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Effect of polymer ratio and calcium concentration on gelation properties of gellan/gelatin mixed gels

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Abstract

Gelation properties of gellan/gelatin mixed solutions were studied using dynamic viscoelastic testing at eight different ratios of gellan (1.6-0.2% w/v) to gelatin (0-1.4% w/v) and seven different calcium levels (0-30 mM). The gelation temperature and gelation rate of the mixed gels were significantly affected by the ratio of gellan to gelatin as well as concentration of calcium. Addition of calcium at low levels resulted in an increase in gelation temperature and gelation rate compared to gels with no added calcium. Further increases in calcium increased the gelation temperature, but caused a decrease in gelation rate of the mixed gels. In addition, the presence of gelatin generally had a negative influence on gelation rate, especially at high proportions and when the solution had a high gelling temperature, probably by physically hindering the growth and development of gellan crosslinks. It appeared that in the presence of calcium, gellan formed the continuous gel matrix, with gelatin present as a discontinuous phase. Gellan/gelatin mixtures can form gels over a wide temperature range by varying the ratio of the two polymers as well as the calcium concentration. \bigcirc 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Gellan; Gelatin; Gel; Gelation temperature; Viscoelasticity; Dynamic testing

1. Introduction

Combining two different types of polymers to achieve desired functional properties is a common practice in the food industry (Sanderson, Bell, Burgum, Clark, & Ortega, 1987). Blending two different polymers can greatly improve the functional properties such as gelling and melting temperature, gel strength and room temperature stability (Duxbury, 1993; Morris, 1990; Tolstoguzov, 1986). The addition of gellan to gelatin can create a wide diversity of textural properties compared to other polysaccharides (Lau, Tang, & Paulson, 2000; Sanderson et al., 1987; Wolf, Beach, La Velle, & Clark, 1989).

Gellan is a microbial polysaccharide produced by the bacterium *Sphingomonas elodea* (formerly referred to as *Pseudomonas elodea*). Low-acyl gellan forms strong, clear and brittle gels with the proper ratio of polymer to cation concentration. The gellan polymer is composed

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of tetrasaccharide repeat units of glucose, glucuronic acid and rhamnose in a molar ratio of 2:1:1 (Sanderson, 1990). A proposed gelling mechanism involves the formation of double-helical junction-zones, followed by aggregation of the double-helical polymers to form a three-dimensional network (Chandrasekaran & Radha, 1995), stabilized by the presence of either monovalent or divalent cations (Sanderson & Clark, 1983). Much lower levels of divalent than monovalent cations are needed to make gellan gels of similar strength. The amount of cation and valency also affect the temperature at which gelation occurs. A study by Sanderson (1990) showed that gellan (0.2-0.4%) with the addition of calcium ions (1-8 mM) formed a gel at temperatures ranging from 25 to 40 °C. A more comprehensive study by Tang, Tung, and Zeng (1997a) showed that gelling temperatures of gellan (0.2-1.8%) were much higher for gellan solutions containing divalent cations than those with the same amount of monovalent cations.

While gellan polymer alone as a gelling agent offers many advantages over traditional gelling agents, extra benefits may be obtained by combinations of gellan and gelatin (Anon., 1992; Lau et al., 2000; Sanderson et al.,

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1987). For many years, gelatin has been used in numerous food applications, especially in the formulation of water-based dessert gels (Johnston-Banks, 1990; Kinsella, 1984). However, gelatin has several disadvantages compared to gellan including lower gelation and melting temperatures, as well as higher polymer concentration needed for gel formation (Johnston-Banks, 1990; Wolf et al., 1989). The addition of gellan has been shown to change these properties. Despite the potential advantages offered by gellan/gelatin mixed gels, studies on these systems have been limited. It has been documented that the gellan/gelatin ratio and cation type and concentration have a profound effect on the gelation properties of gellan/gelatin gels (Papageorgiou, Kasapis, & Richardson, 1994; Wolf et al., 1989). However, most studies to date on gellan/gelatin mixed gels have generally used a small amount of gellan (0.05-0.5%) incorporated into gelatin.

A useful method to study gel network formation is small-strain dynamic rheological testing. Using this method, the gelation process can be monitored by the changes in rheological properties as a function of temperature and/or time (Hamann, 1987; Rao, 1992; Ross-Murphy, 1995; Tang et al., 1997a, b). Large strain destructive testing methods such as instrumental Texture Profile Analysis are most useful for studying the textural properties of finished gels, whereas small-strain dynamic measurements can be used to identify the gel point and make inferences about the molecular structure of the resultant network (Clark, 1992; Fernandes, Goncalves, & Doublier, 1994; Goff, Freslon, Sahagian, Hauber, Stone, & Stanley, 1995; Hamann, 1987). The objective of this study was to use dynamic rheological measurements to evaluate the effect of a wide range of gellan to gelatin ratios and calcium concentration on the gelation behaviour of gellan/gelatin mixed gels.

2. Materials and methods

2.1. Preparation of gellan/gelatin solutions

Deacylated gellan (Nutrasweet Kelco, Monsanto Co., San Diego, CA) and type B gelatin (from bovine skin, approximately 225 Bloom, Sigma Chemical Co., St. Louis, MO) were blended at eight different ratios of gellan (1.6, 1.4, 1.2, 1.0, 0.8, 0.6, 0.4, and 0.2% w/v) to gelatin (0–1.4% w/v) to obtain a total polymer concentration of 1.6% (w/v), as previously described (Lau et al., 2000). The blends were dispersed in deionized distilled water at room temperature with the aid of a magnetic stir bar. The solutions were heated with constant stirring to 90 °C, then calcium chloride (CaCl₂) at seven different concentrations ranging from 0 to 30 mM (0, 2, 4, 6, 10, 20, and 30 mM) was added. Weights of the solution were checked and distilled water at ~80 °C was added to make up evaporative losses. The deacylated gellan powder contained about 0.4% (dry basis) calcium, 0.3% sodium and 4.9% potassium, and gelatin contained about 0.055% calcium, 0.022% magnesium, 0.196% sodium and 0.016% potassium (Nutrasweet Kelco, San Diego, CA).

2.2. Dynamic shear measurements of sol-gel transitions (gelation)

Dynamic shear measurements were performed with a Bohlin controlled stress rheometer (Bohlin, Inc., Cranbury, NJ) using a coaxial cylinder fixture (C25) at an oscillatory frequency of 0.1 Hz and a strain (0.5%)within the linear viscoelastic range. Each hot gellan/ gelatin solution (90 °C) was poured into the preheated coaxial fixture (about 20 °C above the gelation temperature of the sample) and equilibrated for 5 min prior to the dynamic measurements. A temperature sweep for each sample was obtained by ramping the temperature downward at 0.6 °C/min. The temperature was controlled by a circulating water bath and measured by a thermocouple which was in direct contact with the coaxial cylinder fixture. The gelation behaviour was monitored by measuring the changes in dynamic shear storage modulus (G', the recoverable energy per cycle of sinusoidal deformation) as a function of temperature. The rheological measurements were terminated when the G' value showed no further substantial change. Moisture loss and evaporative cooling from the gel sample was avoided by placing a water trap over the fixture (Tang et al., 1997a, b). Each dynamic shear test was carried out in duplicate.

2.3. Gelation temperature and gelation rate determination

Gelation temperature of each solution was determined by linear extrapolation of the straight-line por-

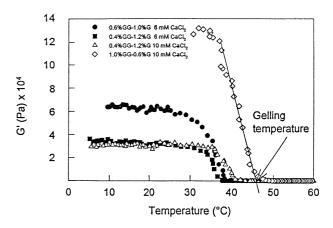


Fig. 1. Typical gelling curves of gellan/gelatin mixed gels when the temperature was decreased at 0.6 $^\circ C/min.$

tion of each cooling curve back to the value G' = 0 from the crossover with the x-axis (temperature) as shown in Fig. 1. This technique was also used by Dahme (1992), Hsieh, Regenstein, and Rao (1993) and Tang et al. (1997a, b). Dahme (1992) compared several different techniques in determining gelling temperature in highmethoxyl pectin gels and indicated that this method was superior to the others tested. Tang et al. (1997b) also found that a linear extrapolation method to determine gelation temperature by dynamic testing correlated well with gelation temperatures determined using visible light spectrophotometry.

Gelation rate was determined by an adaptation of the method of Richardson and Goycoolea (1994) and da Silva and Rao (1995) using the following equation:

$$G'(t) = \frac{\Delta G'}{\Delta \text{temperature}} \times \frac{\Delta \text{temperature}}{\Delta \text{time}} = \frac{\Delta G'}{\Delta \text{time}}$$
(1)

where $\Delta G'/\Delta$ temperature is the slope from the plot of storage modulus versus temperature and Δ temperature/ Δ time is the cooling rate. All the parameters from the above equation were obtained from the linear region of the G' versus temperature curve.

2.4. Visual observation and temperature measurements of gelation temperature

This method was adapted from Tang et al. (1997a, b), where hot gellan/gelatin solutions (90 $^{\circ}$ C) were poured into beakers and a thermocouple was inserted into the solution for temperature measurement. Gel formation was observed visually by gently stirring the solutions while cooling to ambient temperature. The gelation temperature was taken as the temperature where the sol fraction became extremely viscous and difficult to stir. Duplicate measurements were taken.

2.5. Statistical analyses

Multiple regression analysis and analysis of variance were used to examine the effects of gellan to gelatin ratio and calcium concentration on gelation temperature and gelation rate using Minitab 10.5 for Windows (Minitab Inc., State College, PA). Three-dimensional plots were generated by Surfer version 5.01 (Golden Software Inc., Golden, CO).

3. Results and discussion

3.1. Sol-gel transition by dynamic oscillatory testing, and gelation temperature

As the temperature of each hot polymer solution decreased, G' increased abruptly over a temperature

range of about 5 °C from a baseline near zero, followed by a further slight increase and levelling off at lower temperatures (Fig. 1). The abrupt increase in G' was used as an indication of the onset of the sol–gel transition. As indicated by Clark, Gidley, Richardson, and Ross-Murphy (1989), the initial rise in G' has been linked to the formation of a three-dimensional network where most of the sol fraction is converted to a gel.

Gelation temperature of the polymer solutions was significantly affected by the gellan and calcium concentration (P < 0.05). Gelation temperature increased as the proportion of gellan in the mixture increased, and at each combination of gellan and gelatin, gelation temperature increased with increasing calcium ion concentration (Fig. 2).

Tang et al. (1997b) developed a relationship between the reciprocal of absolute gelation temperatures and concentrations of gellan and total calcium. Similar trends were observed for gellan/gelatin mixed gels of the present study. A multiple linear regression was used to fit the experimental data of reciprocal gelation temperature for different levels of gellan to gelation ratio and calcium concentration. The regression equation for gellan/gelatin mixed gels containing calcium is shown below:

$$1/T_{gel} = 3.4 \times 10^{-3} - 2.8 \times 10^{-5} \ln[GG] + 2.4$$
$$\times 10^{-5} \ln[G] - 10^{-4} \ln[Ca^{+2}]$$
(2)

where T_{gel} is gelation temperature (K), [GG] is gellan concentration (%), [G] is gelatin concentration (%) and [Ca⁺²] is added calcium ion concentration (mM; $R^2 = 0.953$, P < 0.001). The coefficients for calcium and the constant value in the equation are close to those reported by Tang et al. (1997b) for gellan solutions. The fact that Eq. (2) fitted the experimental data well suggests that the gelation mechanism for the gellan/gelatin mixed solutions is similar to that of gellan solutions.

A comparison was made between the gelation temperatures of gellan/gelatin gels and gellan-only gels at the same gellan concentration to determine whether gellan or gelatin formed the initial gel network, or if there was an interaction which was reflected in gelation temperature. The gelation temperatures of gellan-only gels from the study by Tang et al. (1997b) and the gellan/gelatin gels of the present study were in quite good agreement (Table 1), with no significant difference (P > 0.05) between the two gelling systems. Therefore, it is likely that the initial three-dimensional network of gellan/gelatin mixed gels was formed by the gellan polymer, with gelatin forming a discontinuous gelled phase upon further cooling. Papageorgiou et al. (1994) concluded that mixed gellan and gelatin polymers (0.5%)gellan; 5% gelatin) undertake conformational changes

 Table 1

 Comparison of gelation temperature of gellan/gelatin mixed gels with gellan gels

Gellan/gelatin mixed gels		Gellan gels ^a		Difference
Polymer and CaCl ₂ concentration	$T_{\rm gel} (^{\circ}{ m C})$	Polymer and CaCl ₂ concentration	$T_{\rm gel}(^{\circ}{ m C})$	
2 mM CaCl ₂		2 mM CaCl ₂		
1.0%GG-0.6%G	34.3	1.0%GG	35.0	0.7
0.6%GG-1.0%G	32.2	0.6%GG	31.5	0.7
0.4%GG-1.2%G	31.2	0.4%GG	30.0	1.2
4 mM CaCl ₂		$4 mM CaCl_2$		
1.0%GG-0.6%G	38.1	1.0%GG	40.0	1.9
0.6%GG-1.0%G	36.2	0.6%GG	36.5	0.3
0.4%GG-1.2%G	35.6	0.4%GG	35.0	0.6
6 mM CaCl ₂		$6 mM CaCl_2$		
1.0%GG-0.6%G	40.5	1.0%GG	44.0	3.5
0.6%GG-1.0%G	38.5	0.6%GG	42.0	3.5
0.4%GG-1.2%G	37.5	0.4%GG	38.5	1.0
20 mM CaCl ₂		$20 mM CaCl_2$		
1.0%GG-0.6%G	55.3	1.0%GG	58.0	2.7
0.6%GG-1.0%G	53.9	0.6%GG	53.5	0.4
0.4%GG-1.2%G	52.5	0.4%GG	51.0	1.5
30 mM CaCl ₂		$30 mM CaCl_2$		
1.0%GG-0.6%G	63.4	1.0%GG	64.0	0.6
0.6%GG-1.0%G	58.9	0.6%GG	59.0	0.1
0.4%GG-1.2%G	55.9	0.4%GG	55.5	0.4

^a Gelation temperature of gellan gels was determined by Tang et al. (1997b).

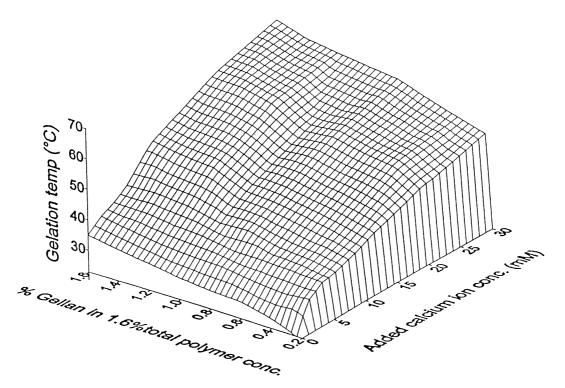


Fig. 2. Gelation temperature of gellan/gelatin gels as a function of gellan to gelatin ratio and different calcium ion levels. The 1.6% total polymer solutions contained gellan (1.6–0.2% w/v) and gelatin (0.0–1.4% w/v).

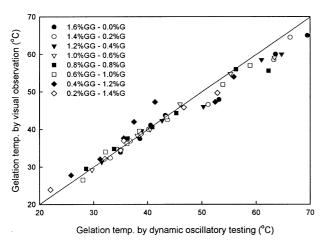


Fig. 3. Relationship between gelation temperature of gellan/gelatin mixed gels from small deformation testing and visual observation/ thermocouple measurements.

independently with cooling and heating, but found that by manipulating gellan/gelatin and NaCl concentration, phase inversion from a gellan-continuous/gelatin-discontinuous gel to one where gelatin formed the continuous phase could be induced. However, this did not appear to be the case in the present study since a gelatin-continuous mixed gel exhibits properties (e.g. gelation temperature) characteristic of gelatin gels, whereas the gellan/gelatin gels of this study had gelation behaviour more characteristic of gellan.

3.2. Visual observation and temperature measurements of gelation temperature

Visual observation/thermocouple measurements of gelation temperature were used in this study to obtain preliminary information regarding the gelation temperatures of gellan/gelatin gels, to help confirm the gelation temperatures obtained by dynamic testing, and to determine the relative utility of this method compared to dynamic testing for gelation temperature of mixed gel systems. The gelation temperature determined by each method was similar up to about 50 °C, above which visual observation/thermocouple measurements gave somewhat lower gelation temperatures than dynamic testing (Fig. 3). Tang et al. (1997a) observed a similar relationship between the two methods for gellanonly gels. Visual observation and small deformation oscillatory measurements were also used by Kasapis, Morris, Norton, and Clark (1993) to determine the gelation time for gelatin/maltodextrin mixed gels. The difference between the gelation temperatures was simply due to the sensitivity of each measurement technique. The small deformation oscillatory measurements corresponded to the initial development of an infinite network. The visual inspection method was less sensitive and might require a greater degree of cross-linking.

Tang et al. (1997a) suggested that deviations in gelation temperature between the two methods at higher than 50 °C (Fig. 3) were mainly caused by the differences in rate and uniformity of cooling, where the visual observation/thermocouple method would be expected to have a faster cooling rate with less temperature uniformity than the dynamic oscillatory testing method. In addition, the visual inspection method, although simple and rapid, was based primarily on a subjective single point measurement of gelation temperature, whereas the dynamic oscillatory testing method used an extrapolation based on a number of data points. Therefore, this study suggests that for both gellan/gelatin and gellanonly gels, dynamic oscillatory testing is more appropriate for gelation temperature measurements above 50 °C, whereas either method would be suitable at lower temperatures.

3.3. Gelation rate

Gelation rate as a function of gellan to gelatin ratio and calcium ion concentration is shown in Fig. 4. With high calcium concentrations, the gelation rate increased as the proportion of gellan in the gels increased, and at high proportions of gellan, gelation rate increased with level of calcium. However, these general trends were not followed at intermediate levels of either calcium or gellan/gelatin ratio where gelation rate increased with calcium ion concentration up to 5 mM calcium, after which the response depended upon the specific polymer concentration. It would appear that the effect of calcium was related to the proportion of gellan in the mixture, where the level of calcium giving the highest gelation rate tended to increase as the proportion of gellan increased. When no calcium was added to the gels, gelation rate increased in an overall linear manner as the proportion of gellan increased (Fig. 5). This effect may have been due to a small amount of ions present in the commercial gellan and gelatin powders, but was low compared to when low to moderate amounts of calcium were added.

3.4. Correlation between gelation temperature and gelation rate

It is clear from Fig. 2 that gelling temperature of mixed gellan/gelatin solutions increased monotonically with added calcium concentration. An examination of the correlation between gelation rate and gelling temperature at various gellan/galatin ratios may help to reveal the gelling mechanism of the mixed gels.

For gels with a high proportion of gellan (i.e. 1.6% gellan–0.0% gelatin and 1.4% gellan–0.2% gelatin), both gelation temperature and gelation rate tended to increase as the level of added calcium increased (Fig. 6a, b). Since gelation of gelatin is independent of salt

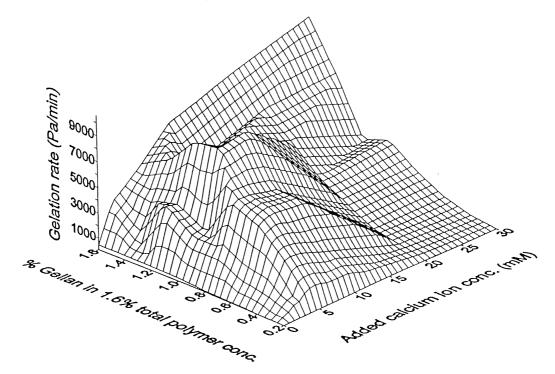


Fig. 4. Gelation rate of gellan/gelatin mixed gels as a function of gellan to gelatin ratio and different calcium ion levels. The 1.6% total polymer solutions contained gellan (1.6-0.2% w/v) and gelatin (0.0-1.4% w/v).

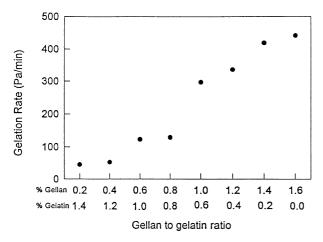


Fig. 5. Gelation rate of gellan/gelatin mixed gels with no addition of calcium ions as a function of gellan to gelatin ratio.

(Johnston-Banks, 1990; Papageorgiou et al., 1994) it would appear that a continuous gellan phase was responsible for this effect. It has been suggested that gelation of gellan can be considered to be a 2-stage reaction involving conversion of random coils to double helices with the aid of Ca^{+2} followed by aggregation of the helices to form junction zones (Chandrasekaran & Radha, 1995; Nakamura, Tanaka, & Sakurai, 1996). Since gelation temperature is associated with the initial onset of gel network formation whereas gelation rate is a measure of how quickly the network forms with cooling, the addition of more calcium ions would increase the chance of earlier initiation of double helix formation, thus increasing the temperature at which the gel matrix formed, but also accellerating the crosslinking of adjacent gellan helices. Based on Table 1, the gelation temperatures of the mixed solutions were determined by gellan and calcium concentrations only, i.e. the presence of gelatin did not affect the initiation of the gel network. This is because only a small number of cross-links need to be developed on long gellan polymer chains of an average of 800 tetrasaccharide repeat units to form an initial 3-dimensional network (Tang et al., 1997b). However, the presence of gelatin tended to have a negative influence on gelation rates, especially when the solutions had high gelling temperatures (Fig. 6c-h). It is likely that gelatin polymers physically hindered the growth of the initiated crosslinks and the development of new crosslinks in the gel firming process. Only when the solution gelling temperatures were less than or close to the temperature at which gelatin polymers could form an ordered structure (e.g. 35-40 °C) did gelatin participate in the development of gel networks. At temperatures of 40 °C and above, gelatin polymers are generally assumed to be random and mobile coils (Ledward, 1986). Thermodynamically, gellan gels and gelatin polymers were not compatible in the mixed solutions with gelling temperatures above 40 °C, and randomly moving gelatin polymers hinder the development of gel networks initiated at the gelling temperatures of gellan gels. The two systems became more compatible when

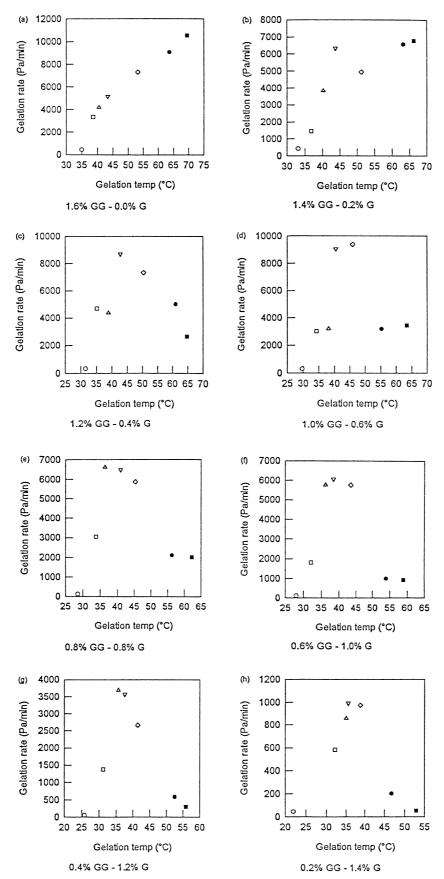


Fig. 6. Correlation between gelation rate and gelling temperature of eight different gellan (GG)/gelatin (G) mixed solutions (\bigcirc , 0; \square , 2; \triangle , 4; \bigtriangledown , 6; \diamondsuit , 10; \bullet , 20; \blacksquare , 30 mM Ca²⁺).

the gelling temperatures of the mixed solutions were at or below about 40 $^{\circ}$ C.

4. Summary and conclusions

Calcium and gellan concentration in gellan/gelatin mixed solutions had a significant (P < 0.05) effect on the gelation temperatures in a similar manner as in gellan solutions. No significant difference (P > 0.05) was found between the gelation temperature of gellan/gelatin mixed gels and gellan gels at the same levels of gellan and calcium. It was concluded that in the presence of calcium, gellan formed the continuous gel matrix, with gelatin present as a discontinuous phase. Gellan/gelatin ratio significantly influenced the gelation rates of the mixed gels. The gelation temperature increased with increasing gellan and calcium concentrations, whereas the effect of calcium concentration on gelation rate was related to the proportion of gellan in the mixture. The gelation rate increased with level of calcium at high proportions of gellan whereas when the proportion of gellan was lower, the gelation rate increased initially with increasing calcium concentration but decreased as more calcium was added. Moreover, the presence of gelatin also appeared to negatively influence gelation rate, especially at high concentrations and when the solution had a high gelling temperature, probably by physically hindering the growth and development of gellan crosslinks. Thus factors that governed the initial formation of the three-dimensional matrix were not necessarily the same as those that influence the rate at which crosslinking occurs or which give the matrix strength and rigidity.

Acknowledgements

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