Gelation Behavior of Concentrated Locust Bean Gum Solutions

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ABSTRACT: The gelation of locust bean gum in concentrated sucrose solutions has been studied as a function of temperature, LBG and sucrose concentrations. A critical gelling concentration of approximately 1% w/w LBG in 60% w/w sucrose was measured. The gelation rate initially increased with decreasing temperature until a maximum in gelation rate was found close to −5 °C. Along with the large temperature hysteresis between the temperature for maximum gelation rate and the melting temperature (>44 °C), it is proposed that the cross-linking was controlled by nucleation and growth processes rather than reversible pairwise cross-linking. The slow evolution of LBG cross-links was detected at lower frequencies through an increase in the storage modulus, while at higher frequencies a decrease in the storage modulus was observed. This decrease at higher frequencies was associated with a reduction in chain entanglements during the initial stages of gelation. In addition to cross-link formation through association of 1,4-linked β-D-mannan regions which were sterically uninhibited by galactose, chain localization may also have been entropically driven due to a reduction in solvent quality as the temperature was decreased toward the gelling temperature. LBG exhibited incompatible behaviour at high sucrose concentrations as a rheological inversion was detected for sucrose concentrations greater than 50% w/w sucrose (at 1.5% w/w LBG), where the polymer rheology changed to that resembling a concentrated sucrose solution. A rheological assessment of the influence of temperature upon the viscoelastic properties of a concentrated LBG/sucrose solution was also performed using the time/temperature superposition principle. The evaluated WLF constants c_1 and c_2 were 7.5 and 50 K for $T_g = 255$ K, similar to those reported for sucrose, glucose, and maltodextrin solutions and several inorganic and organic liquids. These constants did not retain their original meaning, however, owing to the heterogeneous nature of 1.5% w/w LBG, 60% w/w sucrose solution, as reflected by the rheologically derived high glass transition temperature.

I. Introduction

An understanding of the gelation process has led to the greater use and applicability of biopolymer gels within the food industry. Extensive gelation studies have been performed on highly cooperative gelling systems where the starting concentrations are typically semidilute and gelation is relatively rapid once the system is below its gelling temperature, e.g. agarose and carrageenans[1,2] and gellan.[3] Only a limited amount of work has been undertaken studying the gelation of concentrated biopolymer solutions, e.g. high methoxyl pectin,[4,5] nor have there been many investigations of the influence of temperature upon the gelation rate. Such studies would enable a greater understanding of the gelling mechanisms involved.

LBG is widely used as a thickener, and it is well-known to form a gel after a freeze/thaw cycle.[6] Its chemical structure is based on a 1,4-linked β-D-mannan backbone which is solubilized by 1,6-linked α-D-galactose side chain residues. It has been shown that these side chain residues are distributed in a closely random fashion, with a large proportion of galactose-subsitituted couplets (two galactose monomers consecutively substituted along the mannan backbone) but fewer triplets.[7] For gels formed through a freeze/thaw cycle, cross-link formation results from association of galactose uninhibited mannan regions, where the weight-average mannan block length is greater than six monomer units.[8] Its gelation behavior from concentrated solutions is less well-known and understood. A rheological trend toward gelation for a 1.85% weight/weight (w/w) LBG solution after 15 days at 20 °C was measured,[9] and this slow gelation of concentrated LBG solutions is similar to the renaturing process in semidilute solutions observed by Morris.[10] Dea *et al.*[6] also reported that at high LBG concentrations, a gel will form in a low water activity environment such as in a concentrated sucrose solution. Until now, however, there has been no systematic work studying the gelation behavior for concentrated LBG solutions. It is worth noting here a difference in notation between natural and synthetic polymers. Typically, biopolymer chains are inherently more rigid and thus exhibit highly viscous solutions at relatively low concentrations. As a consequence, greater than 1% w/w LBG solutions are termed concentrated solutions here, contrary to the usual definition for many synthetic polymer solutions where much higher levels of solids are required. Higher concentrations greater than 3% w/w are not accessible owing to the limited solubility of LBG in water. Here, a rheological investigation has been undertaken to study the gelation rates of LBG in concentrated sucrose solutions at different temperatures and over a range of LBG and sucrose concentrations. The relatively strain tolerant starting solution and slow gelation rates allowed the sol/gel transition from a concentrated solution to be studied in some detail. In addition to the temperature dependence of gelation, the effects of temperature upon the viscoelastic properties of the system have been assessed and quantified using the Williams−Landel−Ferry (WLF) equation.[11]

II. Experimental Section

Materials. Locust bean gum (LBG) supplied by Meyhall was used for this study. The molecular weight characteristics as determined using high performance size exclusion chromatography with multi-angle laser light scattering (HPSEC-MALLS) were M_n = 288 000, M_w = 370 000, and M_w/M_n = 1.285 (dn/dc = 0.155 mL/g). For the molecular weight evalu-

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ation, LBG solutions were prepared by first dispersing the polymer in a phosphate buffer (pH = 8) and then heating at 80−90 °C for 30 min with continuous stirring. The phosphate buffer solution was chosen to remove any polymer aggregation in a fashion similar to the removal of hyperentanglement for LBG solutions as reported elsewhere.[12,13] The average galactose content of LBG was 20.4% as determined using a method by Taylor and Conrad.[14] LBG was hydrolyzed using 100 μL/mg of 12 M sulfuric acid (cooled on ice) and heated for 1 h at 35 °C. The solution was then diluted with water and heated for 3 h at 100 °C. After neutralization using concentrated ammonium hydroxide followed by dilution to 25 mL, the monosaccharides were analyzed using a high-performance anion exchange column (HPAEC).

Locust bean gum/sucrose solutions were prepared by dry mixing the appropriate amount of LBG and sucrose, followed by addition to a 250 mL glass jar containing deionized water, while stirring. For those systems that did not fully disperse, a mechanical stirrer (Silverson or Ultra-Turrax) was used to fully homogenize the solution. The solution was heated to 90 °C and stirred using a magnetic flea for 30 min. The glass jars were sealed to prevent moisture loss during solvation. Solutions were prepared ranging from 0.9% to 2.0% LBG and from 30 to 65% sucrose (weight by weight). For a 1.5% LBG, 60% sucrose solution held at 90 °C, an RVA (rapid viscosity analyzer) experiment showed that no further viscosity buildup was measured after 30 min, inferring that LBG solvation was complete.

Methods. Dynamic rheological measurements were conducted on a controlled stress Carri-Med CSL-500 rheometer using 4 cm diameter parallel plate geometry. The plates were covered with grade 60 emery paper to prevent slippage. In addition to the Peltier element, a RK20 Lauda cooling bath filled with a 50/50 glycol/water solution was used to reach the required low temperatures (−8 to +20 °C). The accuracy of temperature control was ± 0.2 °C. Samples were covered with a low viscosity oil (mineral oil, Sigma M-3516), and the apparatus was enclosed within a polyethylene bag under a dry nitrogen atmosphere to prevent condensation onto the sample.

To access a broad frequency range, the time/temperature superposition principle[15] was used with −5 °C as the reference temperature. For a 1.5% w/w LBG, 60% w/w sucrose solution, frequency sweeps were performed over 0.01−10 Hz, at 1 Pa stress at 75, 55, 35, 15, and then −5 °C. After 70 h at −5 °C, the frequency sweeps were repeated at −5, 15, 35, 55, and then 75 °C. The samples were allowed to equilibrate at the measurement temperature for 15 min prior to measurement, and the stress sweeps applied were within the linear viscoelastic region.

Dynamic time sweeps were performed over 16 h at 0.5 Hz and at 1 Pa stress (cure curves). For those systems where gelation was very slow, measurements were performed for 60 h. After each cure curve, a stress sweep was run at 0.5 Hz at the same temperature of the curing experiment. A few cure curve experiments were repeated and a temperature sweep executed ranging from the cure temperature to 90 °C at 1 °C/min. Three variables were chosen for studying the gelation of LBG (curing of LBG): temperature, LBG and sucrose concentration. The cure temperature was varied from +10 to −8 °C for a 1.5%/60% LBG/sucrose solution. The LBG concentration was varied from 1.0% to 2.0% w/w at −5 °C and 60% w/w sucrose, and the sucrose concentration was varied from 20% to 65% w/w at −5 °C and 1.5% LBG.

The initial stages of gelation for 1.5% w/w LBG, 60% w/w sucrose at −5 °C were probed by performing multiple frequency sweeps, 0.01−10 Hz at 1 Pa applied stress as a function of time.

III. Results and Discussion

Solution/Gel Rheology of Concentrated LBG/Sucrose System. Figures 1 and 2 display the frequency sweeps for 1.5% LBG, 60% sucrose at different temperatures for the solution and the gel cured at −5 °C for 70 h, respectively. The resultant time/temperature superposition rheology profiles reduced to −5 °C for a 1.5% w/w LBG, 60% w/w sucrose solution initially and after 70 h at −5 °C are shown in the inserts, Figures 3 and 4, respectively. The reference temperature of −5 °C was chosen, and the modulus values were corrected by the factor $T_o\rho_o/T\rho$,[15] where T_o and T are the reference and measurement temperatures and ρ is the density. The slight change in density with temperature was assumed to be negligible, $\rho_o/\rho = 1$. The moduli were

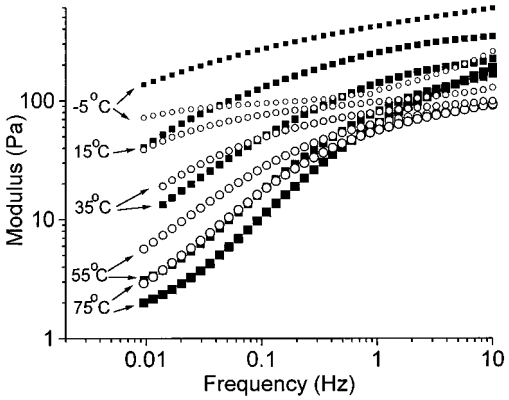

Figure 1. Frequency sweeps (1 Pa) performed at different temperatures for freshly prepared 1.5% LBG, 60% sucrose solution (■ = G'; ○ = G'').

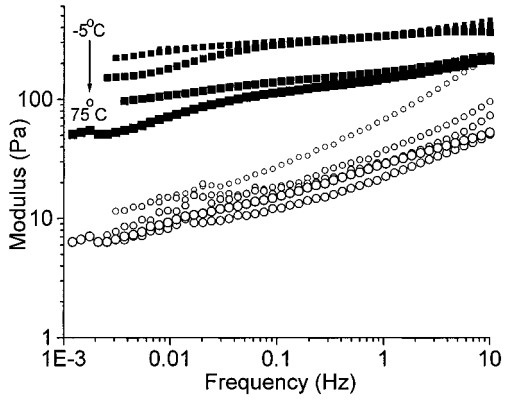

Figure 2. Frequency sweeps (1 Pa) performed at different temperatures for 1.5% LBG, 60% sucrose which has been cured at −5 °C for 70 h (■ = G'; ○ = G'').

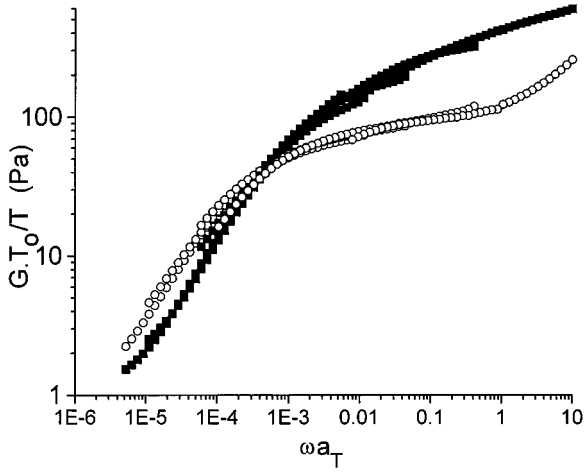

Figure 3. Time/temperature superposition of the frequency sweeps displayed in Figure 1 for 1.5% LBG, 60% w/w sucrose solution (reduced to −5 °C) (■ = G'; ○ = G'').

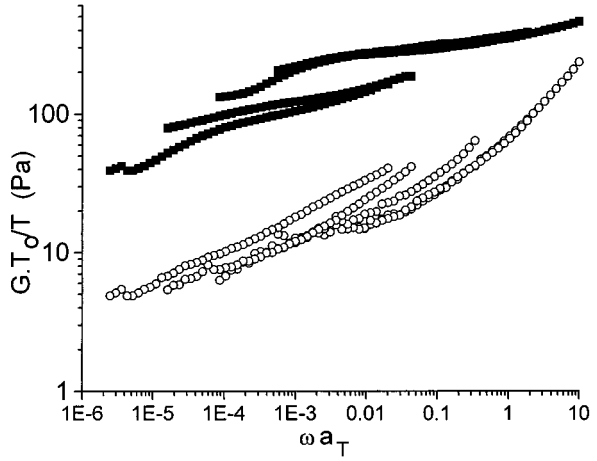

Figure 4. Time/temperature superposition of the frequency sweeps displayed in Figure 2 for 1.5% LBG, 60% w/w sucrose which has been cured for 70 h at −5 °C (reduced to −5 °C) (■ = G'; ○ = G'').

Table 1. Analysis of Time/Temperature Superposition of 1.5%LBG/60% Sucrose (Reduced to −5 °C)

temperature, T (K)	$T - T_o$ (K)	$\Delta(\log a_T)$	$\log a_T$
268	0	0	0
288	20	1.388	−1.388
308	40	0.979	−2.368
328	60	0.572	−2.939
348	80	0.326	−3.266

shifted along the frequency axis producing a master curve with respect to the reference temperature of −5 °C. The temperature shift factor, $\log a_T$ was determined graphically and is displayed in Table 1.

Figure 3 is a typical profile for a concentrated macromolecular solution.[16] Over the frequency range measured using the Carri-Med rheometer, 0.01−10 Hz, at −5 °C, the storage, G', and loss moduli, G'', were relatively frequency independent, due to extensive molecular entanglement, and similar in appearance to the corresponding frequency profile after 70 h at −5 °C; see Figure 2. That is, the solution and gel are in the plateau zone according to Ferry.[15] At lower frequencies, however, the system which has been cured for 70 h at −5 °C does not display a characteristic solution behavior G'', G' crossover at lower frequencies, in the terminal zone. The moduli are relatively frequency independent, even at very low frequencies, indicating that this system has gelled, possessing long-lived cross-links. This frequency independence in addition to G' being an order of magnitude greater than G'', are criteria often used to define a gel.[16] For LBG, the cross-links (junction zones) are formed through hydrogen bonding between galactose uninhibited mannan regions.[6]

The superposition failed at lower frequencies (higher temperatures, 55 and 75 °C) as the gel began to melt. Figure 5 shows a melting profile for this system after only 16 h at −5 °C. The storage modulus increased with increasing temperature up to 44 °C and then gradually decreased to 90 °C. The increase in modulus with temperature is exhibited for rubber-like gels,[17] where the flexible chain segments between cross-links become more dynamic with temperature, contributing an RT component to the storage modulus; see eq 1, where G_e

$$G_e = gvRT \tag{1}$$

is the equilibrium shear modulus, v is the moles of

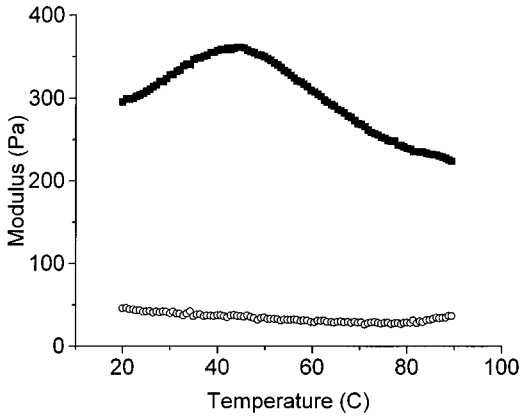

Figure 5. Rheological temperature sweep (1 °C/min, 0.5 Hz, 1 Pa) performed on 1.5% LBG, 60% sucrose which has been cured at −5 °C for 16 h (■ = G'; ○ = G'').

network strands per cm^3, and g is a numerical factor close to unity. Above 44 °C, G' decreased with temperature, indicating the loss of junction zones through melting. This is a very gradual melting process, implying a weakly cooperative association and/or polydisperse junction zone stabilities. The latter point is likely when the polydisperse chemical nature of LBG is considered.[18] The presence of associations at 75 °C is not only indicated by the gradual loss of G' in Figure 5, but also by the frequency independent and high storage moduli at low frequencies in Figure 4. Such high melting temperatures were not observed for aqueous LBG gels formed through a freeze/thaw cycle where melting was found to occur between 60 and 65 °C.[6] A possible reason for this increase in melting temperature is that in our studies, gelation has occurred slowly which has allowed more stable junction zones to be formed. In addition, as observed for agarose and gelatin,[19] the presence of sucrose may have increased the melting temperature of the gel. This would imply that sucrose addition can increase the value of the quotient, $\Delta H/\Delta S$ for cross-link formation,[20] by increasing the strength or length of junction zone and/or a decrease in the entropy change accompanying gelation.

Analysis of the time/temperature superposition shown in Figure 3 for a concentrated solution of LBG and sucrose can lead to a more detailed description of the effect of temperature on the viscoelastic properties of this system. Figure 6 displays the temperature shift factors in Table 1 in the form of the WLF equation[15] (eqn 2), $T - T_o/\log a_T$ vs $T - T_o$.

$$\log a_T = \frac{-c_1^0(T - T_0)}{(c_2^0 + T - T_0)} \tag{2}$$

This plot yielded the following constants, $c_1^0 = 5.95$K and $c_2^0 = 63$K for 1.5% w/w LBG, 60% w/w sucrose reduced to the reference temperature, −5 °C. A linear regression was chosen to determine the constants c_1^0 and c_2^0 in Figure 6, but clearly, a second order polynomial would have given a better fit. Thus, the WLF equation did not exactly describe the influence of temperature upon the viscoelastic properties of 1.5% w/w LBG, 60% w/w sucrose solution, but certainly the temperature dependence of the viscoelastic properties was relatively steep and non-Arrhenius like.[33]

As shown by Ferry,[15] a fixed temperature, T_∞, where $\log a_T$ becomes infinity, can be determined by plotting

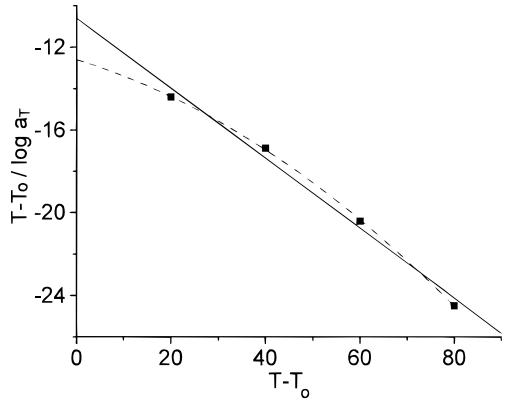

Figure 6. WLF analysis to determine the material specific constants, c_1 and c_2, for freshly prepared 1.5% LBG, 60% sucrose solution.

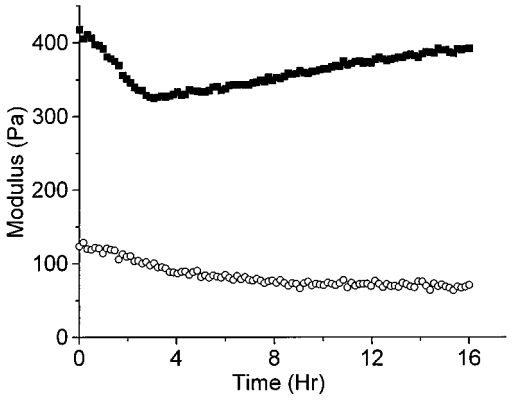

Figure 7. Cure curve profile (1 Pa, 0.5 Hz) for 1.5% LBG, 60% sucrose at −5 °C (■ = G', ○ = G'').

log a_T against $(T - T_o)/(T - T_\infty)$ and choosing T_∞ so that the plot is linear and through the origin. T_∞ was found to be 205 ± 5 K, which gives an estimate for the glass transition temperature, T_g, of 255 K ($T_\infty \approx T_g - 50$ K).[15] This is significantly higher than the expected value for a homogeneous solution of 1.5% w/w LBG, 60% w/w sucrose. For this system, the higher weight fraction of sucrose would dominate the value of T_g, and therefore approximate to that for 60% w/w sucrose alone, 185 K.[21] A possible explanation for this apparent discrepancy is that the system is not homogeneous. LBG rich regions may exist which dominate the rheological response. Consequently, these evaluated WLF constants do not retain their exact original meaning. Nonetheless, the constants were converted to the more useful constants, $c_1^g = 7.5$ K and $c_2^g = 50$ K associated with the glass transition temperature, $T_g = 255$ K, using the following relationships:

$$c_1^0 = c_1^g c_2^g (c_2^g + T_0 - T_g) \quad (3)$$

$$c_2^0 = c_2^g + T_0 - T_g \quad (4)$$

The constants, c_1^g and c_2^g are similar to those determined for several organic and inorganic liquids[10] (c_1 = 17.4 K, c_2 = 51.6 K) and for sucrose, glucose, and maltodextrin[22] ($c_1 = 16.5$ K, $c_2 = 37.5$ K). The fractional free volume, f_g, and the thermal expansion coefficient, α_f, at the glass transition temperature can be calculated from these WLF constants, where $f_g = B/2.303c_1^g$ and α_f, $= B/2.303c_1^g\, c_2^g$ (where B is a numerical constant close to unity).[15] These values, $f_g = 0.058$ and $\alpha_f = 11.6 \times 10^{-4}$ deg^{-1} are relative large when compared to synthetic polymers.[15] According to the concept that the mobility at any temperature depends primarily upon the free volume remaining,[15,23] the high free volume present for LBG may be the source of its maximum gelation rate at low temperatures; see later.

Using these constants, an energy term can be included into the WLF equation:[15]

$$\Delta H_a = R \frac{d(\ln a_T)}{d(1/T)} \quad (5)$$

$$\Delta H_a = \frac{2.303 R c_1^0 c_2^0 T^2}{(c_2^0 + T - Tg)^2} \quad (6)$$

This apparent activation energy, ΔH_a, for viscoelastic relaxation will be considered with respect to LBG gelation at low temperatures in a later section.

Rheology of LBG/Sucrose Gelation. Figure 7 displays a typical cure curve for 1.5% w/w LBG, 60% w/w sucrose at −5 °C. The initial values for G' and G'' are, as expected, relatively large owing to extensive molecular entanglement and contribution from the concentrated sucrose solution at 0.5 Hz. As the solution gelled, the loss modulus decreased over the 16 h period of measurement. For the storage modulus, however, there was a initial decrease followed by a gradual increase with time. Such moduli variations with time are reflected by an initial maximum for tan δ (tan $\delta = G''/G'$), which then decays with time. As expected from Figures 3 and 4, the change in tan δ values at 0.5 Hz from the concentrated solution toward the gelled state was small. In hindsight, a greater change in rheological response (G', tan δ) would have resulted if a lower frequency value was chosen for the cure experiments.

The initial dip in G', at a maximum in tan δ, was not a result of junction zone breakage due to the applied measurement stress. A reduction to a lower applied stress (0.2 Pa) did not produce any change to the cure curve profile shown in Figure 7. In fact, the concentrated LBG/sucrose environment has effectively lowered the stress sensitivity of the LBG network to damage during the initial stages of gelation and thus allowed the sol/gel transition to be measured. In addition, when the measurement stress was delayed by up to 4 h, the subsequent data (data not shown) continued to follow the cure curve profile shown in Figure 7, indicating that the initial loss in G' at 0.5 Hz had occurred even when no measurement stress was applied. Thus, this initial maximum in tan δ represents the rheological changes that have occurred to the gelling LBG, sucrose solution and, as shown in Figures 8 and 9, is a result of monitoring the gel transition from a starting concentrated solution at a fixed, single frequency, 0.5 Hz. Figures 8 and 9 display frequency sweeps after every 44 min during the initial stages of gelation of 1.5% w/w LBG, 60% w/w sucrose at −5 °C. As shown in Figure 8, at 0.5 Hz, there is an initial drop in the storage modulus followed by a gradual increase with time. This is consistent with the cure curve measured at 0.5 Hz; see Figure 7. In Figure 9, at 0.5 Hz, tan δ increased before beginning to decrease with the time of gelation. At 0.01 or 10 Hz, however, a respective gradual decrease or increase in tan δ would have resulted during the cure curve measurement; see Figure 10. Consequently, the

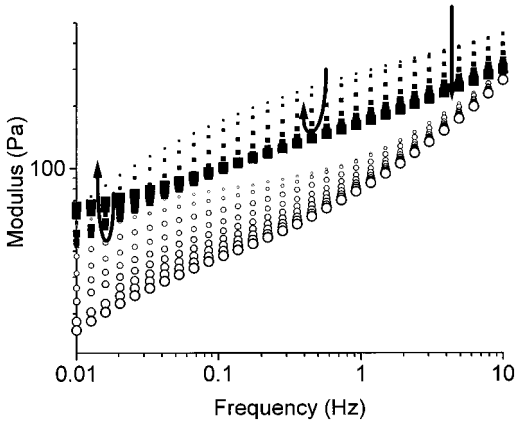

Figure 8. Frequency spectra displaying the storage and loss moduli changes during the initial stages of gelation (44 min intervals, increasing in symbol size with time) of 1.5% LBG, 60% sucrose at −5 °C (■ = G', ○ = G'').

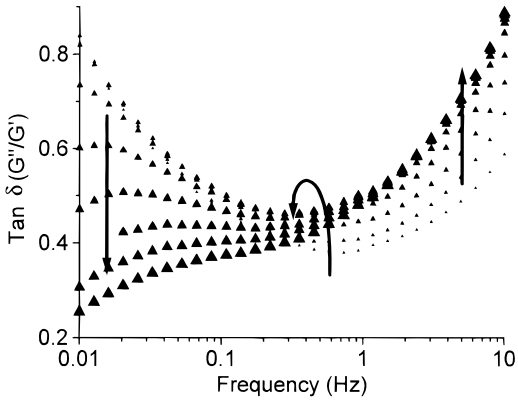

Figure 9. Frequency spectra displaying the loss tangent, tan δ, changes during the initial stages of gelation (44 min intervals, increasing in symbol size with time) of 1.5% LBG, 60% sucrose at −5 °C.

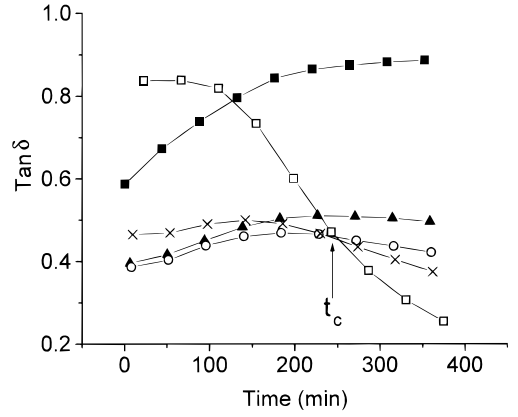

Figure 10. tan δ as a function of time at different measurement frequencies (■ = 10 Hz, ▲ = 1.17 Hz, ○ = 0.45 Hz, × = 0.11 Hz, □ = 0.01 Hz) for 1.5% LBG, 60% sucrose cured at −5 °C.

nature of the cure curve obtained is highly frequency dependent.

Owing to the high proportion of flexible chain segments between point-like mannan/mannan junction zones, it was expected that junction zone formation resulted in a decrease in the loss tangent at very low frequencies as seen in Figures 8 and 9. There also should have been a maximum in tan δ at higher frequencies representing the space scale of vibration of the actual junction zone, as with gelatin for example.[24] This was not detected with the limited frequency range available. It was surprising to detect a decrease in G' over the intermediate frequency range during the initial stages of gelation. This drop in G' represents a reduction in the connectivity of the entangled solution during the initial stages of gelation. It is proposed that this reduction in G' is due to a degree of chain disentanglement driven by an enthalpic driving force for association between galactose free mannan regions on the LBG backbone.[7,25] In addition, as the temperature was decreased toward the gelling temperature, the quality of the solvent would also have decreased and thus contributed to LBG chain-chain localization. Clusters of LBG cross-links are formed resulting in a heterogeneous system. During the initial stages of gelation, the continuous rheological nature of the entangled solution over shorter distance scales is now more dependent upon the un-cross-linked, less entangled LBG/concentrated sucrose solution. Consequently, the storage modulus decreases. Once these clusters begin to network to a higher degree with time, then the LBG network begins to dominate the rheological response and thus G' increases. Such demixing processes prior to gelation have also been detected using other techniques for other biopolymer systems, for example agarose.[26]

Gel Time Method. Typically, the rate of gelation is defined by a gel time which can be determined by either one of the following approaches: the time where a finite number of junction zones are formed resulting in a gel network[27] (measured using an inverted sample vial technique), the time when G' increases to a value where $G' = G''$, tan $\delta = 1$[28] at a low measurement frequency and the time when the following conditions hold (eqs 7 and 8) as proposed by Winter *et al.*[29] for chemical gels and applied to some physical gels:[30,31]

$$G'(\omega) \propto G''(\omega) \propto \omega^{\Delta} \tag{7}$$

$$G''/G' = \tan \delta = \tan(\Delta\pi/2) \tag{8}$$

Δ is a critical exponent which theoretically ranges from 0 to 1.[29] This approach defines a critical gel time, t_c, where G' and G'' exhibit the same power law dependency with frequency over a broad frequency range corresponding to the formation of a continuous network at the percolation threshold.

All three approaches were not applicable to this concentrated LBG/sucrose solution under investigation. The sample vial inversion technique is not an absolute method for monitoring the rate of gelation. The method involves both shear and tensile components, and adhesion to glass will be material specific. It is also only applicable to high modulus systems where the moduli necessary for the gel to maintain its position in the sample vial is low in comparison.[27] The second gel time method was not applicable as the storage modulus was already larger than the loss modulus at 0.5 Hz, for the initial concentrated, highly entangled solution. Third, the Winter and Chambon approach was not possible as no critical point existed for this system over the frequency range measured, see Figure 10, owing to the reduction in G' at higher frequencies. In fact, if the analysis was restricted to the lower frequency range, <0.45 Hz, then a critical crossover with frequency did exist with an exponent, $\Delta = 0.27$ and a critical gel time, t_c, of 250 min. It is noteworthy that this exponent is

Table 2. Analysis of tan δ vs Time at a Range of Temperatures for 1.5%LBG/60% Sucrose and at Different LBG Concentrations at 5 °C in 60% W/w Sucrose

	temp (°C)	P_1	P_3	P_2 (h)	half life, $t_{1/2}$ (h)	rate $1/t_{1/2}$ (h^{-1})
1.5% LBG/60% sucrose	−8	0.153	0.168	7.78	5.39	0.186
	−5	0.245	0.168	4.81	3.33	0.300
	−2	0.247	0.144	6.55	4.54	0.220
	2	0.198	0.197	6.95	4.81	0.208
	5	0.153	0.176	17.1	11.8	0.085
	10	0.086	0.237	61.9	42.9	0.023

	[LBG] % w/w	P_1	P_3	P_2 (h)	$t_{1/2}$ (h)	$1/t_{1/2}$ (h^{-1})
at −5 °C, 60% sucrose	2.0	0.173	0.158	6.64	4.60	0.217
	1.7	0.407	0.202	3.90	2.70	0.370
	1.5	0.245	0.168	4.81	3.33	0.300
	1.4	0.197	0.235	13.6	9.42	0.106
	1.3	0.216	0.311	15.3	10.6	0.094
	1.0	0.207	0.25	93.9	65.1	0.015

similar to those obtained by Michon *et al.*[32] for gelatin and *i*-carrageenan at relatively high concentrations.

Clearly, an alternative gel time method is required for this system. Ideally, a kinetic model representing junction zone formation would be used; however, one is not available, and furthermore, it would be difficult to link the changes in viscoelastic properties at a single frequency with such a model. Consequently, for the gelation of LBG/sucrose solutions, a simple first-order half-life analysis of tan δ vs time was chosen to assess the rate of gelation, similar to the G' vs time approach taken by Lopes da Silva[4,5] for high methoxyl pectin in concentrated sucrose solutions.[4−5] The loss tangent (tan δ) was chosen here as this parameter takes into account the loss contribution and thus provides a more representative description of the gelation process, over G' alone.

Gelation Rate Dependence upon Temperature. Figure 11 displays tan δ cure curves as a function of temperature for 1.5% w/w LBG, 60% w/w sucrose over 16 h of gelation at 0.5 Hz. It is apparent that the rate of gelation increased (tan δ decay) as the temperature decreased. The LBG cure curves at different temperatures were analyzed according to eq 9, where x is the time of gelation and y is the loss tangent, tan δ.

$$y = P_1 \exp(-x/P_2) + P_3 \tag{9}$$

The exponential fit was restricted to the later stage tan δ decay, omitting the initial tan δ maximum. The time required for half of the reduction in tan δ, the half-life $t_{1/2}$, was determined from $t_{1/2} = (P_2 \ln 2)$. Results of the analyses are presented in Table 2, and Figure 12 displays the gelation rate (1/half-life) for gelation of 1.5% w/w LBG, 60% w/w sucrose at different temperatures. Owing to the small change in tan δ over long times and the fact that the gelation was far from completion, the error associated with the half-life and thus gelation rate was relatively large. Interestingly, the half-life and critical gel time determined using Winter's approach[29] for 1.5% w/w LBG, 60% w/w sucrose at −5 °C were very similar.

In Figure 12, the gelation rate increased with decreasing temperature toward an apparent maximum at −5 °C. It is expected that the gelation rate would further decrease at lower temperatures as the gelation mechanism becomes more rate dependent upon molecular chain diffusion, as illustrated in Figure 12. The

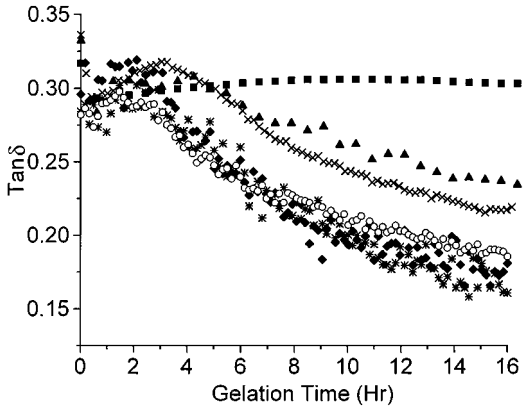

Figure 11. Rheological time sweeps (1 Pa stress, 0.5 Hz) at different temperatures (■ = 10 °C, ▲ = 5 °C, × = 2 °C, * = −2 °C, ◆ = −5 °C, ○ = −8 °C) for 1.5% LBG, 60% sucrose solution.

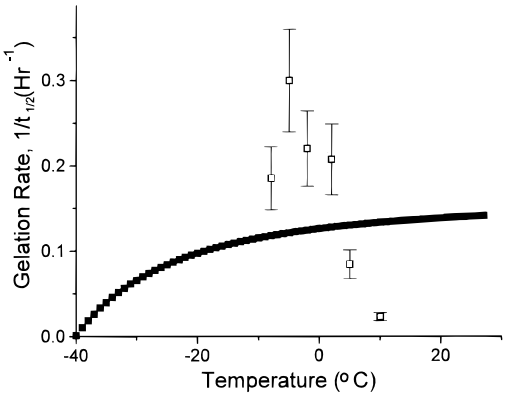

Figure 12. Gelation rate, as determined from a half-life analysis, at different gelation temperatures for 1.5% LBG, 60% sucrose (□). Illustration of the type of gel rate dependence predicted for a diffusion controlled gelation governed by WLF kinetics (■).

form of gelation rate vs temperature for a reaction that is diffusion controlled according to the WLF equation is given. The WLF rates have been arbitrarily shifted onto the same scale as the experimental gelation rates, with the intention of highlighting the relative gelation rate dependencies with temperature. The theoretical gelation rate was calculated using the apparent activation energy for viscoelastic relaxation as determined earlier using eq 6 for $T_g' = 233$ K.[21] As explained by Slade and Levine,[33] as the temperature decreases toward the glass transition temperature, diffusion controlled reactions will exhibit WLF (non-Arrhenius) kinetics. At temperatures greater than −5 °C, the experimentally determined gelation rates deviate from that of WLF kinetics. This maximum in gelation rate and the reduction at higher temperatures is characteristic of an equilibrium process, with both forward and backward reactions. At −5 °C, the backward reaction is at a minimum and thus the gelation rate is at a maximum. At higher temperatures, the backward reaction increases and thus the gelation rate decreases as it approaches the critical melting transition. Owing to the large temperature difference (hysteresis) between the gelation and melting temperatures, the gelation mechanism is more aligned to a frustrated crystallization process where nucleation and growth processes are in equilibrium. This in contrast to reversible pairwise cross-linking where the hysteresis is much smaller. In addition, the formation of nuclei seems consistent with the chain disentanglement process during the early

Table 3. Stress Sweep Results Performed on Gelled LBG/Sucrose Solutions

sucrose % w/w (1.5% LBG, −5 °C)	G'	G''	G* (tan δ = 1)	cure time (h)
40	420	160	650	16
45	540	118	2500	16
50	730	95	2580	16
55	653	103	1940	16
60	483	95	2200	16
65	145	75	570	16
LBG % w/w (60% sucrose, −5 °C)	G'	G''	G* (tan d = 1)	cure time (h)
1.0	125	55	540	16
1.3	275	94	1170	16
1.4	388	85	1880	16
1.5	483	95	2200	16
1.7	720	104	2700	16
2.0	1230	162	3770	16
temp, (°C) (1.5% LBG, 60% sucrose)	G'	G''	G* (tan d = 1)	cure time (h)
10	320	85	2050	60
5	480	90	1770	60
2	407	82	1450	20
−2	550	98	1910	16
−5	483	95	2200	16
−8	647	135	2870	16

[a] Storage and loss moduli have been determined at 200 Pa, within the linear-viscoelastic region.

stages of gelation, as described earlier. It is noted that this mechanistic description is very simple. For instance, nucleation is a metastable process with both formation and destruction of nuclei and not simply a backward reaction. Further investigations probing the gelation mechanism of LBG using high-temperature water soluble (low galactose galactomannans) are in progress.[18]

This low temperature for a maximum in gelation rate may explain LBG's readiness to gel after a freeze/thaw cycle (cryogel).[6] It is proposed that the cryogelation rate of polymers is primarily controlled by two opposing factors: first, the freeze concentration of the polymer solution effectively increasing the rate of gelation and, second, the typical reduction in gelation rate at lower temperatures well below the critical gelling temperature, as cross-link formation becomes diffusion limited. For LBG, the gelation rate does not decrease as the temperature approaches freezing but achieves a maximum at −5 °C.

Table 3 lists all the stress yield data (when tan δ = 1) for the LBG/sucrose systems cured at different temperatures and various LBG and sucrose concentrations. In the case of the cure curves at 5 °C and 10 °C, the systems were cured for 60 h, and consequently, the stress sweeps were performed after 60 h gelation. Although it is difficult to quantify the effect that measurement temperature has upon the yield stress behavior, these values reflected the extent of LBG gelation.

Gelation Dependence upon LBG Concentration. Figure 13 displays tan δ vs time sweeps at −5 °C for a range of LBG concentrations, 1.0−2.0% w/w, at 60% w/w sucrose. At 1.0% w/w LBG/60% w/w sucrose, there was no decrease in tan δ over 60 h. As the concentration of LBG was increased to 1.3% w/w, tan δ increased to a maximum after approximately 7 h and then decayed exponentially. At higher concentrations of LBG, the time over which tan δ reached a maximum decreased,

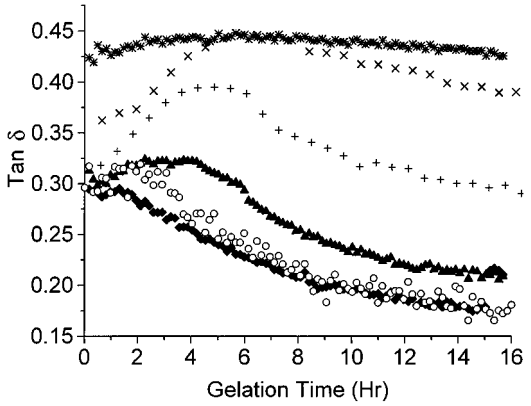

Figure 13. Rheological time sweeps (1 Pa, 0.5 Hz) at −5 °C as a function of LBG concentration (* = 1.0%, × = 1.3%, + = 1.4%, ○ = 1.5%, ?▲ = 1.7%, ◆ = 2.0%) in 60% w/w sucrose.

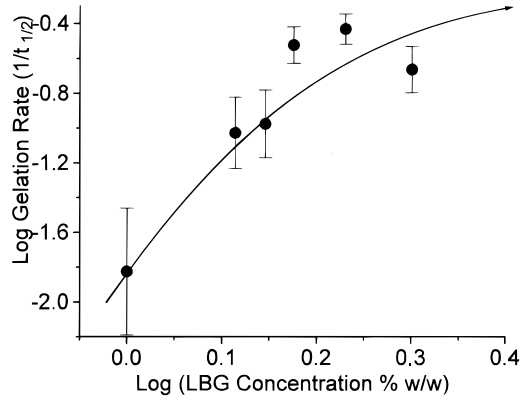

Figure 14. Half-life determined gelation rate as a function of LBG concentration in 60% w/w sucrose at −5 °C.

and the rate of decay increased. Figure 14 displays the gelation rate as a function of LBG concentration at −5 °C in 60% w/w sucrose. The concentration dependence is relatively high at concentrations close to C_o, tending toward unity at higher concentrations. This dependence of gelation rate with concentration is consistent with that predicted by the mean field Cascade formalism[34,35] for gelling polymers. Thus, over the time scale of these measurements, the apparent critical gelling concentration in 60% w/w sucrose was approximately 1.0% w/w, noticeably larger than the 0.2% value in 60% w/v sucrose reported by Dea et al.[6] Furthermore, it is proposed that gelation of LBG through freeze/thawing is a result of achieving this critical LBG gelling concentration in the nonfrozen, freeze concentrated phase.

As shown in Table 3, the yield stress increased as the concentration of LBG increased due to a viscoelastic contribution and a higher extent of gelation at higher LBG concentrations after a fixed time.

LBG Gelation Dependence upon Sucrose Concentration. Figure 15 and Table 3 display tan δ cure curves and the subsequent stress sweeps for 1.5% LBG at −5 °C as a function of sucrose concentration (% w/w), respectively. At 1.5% w/w LBG, over 16 h at −5 °C, sucrose concentrations greater than 40% w/w promoted gelation as detected rheologically. Presumably, the sucrose solution promoted LBG association by reducing the amount of water available for LBG hydration, and through a reduction in solvent quality at these high sucrose concentrations. A maximum in rate of gelation is seen in Figure 15 at 50% w/w sucrose. At higher

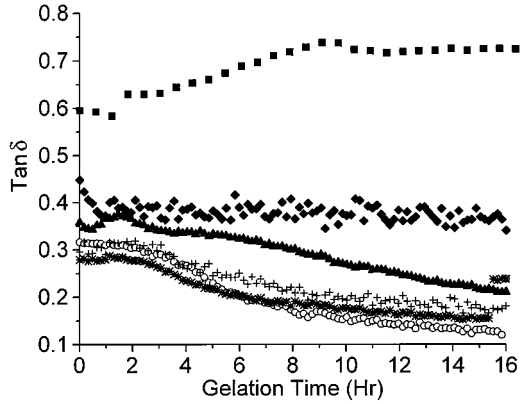

Figure 15. Time sweep (1 Pa stress, 0.5 Hz) at −5 °C for 1.5% w/w LBG in various levels of sucrose concentration (◆ = 40%, ▲ = 45%, ○ = 50%, * = 55%, + = 60%, ■ = 65% w/w).

sucrose concentrations, the gelation rate and the subsequent yield stress values decreased; see Table 3. It is likely that, at these higher sucrose concentrations, gelation of LBG was still promoted by the poor solvent quality of sucrose and the reduction in available water, but a space-filling network was not formed. In fact at 65% sucrose, the rheology resembled more that of a concentrated sucrose solution, presumably with the LBG present as included gel islands. At higher LBG concentrations, it is likely that a space-filling network would be formed at even higher sucrose concentrations, due to an increase in the volume occupied by the gelling polymer. A similar loss in rheological response of the biopolymer at high cosolute concentrations has been reported for gelatin.[10,36]

IV. Conclusions

Locust bean gum behaves as a typical gelling biopolymer. In concentrated sucrose solutions, it has a critical gelling concentration of approximately 1.0% w/w and a maximum in gelation rate close to −5 °C. This maximum in gelation rate with temperature is characteristic of an equilibrium process, and it is proposed that the gelation mechanism of LBG is governed by a frustrated crystallization process with nucleation and growth stages. This mechanism is likely in consideration of the large temperature hysteresis between the maximum gelation rate temperature and the melting temperature. In addition, the initial reduction in storage modulus at higher measured frequencies as the LBG chains disentanglement enabling nuclei formation, is consistent with this model. The storage modulus then increases as the system begins to network. The driving force for association in the initial stages is driven by enthalpic association between galactose free mannan regions on the galactomannan backbone and possibly by a reduction in solvent quality upon cooling to the gelling temperature. Indeed, evidence for the heterogeneous nature of 1.5% w/w LBG, 60% w/w sucrose solution was supplied by a high glass transition temperature of 255 K. Additionally, at higher sucrose concentrations, a tendency for phase inversion was detected as the LBG chains became more included in a concentrated, continuous sucrose solution.

The faster gelling rates for 1.5% w/w LBG, 60% w/w sucrose over previously reported gelation rates for aqueous LBG solutions indicated that sucrose addition effectively decreased the critical gelling concentration of LBG through a poorer solvent quality effect and a reduction in the availability of water. The 1.5% w/w LBG, 60% w/w sucrose solution was successfully characterized using the time/temperature superposition principle. This system did not fit exactly with the WLF model but did give constants, $c_1^g = 7.5$ K and $c_2^g = 50$ K, which were similar to those for other polymeric and low molecular weight systems. These constants, which did not retain their exact meaning due to heterogeneity, nonetheless indicated a high fractional free volume of 5.8% and a high thermal expansion coefficient of 11.6×10^{-4} deg^{-1} at the glass transition temperature. According to the concept that the mobility at any temperature depends primarily upon the free volume remaining, the high free volume present for LBG may be the source of its maximum gelation rate at low temperatures.

The unique maximum in gelation rate at subzero temperatures may explain LBG's readiness to gel after a freeze/thaw cycle. The cryogelation rate of polymers is primarily controlled by two opposing factors: the freeze concentration of the polymer solution, effectively increasing the rate of gelation, and the typical reduction in rate at lower temperatures, owing to a reduction in chain mobility. For LBG, the gelation rate does not decrease as the temperature approaches freezing, but achieves a maximum at −5 °C.

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