm/s until failure. All tests were repeated six times. The engineering stress (σ_{eng} = force/original cross-section area) and strain (ε_{eng} = reduction of length/original length) at failure were obtained from the texture analyzer. True normal strain (ε_t) and stress (σ_t) were obtained by following equations (Tang et al. 1996):

$$\varepsilon_t = -\ln(1 - \varepsilon_{eng}) \tag{1}$$

$$\sigma_t = \sigma_{eng}(1 - \varepsilon_{eng}) \tag{2}$$

The true failure shear strain (γ) and shear stress (σ) were obtained from (Hamann 1983):

$$\gamma = (1 + v)\varepsilon_t \tag{3}$$

$$\sigma = \frac{\sigma_t}{2} \tag{4}$$

A large true failure shear strain γ corresponds to a ductile gel and a large true failure shear stress σ indicates a strong gel.

Gellan gels contain about 99% water and can be reasonably assumed to be incompressible under above test conditions, which means that the Poisson's ratio (ν) in Eq. 3 is 0.5 (Juvinall 1967).

Statistical Analyses

The nonlinear regression procedure of SigmaPlot 4.0 (SPSS Inc. 1997) was used to obtain quantitative relationships between true failure shear stress and gellan polymer and calcium concentrations. A significance level of $P < 0.05$ was chosen. The reproducibility of compression tests for gels made with small amounts of added calcium was generally much better than that with large amounts of added calcium. The standard deviations from six replicates were typically 2-3% for the former and 8-10% for the latter. The means of six replicates were used in the figures and regression analyses.

RESULTS AND DISCUSSION

The Failure Properties of Gels Formed in Citrate Buffers

The effects of calcium concentration on the true failure shear stress at different gellan polymer concentrations are shown in Fig. 1 and 2 for gels formed in pH 3.5 and pH 5.0 citrate buffers, respectively. At both pHs, the gel strength increased linearly with added calcium up to a certain calcium concentration, then decreased linearly with further added calcium. At any calcium concentration, the gel strength increased with increasing polymer concentration. This pattern of change is similar to that observed with gellan gels formed in deionized water (Tang *et al.* 1994, 1995).

The data points for each gellan concentration were separated into two groups, one below an estimated critical calcium concentration (X_{cr}') that corresponds to the maximum failure stress for that gellan concentration and the other above the X_{cr}'. The relationship obtained for gels formed in pH 3.5 citrate buffer is:

$$\sigma = -7.11 + 26.3X_g + 0.636X_c \quad (X_c \leq X_{cr}' \quad R^2 = 0.987) \tag{5}$$

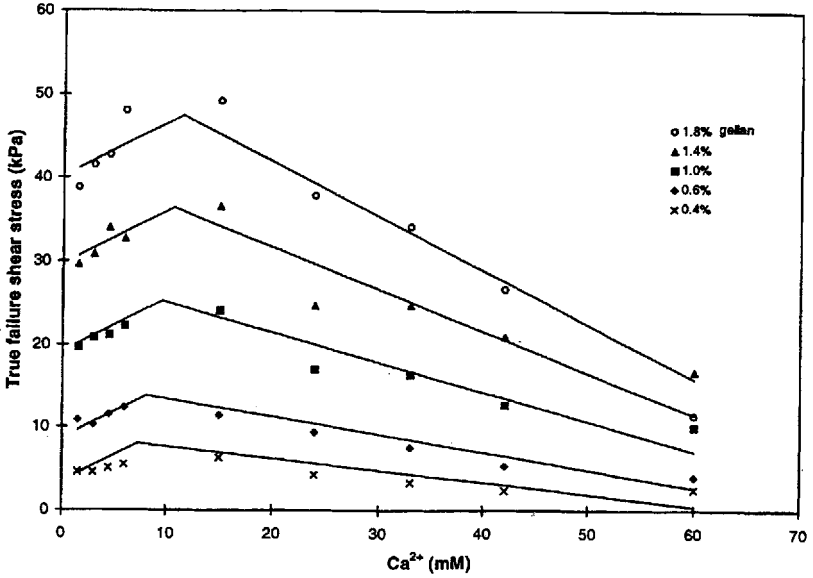$$\sigma = -3.98 + 32.7X_g - 0.360X_gX_c \quad (X_c > X_{cr}' \quad R^2 = 0.964) \tag{6}$$

FIG 1. TRUE FAILURE SHEAR STRESS OF GELLAN GELS FORMED AT pH 3.5
CITRATE BUFFER WITH POLYMER AND CALCIUM CONCENTRATIONS SHOWN
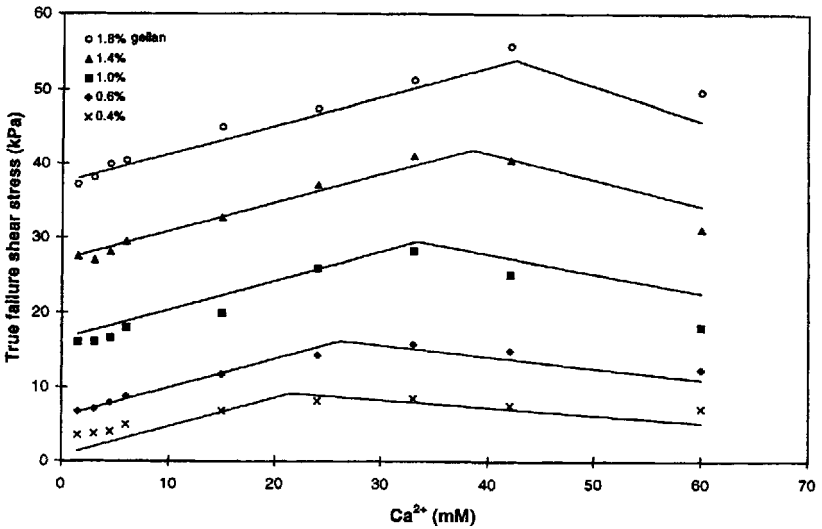ALONG WITH THE FITTED LINES OF EQ. (5-6)

FIG. 2. TRUE FAILURE SHEAR STRESS OF GELLAN GELS FORMED AT pH 5.0
CITRATE BUFFER WITH POLYMER AND CALCIUM CONCENTRATIONS SHOWN
ALONG WITH THE FITTED LINES OF EQ. (7-8)

The relationship obtained for gels formed in pH 5.0 citrate buffer is:

$$\sigma = -9.72 + 26.2X_g + 0.390X_c \quad (X_c \le X'_{cr} \quad R^2 = 0.994) \tag{7}$$

$$\sigma = -6.45 + 44.5X_g - 0.257X_gX_c \quad (X_c > X'_{cr} \quad R^2 = 0.981) \tag{8}$$

where, σ is the true failure shear stress (kPa), X_g is the gellan concentration (% w/v), X_c is the added calcium concentration (mM).

The critical calcium concentration was recalculated as the intersection of each pair of lines described by Eq. 5 and 6 for pH 3.5 or by Eq. 7 and 8 for pH 5.0 at each gellan concentration. X_{cr} and the corresponding peak true failure shear stress (σ_p) are shown in Table 2. Occasionally, the calculated X_{cr} differed greatly from the estimated value (X'_{cr}). In those cases, the data was regrouped and the equations recalculated. After the appropriate X_{cr} was obtained, the X'_{cr} in the limit condition of Eq. 5 to 8 was replaced by X_{cr}.

TABLE 2.
CRITICAL CALCIUM CONCENTRATION (X_{cr}), THE TOTAL EQUIVALENT DIVALENT
CATION CONCENTRATION (X_t), AND CORRESPONDING PEAK TRUE FAILURE
SHEAR STRESS (σ_p) FOR GELS FORMED IN CITRATE BUFFERS AT FIVE
POLYMER CONCENTRATIONS (X_g) AND IN ACETATE BUFFERS AT THREE
POLYMER CONCENTRATIONS

X_g(% w/v)	pH = 3.5			pH = 5.0		
	X_{cr}(mM)	X_t(mM)	σ_p(kPa)	X_{cr}(mM)	X_t (mM)	σ_p(kPa)
			CITRATE	BUFFERS		
0.4	7.3	9.3	8.0	22.0	25.6	9.1
0.6	8.2	10.6	13.9	26.2	30.6	16.2
1.0	9.6	12.7	25.2	33.4	38.5	29.5
1.4	10.6	14.5	36.4	38.5	44.5	42.0
1.8	11.4	16.1	47.4	42.5	49.2	53.9
			ACETATE	BUFFERS		
0.4	3.6	4.5	8.8	6.6	9.7	9.3
1.0	4.1	6.2	27.5	9.6	13.8	29.9
1.8	4.6	8.2	52.3	13.6	19.4	57.3

Tang et al. (1996) showed that the monovalent cations such as sodium and potassium also contributed to gelation of gellan solution, but they were much less effective than divalent cations. Monovalent cation concentration may be converted to an equivalent amount of divalent cation concentration by multiply-

ing a factor of 1/30. Including all cations present in gellan powder and in buffer solution, e.g., 30 mM Na^+ was converted to 1 mM Ca^{2+} according to Tang *et al.* (1996), the total equivalent divalent cation concentration (X_t) corresponding to each X_{cr} were calculated and listed in Table 2.

The peak failure stress σ_p for each gellan concentration is slightly lower (12-15%) at pH 3.5 than that at pH 5.0 (Table 2). This may be explained by the difference in the percentage of the ionized carboxylate groups of gellan polymer in solutions prior to gelation. The glucuronic acid unit in the gellan polymer chain is a weak acid in solution. For a weak acid solution, the relative concentration of ionized species A^- and protonated species HA has following relationship to pH,

$$pH = pK_a + \log\frac{[A^-]}{[HA]} \tag{9}$$

The pK_a of the carboxyl group in the glucuronic acid on the gellan polymer chain is around 3.5 (NutraSweet Kelco Company, 1996). According to Eq. 9, 97% of the total carboxyl groups are in the ionized state at pH 5.0, while only 50% are in the ionized state at pH 3.5. Since only ionized species react with cations to form gel networks, the gels formed at pH 5.0 should be stronger than the gels formed at pH 3.5, provided sufficient calcium concentrations are present.

Since the coefficients for X_g in Eq. 5 and 7 were not different, gellan concentration had the same effect on the strength of the gel at calcium concentrations below the critical calcium levels at both pHs. The critical calcium level, X_{cr}, for each gellan concentration was much larger at pH 5.0 than at pH 3.5 (Table 2). The difference between total equivalent divalent concentrations was even greater. These might have been the result of the difference in the chelating effect of the citrate buffer at different pH. Although the total concentrations of citrate groups in both buffers are the same (0.05M), the mole fraction of different ionized species changes with pH, and so does the chelating ability, because chelating reactions prefer the fully depronated species (Cit^{3-}) or the mono-protonated species ($HCit^{2-}$). The mole fraction of each species of a polybasic acid can be calculated based on the pK_a values (Wolton 1964). For citric acid, $pK_1 = 3.128$, $pK_2 = 4.761$, and $pK_3 = 6.396$ (Bouchard and Merritt 1979). The mole percent of Cit^{3-} and $HCit^{2-}$ was calculated as 2.5% and 61.5% at pH 5.0, and 0.005% and 3.7% at pH 3.5, respectively. The chelating ability of pH 5.0 citrate buffer is much greater than that of pH 3.5. Chelating reduces the available calcium ions in forming gels. This could be the main reason that the critical calcium concentrations at pH 5.0 are much higher than the corresponding values at pH 3.5.

The effect of calcium and polymer concentration on the corresponding true failure shear strain (Fig. 3 and 4) did not follow the pattern of gellan gels formed in distilled water without pH adjustment (Tang *et al.* 1994, 1995). With the latter gels the failure shear strain increased with increasing gellan polymer concentration. The failure strain decreased semilogarithmically with increasing cation concentration from 0.7-1.2 to reach a constant failure shear strains of about 0.4-0.5 (Tang *et al.* 1994, 1995). In the present study, the failure strain of gellan gels in pH 3.5 buffers was equal or less than 0.4-0.5 regardless of calcium and gellan concentrations (Fig. 3). Therefore, only brittle gellan gels can be formed at pH 3.5.
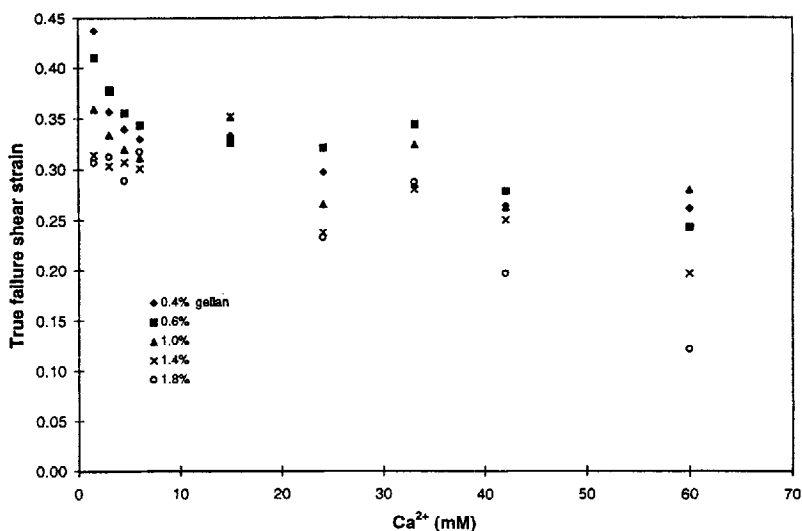
FIG. 3. TRUE FAILURE SHEAR STRAIN OF GELLAN GELS FORMED AT pH 3.5
CITRATE BUFFER WITH POLYMER AND CALCIUM CONCENTRATIONS

It is possible to make ductile gellan gels at pH 5.0 by adding less than 24 mM calcium, as indicated by relatively large failure strains at those low calcium concentrations (Fig. 4). The failure strains decreased linearly with increasing calcium concentration up to approximately 24 mM and then the strain reached a constant value of 0.4-0.5 (Fig. 4).

The failure strain of the gels formed in buffer did not depend on gellan concentration in all buffer systems at all the tested calcium concentrations. This behavior is very different from the gels formed in distilled water.
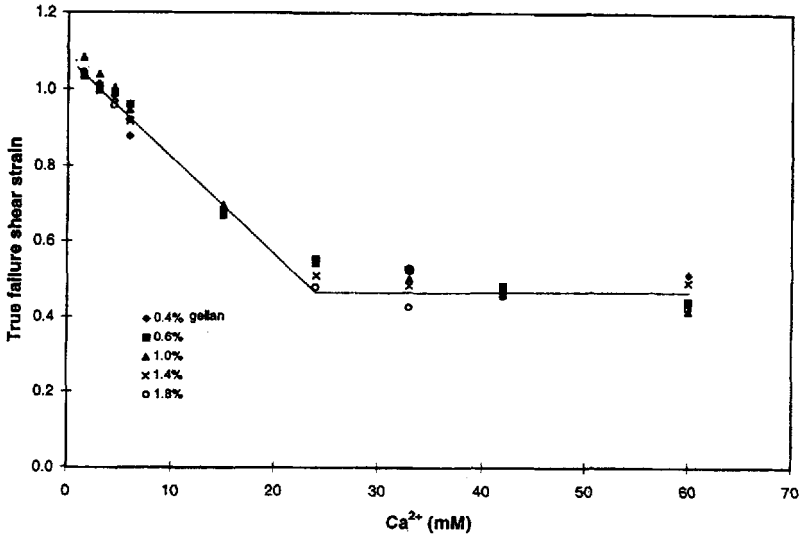
FIG. 4. TRUE FAILURE SHEAR STRAIN OF GELLAN GELS FORMED AT pH 5.0
CITRATE BUFFER WITH POLYMER AND CALCIUM CONCENTRATIONS

The Failure Properties of Gels Formed in Acetate Buffers

In order to separate the chelating effect from the pH change of the citrate buffer system, compression tests were conducted with gellan gels formed in nonchelating acetate buffers of pH 3.5 and pH 5.0. The change of true failure shear stress with polymer and calcium concentrations (Fig. 5 and 6) followed the same pattern as that in gellan gels formed in citrate buffer and the same as gellan gels formed in deionized water (Tang *et al.* 1994, 1995).

The regression of failure shear stress composition relationship for gels formed in pH 3.5 acetate buffer are,

$$\sigma = -5.90 + 30.6X_g + 0.704X_c \quad (X_c \leq X_{cr} \quad R^2 = 0.997) \quad (10)$$

$$\sigma = -3.74 + 32.4X_g - 0.276X_gX_c \quad (X_c > X_{cr} \quad R^2 = 0.992) \quad (11)$$

Similar results for gels formed in pH 5.0 acetate buffer are,

$$\sigma = -8.77 + 29.5X_g + 0.949X_c \quad (X_c \leq X_{cr} \quad R^2 = 0.993) \quad (12)$$

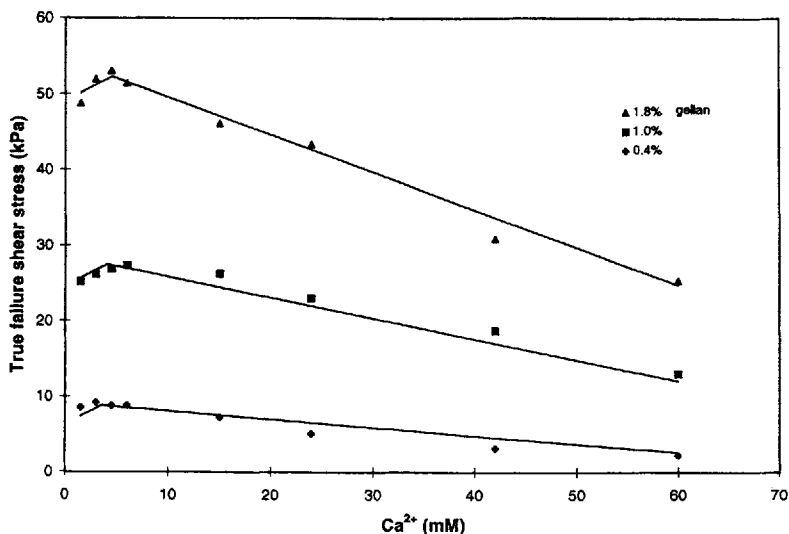$$\sigma = -3.86 + 34.9X_g - 0.126X_c \quad (X_c > X_{cr} \quad R^2 = 0.993) \quad (13)$$

FIG. 5. TRUE FAILURE SHEAR STRESS OF GELLAN GELS FORMED AT pH 3.5
ACETATE BUFFER WITH POLYMER AND CALCIUM CONCENTRATIONS SHOWN
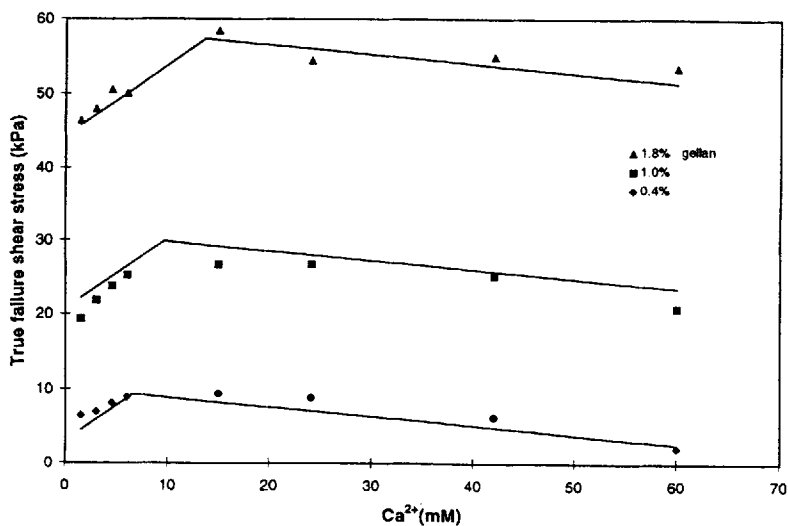ALONG WITH THE FITTED LINES OF EQ. (10-11)

FIG. 6. TRUE FAILURE SHEAR STRESS OF GELLAN GELS FORMED AT pH 5.0
ACETATE BUFFER WITH POLYMER AND CALCIUM CONCENTRATIONS SHOWN
ALONG WITH THE FITTED LINES OF EQ. (12-13)

The critical calcium concentration, the corresponding total equivalent divalent cation concentration, and peak failure shear stress are also shown in Table 2. The critical calcium concentrations of gellan gels formed in pH 5.0 acetate buffer were much lower than gellan gels formed in pH 5.0 citrate buffer because acetate buffer has no chelating ability. When comparing the two acetate buffers, the critical calcium concentrations in gellan gels at pH 3.5 were significantly lower than those at pH 5.0, although both buffers do not have chelating ability. Sime (1990) indicated that an alginate solution at pH 4 required half the level of calcium to form a gel compared with the same alginate solution at pH 6. Alginate is a polysaccharide and the reaction group of its gelation is the carboxyl group in its mannuronic acid and glucuronic acid units. Thus, the need for less cation at acid pH may be a common phenomenon for polysaccharide gelling agents.

The failure shear strains of gels formed in pH 3.5 and 5.0 acetate buffers at selected calcium and gellan concentrations are shown in Fig. 7 and Fig. 8, respectively. This relationship did not follow the pattern of gels formed in deionized water (Tang *et al.* 1994, 1995). Similar to the gels formed in citrate buffers, the failure strain of gels formed in pH 3.5 acetate buffer regardless of calcium and gellan concentrations was generally around or below 0.4-0.5, the lower limit of gels formed in deionized water. For the gels formed in pH 5.0 acetate buffer, the failure strain did not show significant dependency on gellan
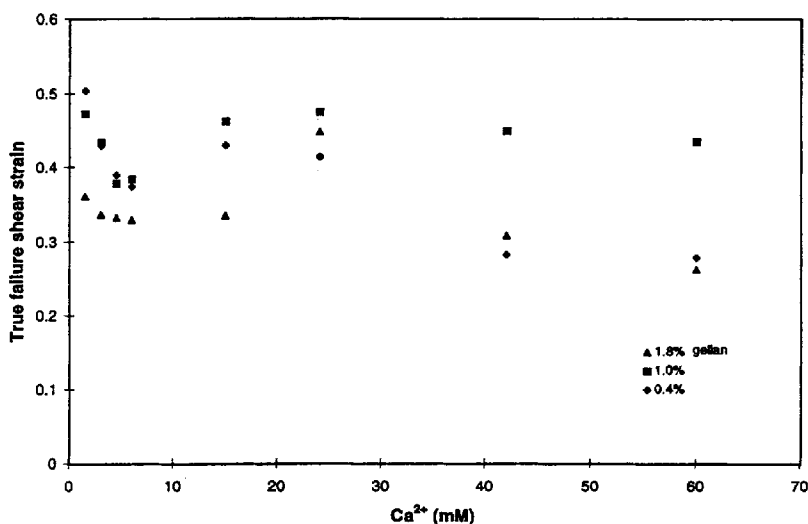

FIG. 7. TRUE FAILURE SHEAR STRAIN OF GELLAN GELS FORMED AT pH 3.5 ACETATE BUFFER WITH POLYMER AND CALCIUM CONCENTRATIONS
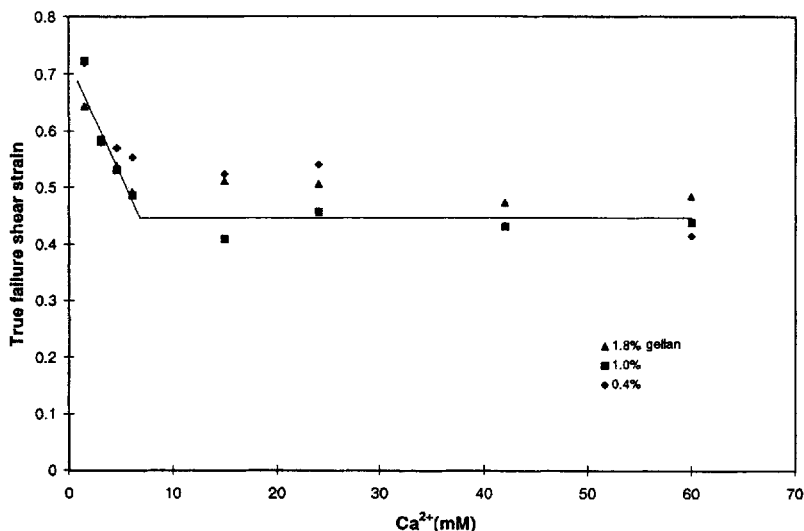
FIG. 8. TRUE FAILURE SHEAR STRAIN OF GELLAN GELS FORMED AT pH 5.0
ACETATE BUFFER WITH POLYMER AND CALCIUM CONCENTRATIONS

concentration and decreased linearly with increasing calcium concentration up
to about 6 mM to reach a constant value similar to gels formed in citrate
buffers. Therefore, only brittle gellan gels can be made at pH 3.5, while it is
possible to make ductile gellan gels at pH 5.0 by controlling the added calcium
not over 6mM, similar to the case of citrate buffer.

Effect of pH and Buffer System

At the same pH, the mechanical properties of gellan gels were significantly
different in different buffer systems. The critical calcium concentrations in pH
5.0 citrate buffer were about 3 times higher than those in pH 5.0 acetate buffer
(Table 2), probably due to the strong chelating effect of the citrate group. When
comparing the critical calcium concentrations of pH 3.5 citrate and acetate
buffers, the former were still twice as high as the latter, although at this pH the
chelating ability of the citrate group was much reduced. It is, therefore, difficult
to make a single general statement on the effect of pH on gel strength and
deformability.   The effect of pH on gellan properties depended upon gel
composition and the buffer system.   For example, when the pH was reduced
from 5.0 to 3.5 in the citrate buffer system, the gellan gels were strengthened
if the added calcium was equal to or less than 15 mM, but the gels were
weakened if the added calcium was equal to or more than 24 mM (Fig. 1 and
2). Similarly, when the pH was reduced from 5.0 to 3.5 in the acetate buffer

system, gellan gels with the same polymer and calcium concentrations were strengthened if the added calcium was below 6 mM and were weakened if the added calcium was higher than 6 mM (Fig. 5 and 6).

CONCLUSION

The failure stress of calcium crosslinked gellan gels formed in pH 3.5 and 5.0 citrate and acetate buffers followed a similar pattern of gellan gels formed with deionized water. The failure stress (indicating gel strength) increased with gellan concentration. It also increased linearly with increasing calcium concentration, but only up to a critical calcium concentration, and then decreased linearly as calcium concentration increased further. Thus, gel strength can be controlled by polymer and cation concentrations. The failure strain (indicating gel deformability) of gels formed in pH 3.5 and 5.0 buffers behaved differently from gellan gels formed with deionized water. It was low for gels formed in pH 3.5 buffer regardless of gellan and calcium concentrations and gellan gels formed at pH 3.5 are always brittle. For gels formed in pH 5.0 buffers, the failure strain can be increased to obtain ductile gellan gels by reducing the calcium concentration. Reducing pH may strengthen gels in some circumstances, and weaken gels in some other circumstances, depending on composition. Different buffer systems had significantly different effects on the mechanical properties of gellan gels. In a buffer system with a chelating effect, e.g. citrate buffer, much more added calcium ion was needed to obtain the maximum gel strength.

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