Molecular Order versus Vitrification in High-Sugar Blends of Gelatin and κ-Carrageenan

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The understanding of synthetic polymer viscoelasticity was applied to the small deformation properties of high-sugar gelatin and κ-carrageenan mixtures. The glass transition zone in sugar/gelatin mixtures exhibited a dominant liquid-like response, which was followed by the method of reduced variables. The glass transition temperature predicted by the WLF/free volume approach coincided with the crossover of storage and loss modulus at the onset of the glassy state. The viscoelastic spectrum was resolved into a basic function of temperature and a basic function of time, thus rationalizing their effect on the vitrification of the mixture. Vitrification was only one in a plethora of viscoelastic properties. Manipulation of the composition of the mixture generated a continuous gelatin matrix or a viscous sugar phase suspending a dispersion of ice crystals. Besides the small-molecule crystallinity, vitrification can be halted by ordered macromolecular helices observed in the κ-carrageenan/potassium system. Thus, the solid-like component of the sugar/κ-carrageenan network remained prevalent over the predicted frequency window. The WLF equation was unable to follow the progress of viscoelastic functions, which were better described by an energetic barrier of rotation from one state to another.

Keywords: *Gelatin; κ-carrageenan; sugar; glass transition; crystallization; free volume*

INTRODUCTION

In the past 40 years or so, the literature on the structural properties of the main industrial polysaccharides in an aqueous/low-solids environment has flourished (Morris, 1990; Whistler, 1993). In cases when gelation is possible, high-modulus structures are formed at low concentrations of the polysaccharide. X-ray diffraction studies, optical rotation, circular dichroism, calorimetry, and rheology have been used to rationalize the conformational transition which, for the most part, involves conversion from disordered coils in solution to double helices in the gel (Dea, 1993). Association of double helices into large aggregates, that is, the structural knots of a network, results in brittle structures that are distinctly turbid (e.g., agarose). Ionic polysaccharides utilize salt at the secondary (e.g., egg box arrangement in the formation of alginate helices) or tertiary (e.g., domain model in the association of κ-carrageenan helices) level of network building (Seale et al., 1982; Robinson et al., 1980). Aggregates remain thermally stable at temperatures higher than those at which helices are formed on cooling with the gels, showing substantial thermal hysteresis (Morris and Norton, 1983).

Recently, the structural properties of commercially important gelling polysaccharides at normal levels of use (i.e., <1%) were examined in the presence of high levels of sugar (glucose syrup or in mixture with sucrose). Differential scanning calorimetry in combination with large deformation compression testing argues that increasing amounts of cosolute induce a transformation from an enthalpic aggregated structure to a lightly cross-linked network of flexible chains (Evageliou et al., 1998). Cooling of the rubbery gels sees an increase in the small-deformation shear moduli of ~5 orders of magnitude. Meanwhile, the viscous response overtakes the solid component of the network as seen in the entropic transition from rubbery to glassy consistency in amorphous synthetic polymers (Al-Ruqaie et al., 1997).

Vitrification was equivalently followed by changing either the temperature or the frequency of measurement (time−temperature superposition principle; TTS), leading to construction of muster curves of viscoelastic functions valid outside the experimental window (Tsoga et al., 1999). Thus, treatment with the combined Williams−Landel−Ferry/free volume theory allows calculation of useful parameters such as the glass transition temperature, fractional free volume, and thermal expansion coefficient as historically exploited to great effect in synthetic polymer science (Ferry, 1980; Kasapis, 1998).

The purpose of the present paper is twofold: first, to conclude the aforementioned series of investigations by documenting that gelatin in the presence of high solids undergoes vitrification as followed by the WLF/free volume approach, and, second, to demonstrate that the process of vitrification in high-sugar/biopolymer mixtures can be readily disturbed by increasing the presence of regularly ordered helices or crystallization of the cosolute/solvent. Besides the scientific interest that this topic stimulates, there are issues of commercial importance related to the confectionery industry. A predictive

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model relating biopolymer conformational characteristics to the vitrification/crystallinity of the product will allow development of formulations for novel rubbery confectioneries containing otherwise brittle polysaccharide networks. A spin-off from this process is the replacement of gelatin with polysaccharides with a view to reducing the polymer concentrations required and circumventing diet and health problems (BSE; vegetarianism; religion).

MATERIALS AND METHODS

Materials. Gelatin was a research sample from Sanofi Bio-Industries, Baupte, Carentan, France. It was the first extract from pigskin produced by acidic hydrolysis of collagen (type A). The isoelectric point was measured by completely deionizing the sample on a bed of ion-exchange resin and then measuring the pH of the eluant. It was found to be pH 8.7. Gel permeation chromatography produced a weight-average molecular mass of 162400 Da, a number-average molecular mass of 68000 Da, and the percent weight of 10 molecular mass classes:

molecular mass $>10^6$	1.17%
molecular mass >540 kDa	4.19%
tetra- plus penta-	5.79%
γ	7.43%
β	21.58%
α	24.38%
subunits 1	12.85%
subunits 2	12.06%
subunits 3	4.98%
subunits 4	5.6%

The Bloom value of the sample was 305 g. It contained 8% moisture, 80 ppm of calcium, 53 ppm of phosphate, 0.16% chloride, and <0.1% sulfate.

The sample of κ-carrageenan was a gift from Hercules, Lille-Skensved, Denmark. ^{1}H NMR analysis showed that ι-carrageenan-like segments, that is, with a sulfate group at position 2 of the 3,6-anhydrite residue, constitute ∼8% of the polymer. An Amberlite IR-120 exchanging resin from BDH was used to prepare the polysaccharide in the potassium form. Details of the procedure have been given previously (Evageliou et al., 1998). Intrinsic viscosity measurements of the chain in the potassium form obtained at 40 °C and a constant ionic strength (0.01 M KCl) yielded a value of 10.5 ± 0.2 dL g^{-1}.

The glucose syrup used was a product of Cerestar, Vilvoorde, Belgium. The dextrose equivalent of the sample was ∼42. The total level of solids was 83%, and glucose syrup compositions in this work refer to dry solids. Gel permeation chromatography showed an extensive degree of polydispersity, which prevents crystallization of the sample at subzero temperatures (Ong et al., 1998).

Rheological Methods. The polysaccharide was dissolved in distilled water at 90 °C using vigorous agitation for 30 min, whereas the solvation temperature of the protein did not exceed 65 °C. Appropriate amounts of sucrose and glucose syrup, or glucose syrup alone, were added, and excess water was heated away to bring the total level of solids in the range of 60−85%. In the case of κ-carrageenan, 20 mM KCl was added to facilitate network formation.

Rheometers used in this work include a controlled stress Carri-Med CSL500, a controlled strain Advanced Rheometric Expansion System (ARES), and a sensitive in-house prototype fitted out with parallel plates ranging in diameter from 50 to 4 mm. The extensive working range allowed accurate characterization of the low-viscosity melts or the viscoelasticity of soft gels at the upper range of temperature while eliminating any inherent machine compliance in the high-modulus glassy state. Thus, we covered a modulus range of 10 orders of magnitude, that is, from about 10^0 to 10^{10} Pa.

Samples were cooled and then heated at 2 °C/min between 90 and −55 °C, taking frequency sweeps at constant temperature intervals. Temperature was controlled with an air convection oven using a dual-element heater for counter-rotating air flow. The measuring gap between the parallel plates was 2 mm, and the exposed edges of the sample were covered with silicone fluid to prevent evaporation. A high-resolution actuator allows application of low deformation (0.0001 unit of strain) so that slippage is avoided when the material turns semicrystalline or glassy. Particular care was taken during sample preparation and loading which ensured that duplicate runs of selected samples reproduced the rubber-to-glass transformation within a 3% margin.

RESULTS AND DISCUSSION

Glassy Phenomena in High-Sugar/Gelatin Mixtures. Much research has been done in the past to unravel the effect of temperature on aqueous gelatin systems (Flory and Weaver, 1960; Harrington and Von Hippel, 1961). It has now been accepted that at low temperatures gelation will occur, which involves the partial regeneration of the helical collagen molecule with the ordered junction zones being linked together by residual soluble, disordered regions. The first step, which is the rate-limiting one, involves the formation of a nucleus. This occurs at a β-bend, bringing together two strands of the same molecule. This "hairpin" structure then collides with a third strand, thereby forming the nucleus (Djabourov et al., 1985; Busnel et al., 1988). The gelatin network supports the water molecules, but at subzero temperatures their inherent mobility leads to ice formation, seen as a sharp increase in the values of storage modulus during an oscillatory cooling run.

In this study, we replaced most of the water molecules with sugar (80%) and recorded the response of the gelatin network in the high-solids environment. Preliminary experiments showed that the lifetime of relaxation processes is determined primarily by the applied cooling regime. Thus, at a fixed temperature within the glass transition region practically no configurational changes occur within the period of deformation. Furthermore, mechanical spectra were recorded at constant temperature intervals of 4 °C during heating from the glassy to the rubbery state, which is an approximate equilibrium process. It turns out that the mechanical spectra within the temperature range of 9 to −55 °C superimpose into a single muster curve. In doing so, 9 °C was chosen arbitrarily as the reference temperature (T_0) and the remaining mechanical spectra of storage (G') and loss (G'') modulus were shifted horizontally along the log frequency axis of Figure 1.

Experimental frequency sweeps typically cover 3 or 4 decades between 10^{-2} and 10^2 rad s^{-1}, but, as illustrated in Figure 1, the above treatment generates results that spread over 14 orders of magnitude. The high-sugar/gelatin mixture shows a transformation from the low-modulus plateau region of the rubbery gel at the low range of frequency to the glass transition, at which the viscous component of the network (G'') becomes dominant. A fully developed glassy state was obtained at frequencies $>10^8$ rad s^{-1}, with the solid-like response (G') dominating once more and achieving values around 10^{10} Pa. Thus, the muster curve emanating from the method of reduced variables (otherwise known as TTS) predicts the small deformation properties of the system beyond the narrow frequency range available at a single temperature (9 °C in Figure 1). If desired, the time−temperature superposition can be implemented at the opposite temperature end, thus unveiling the flow properties of a sample over several
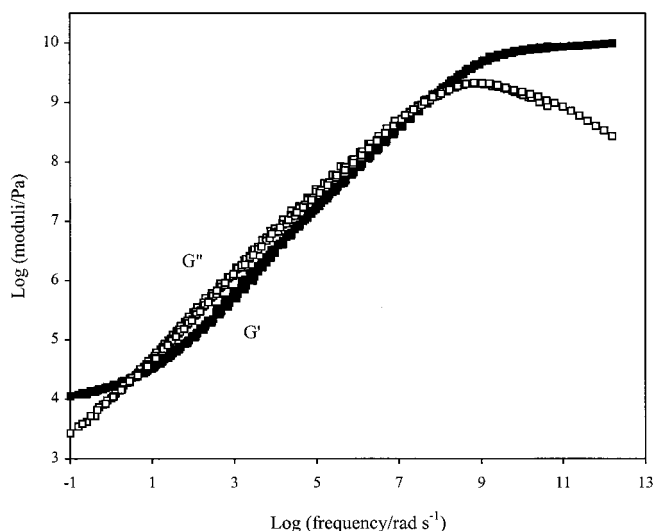
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Figure 1. Muster curve of shear moduli for 5% gelatin in the presence of 50% glucose syrup and 30% sucrose. Frequency sweeps covered the temperature range from 9 to −55 °C and were superimposed at 9 °C.
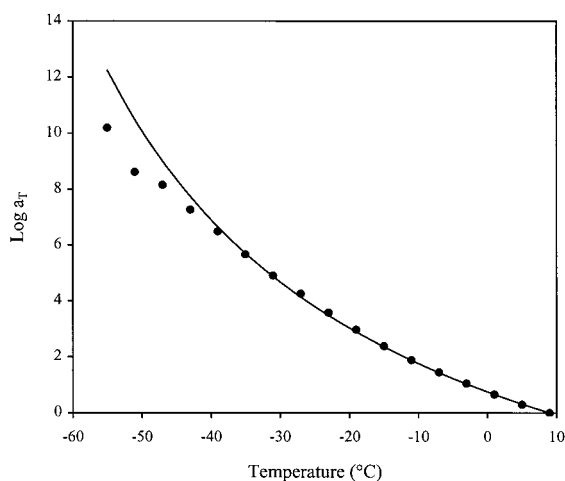

Figure 2. Development of shift factors as a function of temperature with the exponential reflecting the WLF fit. Sample and conditions were as in Figure 1.

decades of storage time [e.g., up to 10^{13} s at −55 °C since frequency (Hz) = 1/time (s)].

Functions of Time and Temperature in the Vitrification of High-Sugar/Gelatin Mixtures. The adjacent frequency sweeps used to create the muster curve of Figure 1 showed significant overlapping and good matching of shape. Furthermore, the same extent of shifting superposed all of the viscoelastic functions of the mixture, thus generating a single shift factor (a_T) for both the G' and G'' traces. In the absence of a single thermal effect on the long and short retardation contributions to the viscoelastic spectrum, attempts to force a fit may generate a set of shift factors for G' and another one for G'' or result in a diagonal superposition of the mechanical spectra, but the link between that type of analysis and fundamental understanding is rather tenuous. Figure 2 illustrates how much the frequency scale has been shifted with cooling starting from the reference temperature of 9 °C (shift factor is equal to 1).

In agreement with work on amorphous synthetic polymers, the combined WLF/free volume approach produced the best fit for the data. Voids between single
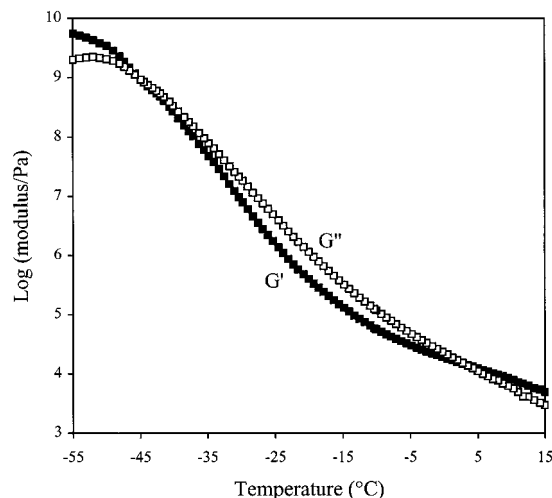

Figure 3. Thermal runs of storage and loss modulus for the sample of Figure 1 (frequency = 1 rad/s; scan rate = 2 °C/min).

or polymeric molecules due to thermal oscillations or packing irregularities constitute the free volume, which at the glass transition temperature (T_g) collapses to a very small amount of the total volume of the molecule (Shen and Eisenberg, 1967). The WLF equation is able to follow the change in free volume with temperature in the following form (Williams et al., 1955):

$$\log a_T = G(T)/G(T_o) = -C_1^o(T - T_o)/(C_2^o + T - T_o) \quad (1)$$

where $C_1^o = B/2.303f_0$ and $C_2^o = f_0/\alpha_f$. The fractional free volume, f_0, is the ratio of free to total volume of the molecule, α_f is the thermal expansion coefficient, and B is usually set to one. Ferry and colleagues fit the shift factors by plotting $1/\log a_T$ against $1/(T - T_o)$ and obtaining the two constants (C_1^o and C_2^o) from the slope and intercept of the resulting straight line. Today, proprietary software can be purchased with the rheometer to implement the WLF fit.

The corresponding parameters for the vitrification of the high-sugar/gelatin mixture are T_g = −46 °C and $\alpha_f = 5.2 \times 10^{-4}$ deg^{-1}. At the glass transition temperature, f_g, C_1^g and C_2^g are equal to 0.026, 17.1, and 50 deg, respectively. Thus, the fractional free volume at the glass transition temperature is 2.6% of the total volume of the mixture and agrees well with predictions for synthetic polymers and diluted systems [between 1.7 and 3.9% in Ferry (1980)]. Continuous cooling or heating of the sample at a constant rate of 2 °C/min reproduces the passage from the rubbery to the glassy state through the glass transition, shown in Figure 1 as a function of frequency of oscillation. Thus, in Figure 3, the second crossover of moduli occurs at ∼ −44 °C, that is, close to the value of T_g predicted by the WLF fit. It appears, therefore, that the onset of the glassy state represents an accurate index of the glass transition temperature, albeit a frequency-dependent one, where the free volume collapses and the only residual contraction is of solid-like character (G' becomes higher than G''). As depicted in Figure 2, the WLF fit follows the development of shift factors throughout the glass transition region but increasingly fails to do so at temperatures < −44 °C (i.e., within the glassy state). Therefore, the T_g constitutes a true boundary between molecular adjustments occurring within the normal experimental times of observation or beyond, thus giving rise to the glassy state.
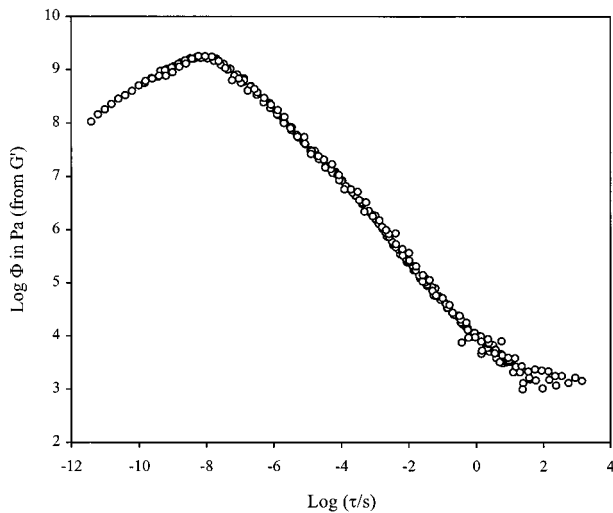
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Figure 4. Distribution function of relaxation times as a function of time scale of observation. Sample and conditions were as in Figure 1.
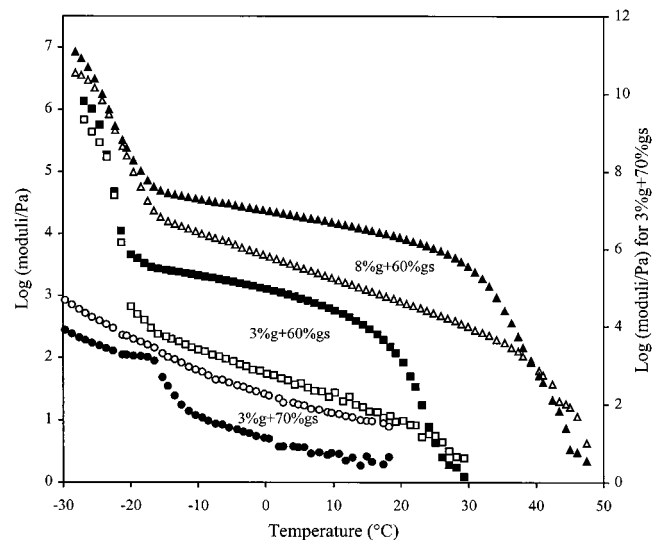

Figure 5. Temperature variation of G' (solid symbols) and G'' (open symbols) for three gelatin samples in the presence of glucose syrup. The sample of 3% gelatin plus 70% glucose syrup is shown on the right y-axis (cooling rate = 1 °C/min; frequency = 1 rad s^{-1}).

In theories of the temperature dependence of viscoelasticity, an attempt is made to identify the energy required (ΔH_a) for the relaxation of points of entanglement (Ferry, 1980). In the case of vitrification, the parameter ΔH_a is associated with the difficulty for large-scale vibrational motions of macromolecules (requiring free volume) to occur and can be calculated by the WLF equation as follows:

$$\Delta H_a = R \, \mathrm{d} \ln a_T / \mathrm{d} \, (1/T) =$$
$$2.303 R C_1^o C_2^o T^2 / (C_2^o + T - T_o)^2 \quad (2)$$

Application of our data to the above equation yields a vitrification energy, which increases rapidly with decreasing temperature (graph is not shown). This, of course, is against the predictions of reaction-rate theory, which advocates a linear relationship of the logarithm of shift factors with temperature, hence, a temperature-independent gradient of energy of activation. At the glass transition temperature, the value of ΔH_a ($\sim$80 kcal) is consistent with experience from the synthetic polymer work (of the order of 87 kcal for the same T_g; Ferry, 1980), a result that further emphasizes the predominance of free volume effects regardless of the chemical structure in the glass transition region.

For comparing the effect of time on various systems, the distribution function of relaxation times (Φ) is preferred because it can be derived from dynamic oscillatory or stress relaxation data. It is calculated as follows (Ferry and Fitzgerald, 1953):

$$\Phi(-\ln \omega) = G' \, (\mathrm{d} \log G' / \mathrm{d} \log \omega) =$$
$$G'' \, (1 - \mathrm{d} \log G'' / \mathrm{d} \log \omega) \quad (3)$$

The storage and loss moduli are independent at a single frequency, but a carefully performed experiment should yield the same values of Φ for both G' and G'', which affords an internal check of the validity of the applied methodology. The storage and loss moduli of the frequency sweeps used for the construction of the muster curve in Figure 1 yield, indeed, similar values for the distribution function.

Figure 4 illustrates the logarithmic plot of Φ versus experimental time for the high-sugar/gelatin mixture. As far as we are aware, this is the first distribution function of relaxation times for a high-solids/biopolymer system covering three of four regions of the viscoelastic spectrum. The main part of the curve is a steep portion where the consistency of the sample is changing with increasing time from a hard glass to a soft rubber. The flat region corresponds to configurational movements of entire molecules which are capable of holding together a three-dimensional structure within the time scale of observation. At extremely short times, only stretching and bending of chemical bonds is allowed, which leads to a flat G' trace in the glassy state (Frick and Richter, 1995). Thus, the contribution to instantaneous rigidity between $\ln \tau$ and $\ln \tau + \mathrm{d} \ln \tau$ becomes negligible and the values of Φ must fall. This is shown in Figure 4 at times <10^{-8} s.

Development of Molecular Order in High-Sugar Gelatin and κ-Carrageenan Mixtures. In the present section we have explored the effect of biopolymer/cosolute composition on the structural properties of a mixture. It, soon, transpired that vitrification was only one of the many facets of this question. In general, reduction of the total amount of solids in the system increases the molecular mobility of individual species, with the behavior of the solvent being prevalent at subzero temperatures. Figure 5 shows the changes in shear moduli as a function of cooling observed for gelatin/glucose syrup samples with 1.8–2.5 times more water than for the preparation of Figure 1. In the case of 8% gelatin plus 60% glucose syrup, development of structure commences at relatively high temperatures, leading to a gel with a solid component approaching 10^5 Pa. The process of consolidating the gelatin network is interrupted at −15 °C by a second step of structure formation.

Reduction in the protein content to 3% delays the onset of network formation to 24 °C, as judged by the frequency-dependent marker of G' overtaking the G'', whereas in the presence of 8% gelatin it occurs at 38 °C. There is also a considerable reduction in the values of storage modulus of the gelatin network that do not exceed 10$^{3.5}$ Pa. Nevertheless, the second transition re-emerges at the same subzero temperatures and generates substantial mechanical strength in the mixture.
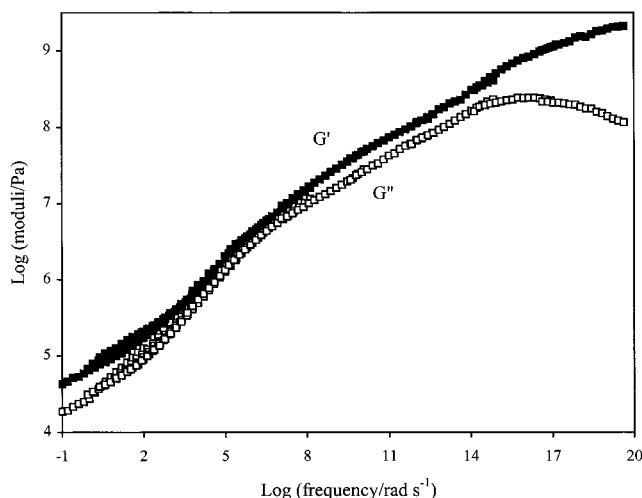
Figure 6. Muster curve of shear moduli for 1% κ-carrageenan in the presence of 84% glucose syrup and 20 mM KCl. Frequency sweeps covered the temperature range from 90 to -30 °C and were superimposed at 90 °C.

The absence of substantial order in the preparation of Figure 1 and its subsequent vitrification is a consequence of the immobility/high viscosity of the solute. In Figure 5, on the other hand, solute molecules can move out of the way, thus allowing water to freeze and ice crystals to grow. Finally, an increase of 10% in the content of glucose syrup results in a phase inversion from a gelatin network imbedded with ice crystals to a cosolute continuous phase. The mixture now shows an overall liquid-like response, but water freezing still occurs in the form of ice crystals suspended within the viscous phase of glucose syrup (observe the rise in values of G' at ≈ -13 °C).

Besides the small-molecule crystallinity, macromolecular order should be able to prevent the formation of a true glass. This is discussed for the high-sugar/κ-carrageenan mixtures. In an earlier paper, we verified that cooling of 0.5% κ-carrageenan solutions in the presence of 10 mM KCl resulted in strong enthalpic-aggregated gels (Evageliou et al., 1998). Ions of potassium support the formation of aggregates, which are further stabilized by a hydration layer around them. Incorporation of 85% glucose syrup altered dramatically the physical properties of the system that formed a partially cross-linked gel of reduced strength. Thus, the scarcity of water molecules prevents extensive formation of ordered associations of κ-carrageenan at 10 mM KCl. The entropic network exhibited vitrification properties along the lines described in Figure 1, a result that further argues for the universality of the WLF/free volume approach in high-sugar/biopolymer mixtures.

Results reported in Figure 6 are for a high-solids system with double the amount of polysaccharide and added salt, that is, 1% κ-carrageenan in the presence of 20 mM KCl. This time, the abundance of polymer and counterions offsets the scarcity of stabilizing water molecules and generates additional intermolecular associations, which are able to form a cohesive gel at 90 °C. Frequency sweeps were recorded at constant temperature intervals of 10 °C to construct a muster curve. The extensive temperature range of observation (from 90 to -30 °C) allows elucidation of the frequency/time function over 21 orders of magnitude. The time–temperature superposition uses 90 °C as the reference
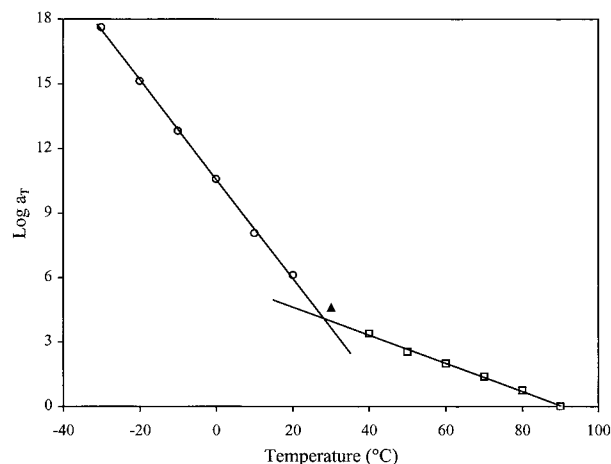

Figure 7. Development of shift factors as a function of temperature with the straight lines reflecting Arrhenius fits. Sample and conditions were as in Figure 6.

temperature and demonstrates a disproportionate increase in the viscous component of the network within the frequency range of $10^{-1}-10^4$ rad s^{-1}. However, the expected crossover of moduli, which signifies the onset of the glass transition, does not materialize, with both traces running for most of the spectrum parallel to each other. Eventually, the mechanical response is that of a hard solid with the values of G' approaching $10^{9.5}$ Pa.

To identify the temperature dependence of all relaxation times in our system, the factor a_T was plotted against temperature in Figure 7. Clearly, the extent of how much the frequency scale shifts with temperature follows two distinct profiles. The WLF equation was unable to provide a basic function of behavior for the entirety or the two subsets of data. For each part of the spectrum, however, the factor a_T was an exponential function of the reciprocal absolute temperature expressed in the familiar form:

$$a_T = K \exp(\Delta H_a/RT) \qquad (4)$$

Equation 4 was used to calculate numerical estimates for the energy of activation, which is independent of temperature. These were 142.4 and 315.5 kJ/mol for the top (40–90 °C) and bottom (-30 to 20 °C) temperature ranges, respectively.

Values are of similar magnitude to the prediction for the intermolecular associations of 1% high-methoxy pectin plus 77% glucose syrup [122.4 kJ/mol in Kasapis (1999)]. The former should be associated with relaxation processes that dissipate energy and contribute mainly to liquid-like character (convergence of G' and G'' traces in Figure 6). Overall, however, conformational freedom is substantially curtailed by the presence of ordered helices of carrageenan assisted by the increasing levels of counterion in the mixture (G' remains above G''). Finally, the estimate of 315.5 kJ/mol should reflect the increasing difficulty in overcoming an energetic barrier for the occurrence of local rearrangements from one state to the other.

LITERATURE CITED

Al-Ruqaie, I. M.; Kasapis, S.; Richardson, R. K.; Mitchell, G. The Glass Transition Zone in High Solids Pectin and Gellan Preparations. *Polymer* **1997**, *38*, 5685–5694.

Busnel, J. P.; Clegg, S. M.; Morris, E. R. Melting Behaviour of Gelatin Gels: Origin and Control. In *Gums and Stabilisers for the Food Industry IV*; Phillips, G. O., Wedlock, D. J., Williams, P. A., Eds.; IRL Press: Oxford, U.K., 1988; pp 105−115.

Dea, I. C. M. Conformational Origins of Polysaccharide Solution and Gel Properties. In *Industrial Gums*; Whistler, R. L., BeMiller, J. N., Eds.; Academic Press: San Diego, CA, 1993; pp 21−52.

Djabourov, M.; Maquet, J.; Theveneau, H.; Leblond, J.; Papon, P. Kinetics of Gelation of Gelatin Solutions. *Br. Polym. J.* **1985**, *17*, 169−174.

Evageliou, V.; Kasapis, S.; Hember, M. W. N. Vitrification of κ-Carrageenan in the Presence of High Levels of Glucose Syrup. *Polymer* **1998**, *39*, 3909−3917.

Ferry, J. D. Dependence of Viscoelastic Behavior on Temperature and Pressure. In *Viscoelastic Properties of Polymers*; Wiley: New York, 1980; pp 264−320.

Ferry, J. D.; Fitzgerald, E. R. Mechanical and Electrical Relaxation Distribution Functions of Two Compositions of Polyvinyl Chloride and Dimethylthianthrene. *J. Colloid Sci.* **1953**, *8*, 224−242.

Flory, P. J.; Weaver, E. S. Helix-Coil Transitions in Dilute Aqueous Collagen Solutions. *J. Am. Chem. Soc.* **1960**, *82*, 4518−4525.

Frick, B.; Richter, D. The Microscopic Basis of the Glass Transition in Polymers from Neutron Scattering Studies. *Science* **1995**, *267*, 1939−1947.

Harrington, W. F.; Von Hippel, P. H. Formation and Stabilisation of the Collagen-Fold. *Arch. Biochem. Biophys.* **1961**, *92*, 100−113.

Kasapis, S. Structural Properties of High Solids Biopolymer Systems. In *Functional Properties of Food Macromolecules*; Aspen Publishers: Gaithersburg, MD, 1998; pp 227−251.

Kasapis, S. Viscoelasticity of oxidised starch and low methoxy pectin in the presence of glucose syrup. *Agric. Sci.* **1999**, *4*, 63−71.

Morris, E. R. Mixed Polymer Gels. In *Food Gels*; Harris, P., Ed.; Elsevier: London, U.K., 1990; pp 291−359.

Morris, E. R.; Norton, I. T. Polysaccharide Aggregation in Solutions and Gels. In *Aggregation Processes in Solution*; Wyn-Jones, E., Gormally, J., Eds.; Elsevier: Amsterdam, The Netherlands, 1983; pp 549−593.

Ong, M. H.; Whitehouse, A. S.; Abeysekera, R.; Al-Ruqaie, I. M.; Kasapis, S. Glass Transition-Related or Crystalline Forms in the Structural Properties of Gelatin/Oxidised Starch/Glucose Syrup Mixtures. *Food Hydrocolloids* **1998**, *12*, 273−281.

Robinson, G.; Morris, E. R.; Rees, D. A. Role of Double Helices in Carrageenan Gelation: The Domain Model. *J. Chem. Soc., Chem. Commun.* **1980**, 152−153.

Seale, R.; Morris, E. R.; Rees, D. A. Interactions of Alginates with Univalent Cations. *Carbohydr. Res.* **1982**, *110*, 101−112.

Shen, M. C.; Eisenberg, A. Glass Transitions in Polymers. In *Progress in Solid State Chemistry*; Reiss, H., Ed.; Pergamon Press: Oxford, U.K., 1967; pp 407−481.

Tsoga, A.; Kasapis, S.; Richardson, R. K. The Rubber-to-Glass Transition in High Sugar Agarose Systems. *Biopolymers* **1999**, *49*, 267−275.

Whistler, R. L. Introduction to Industrial Gums. In *Industrial Gums*; Whistler, R. L., BeMiller, J. N., Eds.; Academic Press: San Diego, CA, 1993; pp 1−19.

Williams, M. L.; Landel, R. F.; Ferry, J. D. The Temperature Dependence of Relaxation Mechanisms in Amorphous Polymers and Other Glass-forming Liquids. *J. Am. Chem. Soc.* **1955**, *77*, 3701−3707.