

Kinetics of a Bioactive Compound (Caffeine) Mobility at the Vicinity of the Mechanical Glass Transition Temperature Induced by Gelling Polysaccharide

Bin Jiang[†] and Stefan Kasapis^{*,†}

[†]Department of Chemistry, National University of Singapore, Science Drive 3, Singapore 117543

^{*}School of Applied Sciences, RMIT University, City Campus, Melbourne, Vic 3001, Australia

ABSTRACT: An investigation of the diffusional mobility of a bioactive compound (caffeine) within the high-solid (80.0% w/w) matrices of glucose syrup and κ -carrageenan plus glucose syrup exhibiting distinct mechanical glass transition properties is reported. The experimental temperature range was from 20 to -60 °C, and the techniques of modulated differential scanning calorimetry, small deformation dynamic oscillation in shear, and UV spectrometry were employed. Calorimetric and mechanical measurements were complementary in recording the relaxation dynamics of high-solid matrices upon controlled heating. Predictions of the reaction rate theory and the combined WLF/free volume framework were further utilized to pinpoint the glass transition temperature (T_g) of the two matrices in the softening dispersion. Independent of composition, calorimetry yielded similar T_g predictions for both matrices at this level of solids. Mechanical experimentation, however, was able to detect the effect of adding gelling polysaccharide to glucose syrup as an accelerated pattern of vitrification leading to a higher value of T_g . Kinetic rates of caffeine diffusion within the experimental temperature range were taken with UV spectroscopy. These demonstrated the pronounced effect of the gelling κ -carrageenan/glucose syrup mixture to retard diffusion of the bioactive compound near the mechanical T_g . Modeling of the diffusional mobility of caffeine produced activation energy and fractional free-volume estimates, which were distinct from those of the carbohydrate matrix within the glass transition region. This result emphasizes the importance of molecular interactions between macromolecular matrix and small bioactive compound in glass-related relaxation phenomena.

KEYWORDS: κ -Carrageenan, glucose syrup, caffeine, mechanical T_g

INTRODUCTION

Vitrification or glass transition is a kinetic process originating from limitations on the rates of internal molecular adjustments due to variations of temperature, time scale of observation, mechanical stress, or hydrodynamic pressure.¹ Molecules are unable to rearrange themselves into an ordered or crystalline form but, instead, become “frozen” in the molecular arrangement of a liquid with a very high viscosity (about 10^{12} Pa s), which virtually stops them from flowing within experimental constraints. A connection between high viscosity and limited molecular mobility in glassy systems was readily made, and the concept of glass transition temperature (T_g) was proposed to be the temperature that controls the rate of chemical, enzymatic, and biological processes below which these processes are thought to proceed extremely slowly.^{2,3}

Applications of the concept of the glass transition temperature have been well documented in synthetic polymers and organic solids used as common glasses and plastics and in biomaterials such as starches and their hydrolysates, dietary fiber, and proteins. These have been used to deliver a range of properties including texture, processability, storage stability, delivery control, etc.^{4,5} In food-related processes where water and/or sugar act as plasticizers of proteins and polysaccharides, modulated differential scanning calorimetry (MDSC) has been the technique of choice to measure T_g .⁶ Increasingly, mechanical spectroscopy in the form of small deformation dynamic oscillation and stress relaxation has been employed to also provide estimates

of the glass transition temperature in single or mixed preparations of macromolecules in the presence of cosolute.⁷

The mechanical approach provides viscosity values for materials via the well-known Stokes–Einstein equation, which is coupled to rates of translational diffusion in fluids and the temperature dependence of free volume, as described by the mathematical expression of Williams, Landel, and Ferry.⁸ For example, the combined theoretical framework of free volume and WLF equation has been used to monitor the glass transition region kinetics of the translational diffusion of fluorescein in amorphous mixtures or sucrose–water and maltose–water.^{9,10} Relatively complex chemical reactions at the vicinity of the glass transition temperature were also evaluated, including nonenzymatic browning, aspartame degradation, high-methoxy pectin de-esterification, etc.^{11–15}

Clearly, chemical reaction pathways and enzymatic processes are critical for the improvement of stability and quality control of processed foods. However, there have been drawbacks in understanding the molecular dynamics of carbohydrates and proteins as stabilization or delivery matrices at the vicinity of T_g . Application of the free volume theory utilizing the amplitude of the difference between glass transition and experimental temperature

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($T - T_g$) in an effort to monitor reaction progress often has been met with erroneous results. The main reason for this appears to be the inability of the so-called “universal” C_1 and C_2 parameters of the WLF equation to describe the structural complexity of various biopolymers, thus reporting in the literature negative estimates or physically unrealistic high values of free volume. Within this context, a recent communication utilized the viscoelastic approach to produce theoretically valid estimates of WLF C_1 and C_2 parameters that relate to free volume characteristics of specific delivery matrices.¹⁶

Another and increasingly critical constraint is the inability to make a link between recent advances in fundamental knowledge and the direct application to product situations with a growing need for scientific input. Utilization of single systems of small polyhydroxyl compounds, for example, sugars, sugar alcohols, or trehalose, does not meet fully the need for technology transfer from the materials science of (aqueous) solid solutions to the functional attributes of established and yet complex or novel foodstuffs with their multifaceted challenges. In an effort to address aspects of controlled delivery in model food materials with superior functionality, the present investigation introduces a gelling biopolymer (κ -carrageenan) to a liquid carbohydrate environment (glucose syrup) and combines mechanical and UV spectroscopy in the experimental protocol. As far as we are aware, this is the first time that a binary composite of gelling biopolymer/cosolute is used to provide mechanistic understanding of the kinetics of diffusional mobility in a glassy matrix that incorporates caffeine as the bioactive compound.

EXPERIMENTAL PROCEDURES

Materials. κ -Carrageenan was a gift from Hercules (Lille Skensved, Denmark). ¹H NMR analysis showed that ι -carrageenan-like segments (i.e., with a sulfate group at position 2 of the 3,6-anhydride residue) constitute about 8.0% of the polymer. An Amberlite IR-120 exchanging resin from BDH was used to prepare the polysaccharide in the potassium form, with details of the ion-exchange method having been reported earlier.¹⁷ κ -Carrageenan in the potassium form was characterized with intrinsic viscosity measurements, $[\eta]$, at constant ionic strength (0.01 M KCl) and 40 °C yielding $[\eta] = 10.5 \pm 0.2$ dL/g.

Glucose syrup was a gift from Cerestar (Mechelen, Belgium). It is a nonsweet saccharide system containing glucose sequences linked by α -(1 \rightarrow 4) and α -(1 \rightarrow 6) glycosidic linkages. Dextrose equivalent (DE) gives the content of reducing end groups relative to glucose as 100, with the DE of our sample being 42. The level of solids is 83.0% (w/w), and the water content of glucose syrup was taken into account in the calculation of the total level of solids for sample preparation. GPC analysis provided by the manufacturer characterized the relationship between degree of polymerization (DP) and surface area (%) of the glucose syrup spectrum, and this molecular weight distribution has been published earlier.¹⁸ The material is a clear and stable solution at temperatures above 0 °C and becomes a transparent glass on further cooling.

Caffeine [1,3,7-trimethyl-1H-purine-2,6(3H,7H)-dione; $C_8H_{10}N_4O_2$] is a drug that acts as a psychoactive stimulant and diuretic in humans. It was purchased from Sigma-Aldrich (St. Louis, MO) in the form of a white powder at a ReagentPlus grade.

Dichloromethane (DCM, CH_2Cl_2) is widely used as a solvent since it is one of the less harmful of chlorocarbons. Melting and boiling points are -96.7 and 39.8 °C, respectively, thus facilitating experimentation within the temperature range of the UV spectroscopy investigation (-50 to 20 °C). It was purchased as an anhydrous colorless liquid from Sigma-Aldrich.

Sample Preparation. Glucose syrup preparations at 80% (w/w) solids were made by diluting the purchased material of 83.0% (w/w) solids with Milli-Q water while stirring gently and heating at 70 °C. Glucose syrup/caffeine preparations were made by dispersing appropriate amounts of 1.0% (w/w) caffeine solution to diluted glucose syrup solutions at 50 °C with gentle stirring to achieve a final concentration of 79.6% (w/w) carbohydrate and 0.4% (w/w) bioactive compound (80.0% w/w total solids).

κ -Carrageenan/glucose syrup preparations were made by dissolving first the polysaccharide in deionized water at 80 °C with gentle stirring for 10 min. The temperature was then dropped to 70 °C for the addition of appropriate amounts of cosolute (glucose syrup). To the homogeneous mixture, a counterion solution was introduced to obtain 10 mM KCl in the final system. In doing so, excess water was evaporated off to bring the total level of solids to 80.0% (w/w) comprising 0.5% (w/w) κ -carrageenan and 79.5% (w/w) glucose syrup.

Finally, high-solid κ -carrageenan/glucose syrup preparations in the presence of caffeine were made as in the preceding paragraph and by adding appropriate amounts of the bioactive compound to obtain a final mixture of 0.5% (w/w) κ -carrageenan (10 mM KCl) with 79.1% (w/w) glucose syrup and 0.4% (w/w) caffeine (80.0% w/w total solids).

Measurements. *MDSC.* MDSC was used to analyze the four types of samples described in the preceding section. These were weighted to about 15 mg, sealed hermetically in Alod-Al pans, and subjected to MDSC measurements (Q1000 DSC, TA Instruments, New Castle, DE). An empty sample pan was used as a reference, and nitrogen was supplied as a purge gas at the flow rate of 50 mL/min. T_{zero} calibration was performed by heating the cells without pans in the temperature range of interest. Cell constant and temperature calibration was performed with indium and Milli-Q water at the heating rate of 2 °C min⁻¹, and heat capacity was calibrated using sapphire. Samples were cooled from 20 to -90 °C at the underlying rate of 2 °C min⁻¹ and then heated up to 0 °C at the same scan rate with a modulation period of 60 s and amplitude ± 1 °C. Measurements were carried out in triplicates, yielding effectively overlapping traces.

Small Deformation Dynamic Oscillation Measurements in Shear. Small deformation dynamic oscillation measurements in shear were performed with the Advanced Rheometrics Expansion System (ARES), which is a controlled strain rheometer (TA Instruments). A parallel plate geometry of 8 mm diameter was used, and the gap was kept at 2 mm. Samples were loaded onto the preheated platen of the rheometer at 60 °C immediately after preparation, and their exposed edges were covered with a silicone fluid from BDH (50 cS) to minimize water loss. They were subjected to a controlled cooling or heating run at 2 °C min⁻¹, frequency of 1 rad s⁻¹, and a strain amplitude that varied from 0.00072% in the glassy state to 2.0% in the rubbery plateau or flow region to accommodate the huge changes in the measured stiffness of the sample. The experimental temperature range was between 20 and -60 °C. Analysis provides the temperature variation in storage (G') and loss (G'') modulus, which are the elastic and viscous components of the network that forms upon cooling and softens upon heating.

In a second set of experiments, isothermal frequency dependence of dynamic oscillatory moduli was assessed by recording mechanical spectra from 0.1 to 100 rad s⁻¹ throughout the experimental temperature range of interest. This extended from -55 to -4 °C to cover the region of glassy consistency and data were recorded at constant temperature intervals of 3 °C. Three replicates were analyzed for each experimental preparation, with the rubber-to-glass transition being readily reproducible within a 3% error margin as a function of temperature or time scale/frequency of observation. Nonlinear regression analysis of experimental data was carried out using GraphPad Prism Version 4.03.

UV Spectroscopy. Kinetic studies of caffeine diffusion in the high-solid glucose syrup and κ -carrageenan/glucose syrup matrices of this investigation were carried out using UV spectroscopy. In doing so, 4 mL

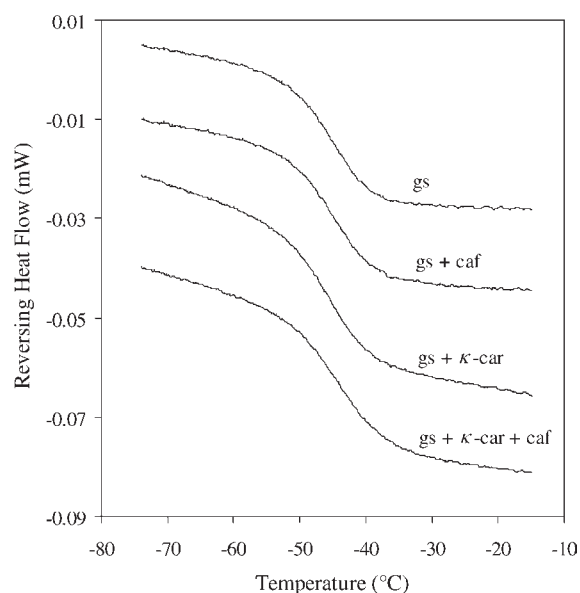


Figure 1. Variation of reversing heat flow as a function of temperature for the following samples from top to bottom in the graph: 80.0% glucose syrup, 79.6% glucose syrup with 0.4% caffeine, 79.5% glucose syrup with 0.5% κ -carrageenan (10 mM KCl), and 79.1% glucose syrup with 0.5% κ -carrageenan (10 mM KCl) plus 0.4% caffeine obtained using MDSC at a heating rate of $2\text{ }^{\circ}\text{C min}^{-1}$.

portions of caffeine-containing samples were transferred into a 30 mL glass vial using a syringe. Care was taken to avoid material spillage on the internal sides of the vial and bubble formation during transfer. Sample and a 10 mL portion of DCM were kept separately at the desired temperature of measurement to equilibrate for 30 min. Following this, DCM was transferred swiftly (as a separate phase on top of the sample) to the vial, which was promptly capped and returned to the measuring temperature. At prearranged time periods of observation, the DCM portion was immediately transferred out, and absorbance readings were taken using a UV-1650PC spectrophotometer (Shimadzu, Tokyo, Japan). Nitrogen gas was purged into the measuring chamber of the instrument to prevent fogging of the cuvette due to atmospheric condensation while operating at subzero temperatures.

Caffeine diffusion from the high-solids matrix to DCM was monitored in the form of absorbance since it relates directly to caffeine's concentration. The maximum absorbance was read at λ_{max} (275 nm) using 11 temperatures of observations (20, 10, 5, 0, -5, -10, -15, -20, -30, -40, and -50 $^{\circ}\text{C}$), which were supported by a cold bath (Thermo Scientific Neslab-CB80, Newington, NH). Absorbance readings were taken at time intervals as short as 60 s and up to 240 min. Glucose syrup or κ -carrageenan/glucose syrup preparations at 80% (w/w) solids served as the control. Experiments were carried out in five replicates, and average values are reported. Averaged means of caffeine diffusion in DCM were modeled using Shimadzu's proprietary software (Tm Analysis System TMSPC-8) and by considering the initial part of the absorbance curve as a function of time scale of observation.

RESULTS AND DISCUSSION

Thermomechanical Characterization of Single and Mixed Systems of Glucose Syrup and κ -Carrageenan. Sample preparation in this work via thorough hydration of glucose syrup or κ -carrageenan results in stable solutions at high temperature, which readily transform in rubbery gels or transparent glasses upon subsequent cooling. At this fixed moisture content

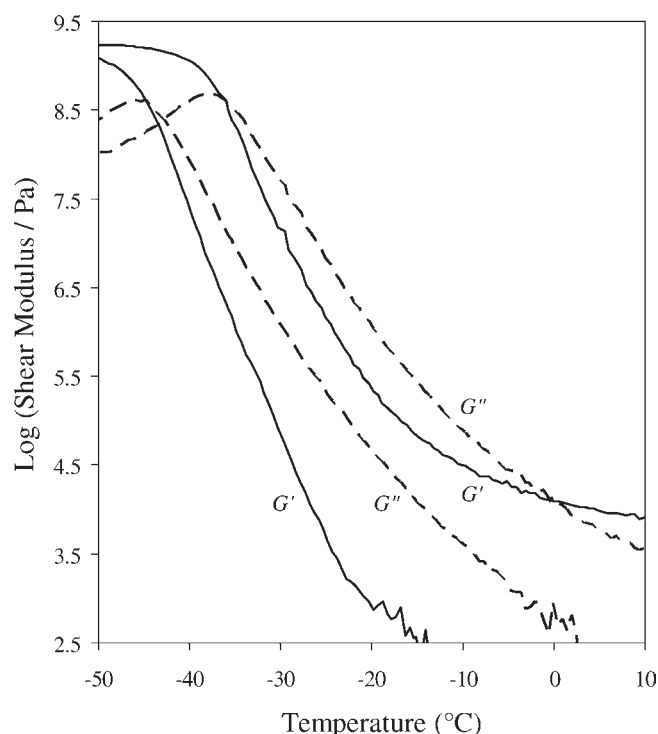


Figure 2. Storage (G') and loss (G'') modulus variation as a function of temperature (heating or cooling scan) for a material of 80.0% glucose syrup (left spectrum on the graph) and a second sample of 79.5% glucose syrup with 0.5% κ -carrageenan (10 mM KCl) scanned at a rate of $2\text{ }^{\circ}\text{C min}^{-1}$, frequency of 1 rad s^{-1} , and strain that varied from 2.0 to 0.00072% in the glassy state.

(20% w/w), crystal formation is avoided, and the concept of vitrification can be considered presently as the governing mechanism of molecular processes. Typically, work in high-solid systems (broadly defined as those with solid levels above 70%) is carried out by differential scanning calorimetry (DSC), which measures as a function of temperature the difference in energy inputs into a substance and its reference.¹⁹ Our samples were subjected to MDSC measurements to increase the sensitivity and resolution of thermal analysis by resolving thermograms into “reversing” and “non-reversing” heat flow.

Figure 1 reproduces thermograms obtained for glucose syrup and glucose syrup plus κ -carrageenan preparations with or without caffeine. Materials were cooled relatively slow at $2\text{ }^{\circ}\text{C min}^{-1}$ and then heated at the same scan rate from temperatures well below the glass transition region, thus recording a “pseudo-equilibrium” relaxation response to the changing thermal regime. In these experiments, the heat flow curve begins at the top (about $-75\text{ }^{\circ}\text{C}$), and this sigmoidal change that ends at $-15\text{ }^{\circ}\text{C}$ (endothermic down) is construed as evidence of vitrification phenomena. The midpoint of this thermal event is readily detectable and is considered presently as the empirical glass transition temperature obtained from the MDSC thermograms. Often, this is referred to as T_{g2} in the literature, with researchers also reporting values for the beginning (T_{g1}) and completion (T_{g3}) of the heat capacity curve.²⁰

Values of the midpoint T_g for our samples are $-46 \pm 1.0\text{ }^{\circ}\text{C}$, an outcome that strongly argues that small additions of polymer or bioactive compound to liquid carbohydrate at this level of solids have no effect on the thermal dependence of vitrification

patterns. Similar values of the glass transition temperature obtained from DSC were recorded for small polyhydric compounds from the state diagrams, for example, of glucose, fructose, and sucrose.²¹

Calorimetry elucidates micromolecular aspects of vitrification phenomena, whereas mechanical measurements should be able to probe macromolecular effects of the addition of 0.5% κ -carrageenan (10 mM KCl) to concentrated (79.5%) glucose syrup preparations. Work of this nature is inspired from the “synthetic polymer approach”, which, in addition to DSC, utilizes small deformation dynamic oscillation or stress relaxation to follow the viscoelastic properties of three-dimensional structures.²² Figure 2 depicts the variation in storage (G') and loss (G'') modulus for thermally reversible cooling and heating profiles of single glucose syrup samples and glucose syrup/ κ -carrageenan mixtures. There is a strong temperature effect on the behavior of both systems over the temperature range of 10 to -50 °C, with values of shear modulus increasing almost 7 orders of magnitude.

In the case of glucose syrup, values of G'' remain well above those of G' at the upper range of temperatures; hence, the system is a viscoelastic liquid. A rapid increase in both moduli is observed on further cooling, but the disproportionate development of the solid component eventually overtakes the trace of the viscous component of the material at about -45 °C. This crossover is close to the midpoint glass transition temperature noted earlier for DSC and signifies the formation of a very strong solid in the glassy state ($G' > 10^{8.5}$ Pa).

In contrast, the addition of κ -carrageenan to glucose syrup results in the formation of a rubbery plateau ($G' > G''$) at temperatures above 0 °C because of the three-dimensional structure of the polysaccharide. The second thermal transition in the mixture when G'' becomes greater than G' at subzero temperatures demarcates the onset of a prolonged glass transition region that runs for most of the experimental routine. Eventually, G' manages to overtake G'' once more at about -34 °C, with the values of the solid component approaching $10^{9.5}$ Pa at the very end of the cooling run (-50 °C). Clearly, the rheological rubber-to-glass transition is displaced at higher temperatures as compared to the solution glass transformation of glucose syrup in Figure 2 and the T_g predictions from calorimetry in Figure 1. Network formation is a process that rheology is extremely well qualified to follow, and this is documented in the acceleration of the mechanical glass transition of glucose syrup/ κ -carrageenan mixture. On the other hand, it appears that calorimetry provides information primarily on the mobility of the small molecules of liquid carbohydrate, with small additions of the biopolymer being a mere cross-contamination.

Mechanistic Considerations in the Viscoelastic Behavior of Single Glucose Syrup Systems and Glucose Syrup/ κ -Carrageenan Mixtures. To progress from qualitative discussions of pictorial rheology in the preceding figure, we evolved mechanistic explanations via the time–temperature superposition principle (TTS). This approach provides a means of identification of the molecular dynamics involved in processes that do not involve first-order thermodynamic transitions.²³ Glass formation does not entail enthalpy release, which results in a change in “phase”, but instead, a gradual transformation takes place from a solidlike to a liquidlike “state”.

To carry out TTS in the present work, a series of mechanical spectra were recorded within the accessible frequency range of 0.1 – 100 rad s^{-1} . Figure 3a,b reproduces these frequency sweeps of G' and G'' taken at constant temperature intervals of 3 °C

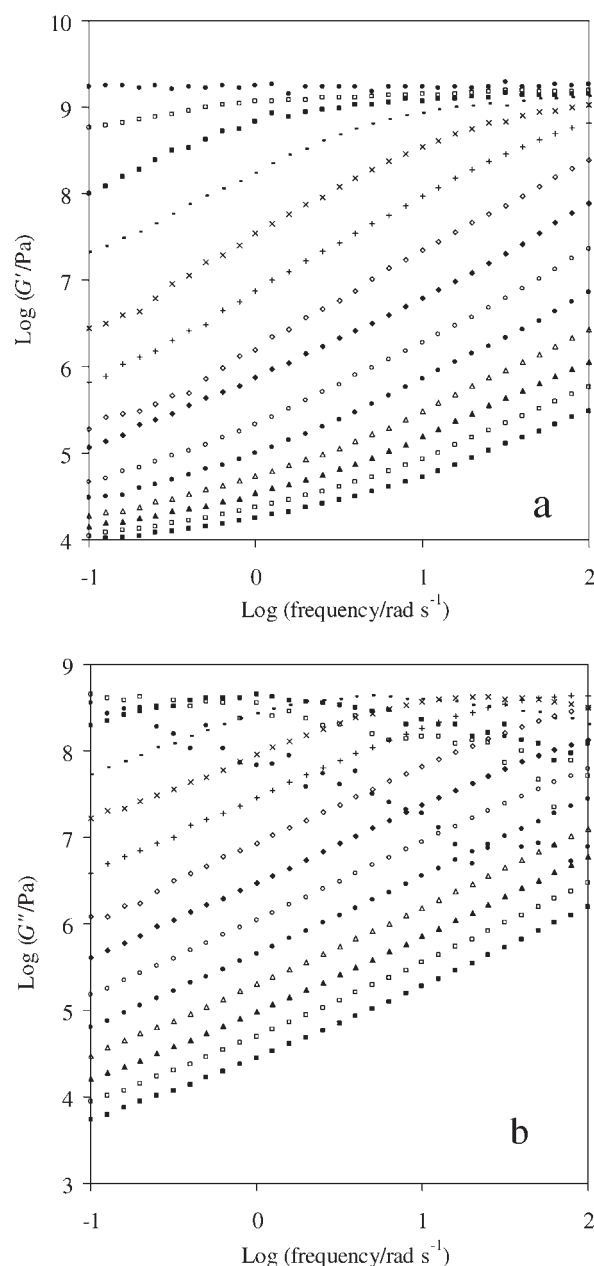


Figure 3. Frequency variation of (a) G' and (b) G'' for the sample of 79.5% glucose syrup with 0.5% κ -carrageenan (10 mM KCl). The bottom curve was taken at -4 °C (■), other curves successively upward were taken at -7 (□), -10 (▲), -13 (△), -16 (black octagon), -19 (○), -22 (◆), -25 (◇), -28 (+), -31 (×), -34 (—), -37 (■), -40 (□), and -49 (black octagon) °C. Data at -43 and -46 °C are not plotted to avoid clutter.

upon heating the glucose syrup/ κ -carrageenan sample from -49 to -4 °C (corresponding data for glucose syrup are not shown). Mechanical spectra remain relatively flat at the lower range of temperature, for example, -40 and -49 °C, but this is transformed into a steep drop in moduli with decreasing frequency at higher temperatures, for example, -28 and -22 °C.

Following this, an arbitrary point within the glass transition region was taken as the reference temperature ($T_0 = -20$ °C), and remaining mechanical spectra were shifted horizontally along the log frequency axis. In doing so, the viscoelasticity at any stage

of the heating or cooling run can be related to that of the reference temperature as long as the frequency of the former is multiplied by a shift factor, a_T . If the long and short relaxation times (contributing to G' and G'' spectra, respectively) generate distinct shift factors or do not superpose thoroughly, rearrangements are the outcome of more than one processes, the basic form of the TTS is not applicable and should be rejected.²⁴

As illustrated in Figure 4, good superposition of adjacent mechanical spectra was achieved for the glucose syrup/ κ -carrageenan sample. Similar quality of superposition was also obtained for glucose syrup, but data are not shown here (generated shift factors between successive temperature steps will be dealt with in the following paragraphs). This is the composite or master curve of viscoelasticity that spans over nine decades of frequency, that is, from 10^{-3} to 10^6 rad s⁻¹ in Figure 4. The terminal part of the rubbery plateau ($G' > G''$) is captured at frequencies below 10^{-2} rad s⁻¹. Next, the onset of the glass transition region is seen at frequencies higher than 10^{-2} rad s⁻¹ when the rising values of G'' exceed those of G' . The entirety of the glass transition region is recorded with increasing frequency of oscillation, and the onset of the glassy state is unveiled at frequencies higher than 10^3 rad s⁻¹ where G' becomes once more greater than G'' . Overall, the master curve in Figure 4 is the time (or frequency) analogue of the temperature profile discussed for the glucose syrup/ κ -carrageenan mixture in Figure 2.

The construction of a smooth master curve of viscoelasticity has generated a series of shift factors at a convenient reference temperature within the glass transition region. For a number of chemical and physical reactions, the extent of temperature dependence of molecular parameters was found to follow the Arrhenius rate law, which advocates that the reaction rate is proportional to $\exp(E_a/RT)$, where E_a is the activation energy of the reaction. In the case of shear moduli and for two different temperatures, this recasts to

$$\log a_T = \frac{E_a}{2.303R} \left(\frac{1}{T} - \frac{1}{T_0} \right) \quad (1)$$

where R is the gas constant.²⁵ If the dependence of all relaxation times on temperature follows the Arrhenius equation, we will obtain a straight line with the gradient reflecting the activation energy of the system. As shown in Figure 5, good linear fits are obtained for the temperature dependence of factor a_T for glucose syrup and glucose syrup/ κ -carrageenan samples at temperatures below -46 and -34 °C, respectively. A cursory inspection of Figure 2 documents that these temperature ranges lie within the glassy state of our samples.

At higher temperatures, however, progress in viscoelasticity is increasingly deviating from the Arrhenius prediction. This outcome is congruent with a gradual shift in the viscoelastic response of Figure 2 from the glassy state to the kinetics of the glass transition region for the glucose syrup and glucose syrup/ κ -carrageenan preparations. The theory of free volume is often used to rationalize mechanical properties observed for molecular events in the glass transition region. This approach dictates that the space available to a group of molecules is the sum of the volume actually taken by the molecules (occupied volume due to van der Waals radii and molecular oscillations) and that from large scale vibrations.²⁶ The latter is known as the free volume and at the glass transition temperature is expected to collapse to something between 2 and 4% of the total volume. Experimentally, Williams, Landel, and Ferry found an excellent fit to the observed shear

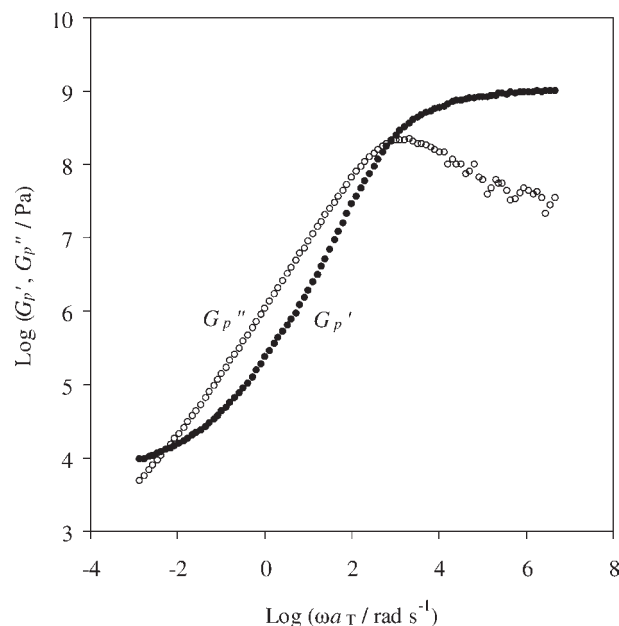


Figure 4. Master curve of reduced shear moduli (G_p' and G_p'') for the sample of 79.5% glucose syrup with 0.5% κ -carrageenan (10 mM KCl) as a function of reduced frequency of oscillation (ωa_T) based on the frequency sweeps of Figure 3 and utilizing a reference temperature of -20 °C.

modulus values in the glass transition region of amorphous synthetic polymers and diluted systems using the following mathematical expression:²⁷

$$\log a_T = - \frac{C_1^0(T - T_0)}{C_2^0 + T - T_0} \quad (2)$$

where C_1^0 and C_2^0 are the WLF constants at T_0 . These are related to the free volume theory as follows:

$$C_1^0 = \frac{B}{2.303f_0} \text{ and } C_2^0 = \frac{f_0}{\alpha_f} \quad (3)$$

where f_0 is the fractional free volume at T_0 , α_f is the thermal expansion coefficient, and B is usually set to one.

Application of the combined WLF/free volume framework to the thermal dependence of shift factors at the upper range of temperatures in Figure 5 yields good fits for both samples, an outcome that emphasizes the change in prevailing molecular dynamics at the glass transition region. The temperatures of -46 °C for glucose syrup and -34 °C for glucose syrup/ κ -carrageenan can be considered as the mechanical glass transition temperature for these systems. The concept acquires physical significance since it describes a threshold where large configurational adjustments that contribute to changes in free volume at the glass transition region give way to a constant activation energy of processes associated with a barrier to rotation in the solidlike environment of the glassy state. Equations 2 and 3 allow in both samples calculation of the fractional free volume at the glass transition temperature ($f_g \sim 0.030$) and thermal expansion coefficient ($\alpha_f = 6.10 \text{ deg}^{-1} \times 10^{-4}$), which are congruent with estimates reported earlier for research in synthetics.²⁸

Theoretical modeling further supports the experimental observations in Figure 2 arguing that the mechanical profile of the rubber-to-glass transition is strongly influenced by the polysaccharide

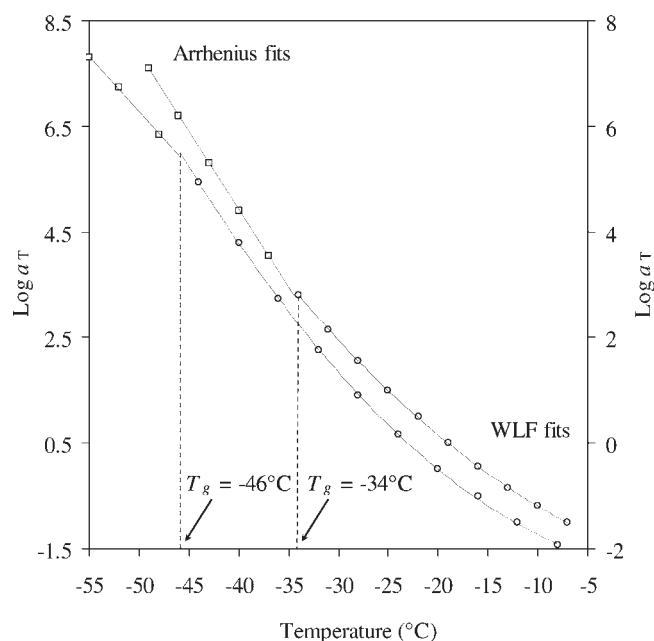


Figure 5. Logarithmic shift factors a_T for a sample of 80.0% glucose syrup (trace on the left y-axis) and 79.5% glucose syrup with 0.5% κ -carrageenan plus 10 mM KCl (trace on the right y-axis). Horizontal shifting in both cases was implemented at the reference temperature of -20°C . Mechanistic modeling of WLF or Arrhenius fits and the mechanical glass transition temperature are also shown.

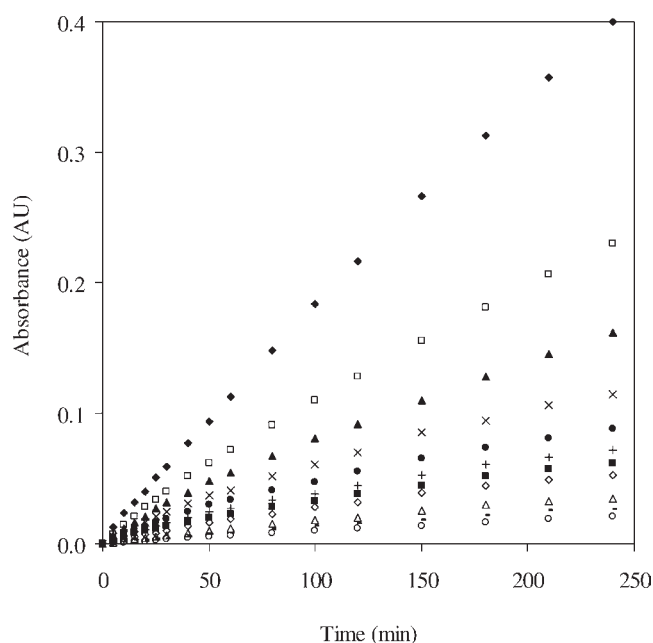


Figure 6. Absorbance of 0.4% caffeine upon diffusion from a sample of 79.1% glucose syrup with 0.5% κ -carrageenan (10 mM KCl) to DCM as a function of time of observation at -50°C (\circ), -40°C (\square), -30°C (\triangle), -20°C (\diamond), -15°C (\blacksquare), -10°C ($+$), -5°C (black octagon), 0°C (\times), 5°C (\blacktriangle), 10°C (\square), and 20°C (\blacklozenge) obtained at 275 nm.

particularly if it is network forming. It was possible in this work to represent the magnitude of κ -carrageenan contribution to rheology by a mechanical (or “network”) T_g that differs from the

glucose syrup T_g obtained using rheology and calorimetry due to the influence of the polymeric network on the mixture.

Diffusion Kinetics of Caffeine in the High-Solid Matrices of Glucose Syrup and Glucose Syrup/ κ -Carrageenan. High-solid environments of biopolymer with cosolute are of great importance to the food industry and have other applications, for example, preservation and delivery of bioactive compounds, flavor encapsulation, color retention, etc. in glassy carbohydrate matrices.²⁹ As shown in this work, fundamental studies are critical to understand the structural functionality of these materials for the development of new uses on a sound technological basis. We have also made a start with caffeine as the bioactive compound, since many foodstuffs like tea, coffee, sodas, and chocolate contain the compound at levels of mild stimulation.³⁰

UV spectroscopy was chosen to provide a sensitive method for quantification of the diffusion patterns of caffeine from the carbohydrate matrix to DCM. It was found that at the various experimental temperatures of interest in this study, the UV absorption spectrum of the bioactive compound exhibited maximum intensity (λ_{max}) at 275 nm. It was further verified that the absorbance at 275 nm had a high-quality linear relationship with caffeine concentration in DCM solutions within the absorbance range of 0–2 AU according to the Beer–Lambert law (UV spectra and calibrations curves are not shown presently).

Figure 6 shows a typical example of the absorbance data obtained for 0.4% caffeine diffused from a sample of 79.1% glucose syrup with 0.5% κ -carrageenan (10 mM KCl) to DCM over 4 h of observation. Experimental temperatures varied from -50 to 20°C , thus covering the entirety of the rubber-to-glass transition recorded for mechanical properties in Figure 2 and the temperature range of interest for heat flow variation in Figure 1. The smooth absorbance behavior is evident within the recording time/temperature framework producing considerable change in spectral intensity. At the end of the experimental routine, for example, temperature variation from -50 to 20°C results in a 20-fold increase in caffeine absorbance.

A similar type of analysis and comparable results were obtained for the diffusion of 0.4% caffeine from the matrix of 79.6% glucose syrup to DCM. Both sets of results were then replotted as a function of temperature for selected diffusion times to further discuss the effect of matrix vitrification on caffeine mobility. This school of thought follows the example of considering fundamental parameters like volume or free energy in glass-forming liquids and looking for changes in magnitude with respect to temperature at the vicinity of T_g .³¹ As shown in Figure 7a,b, the diffusional mobility of the bioactive compound increases considerably for both systems at high experimental temperatures; that is, the farther readings are taken from the glass transition temperature.

An interesting point of comparison is the temperature of -40°C , which lies midway between the glass transition temperatures of glucose syrup and glucose syrup/ κ -carrageenan preparations observed experimentally in Figure 2 and predicted via theoretical modeling in Figure 5. Within the 4 h window of observation, diminishing caffeine mobility is recorded in the polysaccharide matrix, which is almost three times lower than for glucose syrup at the same total level of solids (80.0%). It appears, therefore, that the presence of gelling polysaccharide reduces free volume, accelerates vitrification, and slows down diffusional mobility of the bioactive compound. This observation makes the network glass transition temperature an essential indicator of quality control in processed biomaterials in addition to traditional considerations based on the calorimetric T_g .

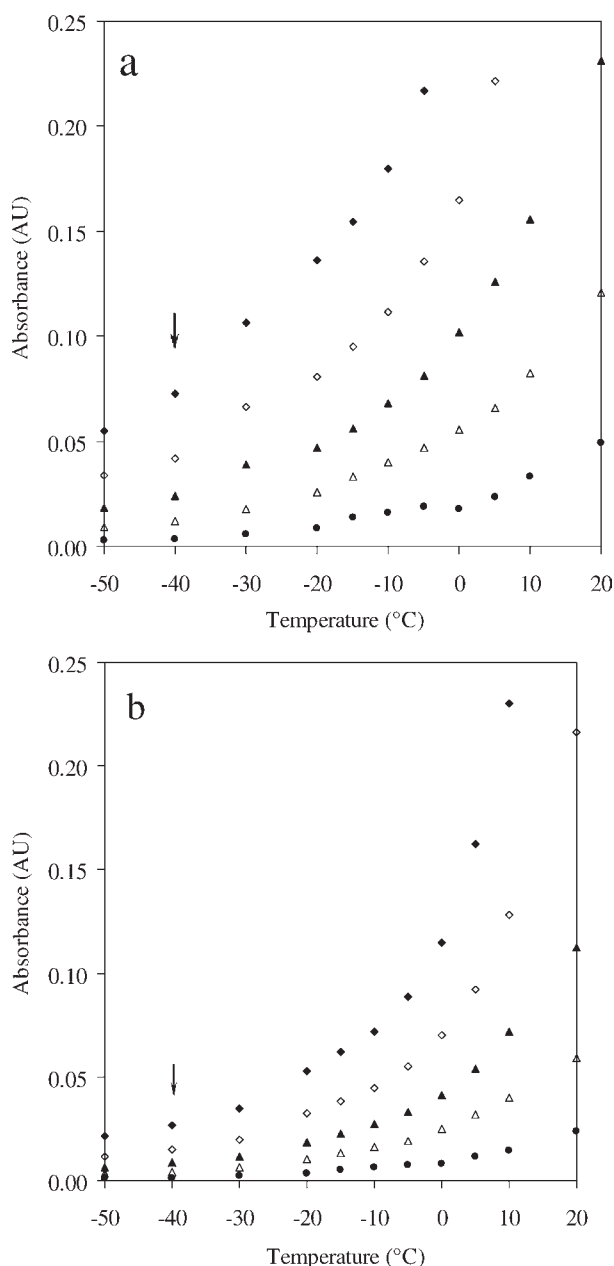


Figure 7. Absorbance of 0.4% caffeine upon diffusion from a sample of (a) 79.6% glucose syrup and (b) 79.1% glucose syrup with 0.5% κ -carrageenan (10 mM KCl) to DCM as a function of experimental temperature for the time periods of 10 (black octagon), 30 (Δ), 60 (\blacktriangle), 120 (\diamond), and 240 (\blacklozenge) min obtained at 275 nm.

Once the utility of adding hydrocolloid to a high-solid formulation is discussed, the question arises as to the nature of the kinetics of bioactive compound mobility in relation to that of the solidlike matrix in the glassy state. Figure 8 summarizes our efforts in this respect by depicting the interplay of predictions from the reaction rate and free volume theories around the mechanical T_g (discussed earlier) and adding to that kinetic patterns of caffeine diffusional mobility. The latter set of results was obtained by considering a very acceptable linear relationship observed in the first section (30 min) of absorbance–time data in Figure 6. This section of the spectrum can be treated as a zero-order reaction with the gradient being the rate constant at $k = dx/dt$.

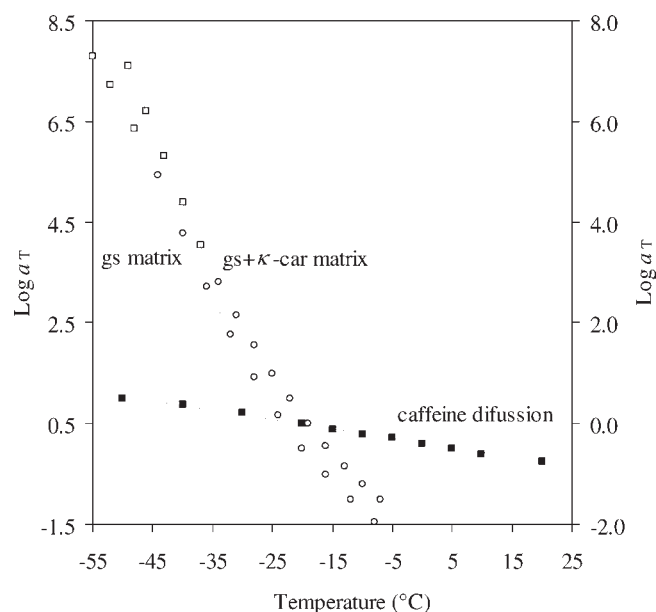


Figure 8. Logarithmic shift factors a_T as a function of temperature for caffeine diffusion in the glucose syrup plus κ -carrageenan matrix at the reference temperature of $-20\text{ }^\circ\text{C}$ (both traces on the right y-axis), with the vitrification of the glucose syrup matrix also shown (trace on the left y-axis).

For each experimental temperature, we then developed a spectroscopic shift factor $[\log(k_o)/k]$, where k_o is the rate constant at the reference temperature of $-20\text{ }^\circ\text{C}$. The outcome is plotted in Figure 8 covering the temperature range of 20 to $-50\text{ }^\circ\text{C}$, as for the corresponding treatment on mechanical data of carbohydrate matrices.

Direct comparison of molecular dynamics between carbohydrate matrices with glassy consistency and diffused bioactive compound can now be made. The temperature dependence of shift factors for caffeine was much lower than for carbohydrate and, upon treating with eq 1, produced a high-quality linearity with a small variation in the energy of activation (depending on matrix) from 0.23 to 0.47 kJ/mol. This estimate is considerably below corresponding values ($E_a = 98\text{--}140\text{ kJ/mol}$) for the glassy state of glucose syrup, glucose syrup/ κ -carrageenan in Figure 8, and previously reported amorphous sucrose,³² hence reflecting the high freedom of caffeine mobility in our systems.

The low E_a value of caffeine mobility is a sign of comparatively high levels of free volume, and our work pursued this by employing the mathematical expression of WLF/free volume theory at temperatures near T_g . As mentioned earlier, the fractional free volume estimate at the glass transition temperature of the glucose syrup/ κ -carrageenan mixture is about 3% of its total volume. In contrast, a WLF fit on the kinetic data of caffeine yields at $-34\text{ }^\circ\text{C}$ an f_o value of about 13%, which indicates conditions of relative diffusional mobility in comparison with the retarded molecular relaxation of the carbohydrate glass. Therefore, the diffusion of caffeine is slowed down by the polysaccharide network, but it does not follow entirely the progression of viscoelasticity in the softening dispersion of glucose syrup/ κ -caffeine manifest in WLF kinetics.

Decoupling between polymer viscoelasticity and translational diffusion of small “solute” molecules is a theoretically acceptable position. This is indicated, for example, in the well-known Stokes–Einstein relation that underestimates the diffusive mobility of small

molecules experiencing lower viscosity than the macroscopic viscosity of the supporting phase.³³ Consequently, there are two issues of particular interest for future research in this area: (i) variation of the network glass transition temperature in relation to the physicochemical environment of the matrix (added counterions, biopolymer concentration, and molecular weight, etc.) and (ii) manipulation of the chemical affinity and molecular interactions between biopolymer and bioactive compound, so that translational motion of the supporting medium and mobile compound is controlled in the glassy state for added value applications.

AUTHOR INFORMATION

Corresponding Author

*Tel: + 61 3 9925 5244. Fax: + 61 3 9925 5241. E-mail: stefan.kasapis@rmit.edu.au.

REFERENCES

- (1) Badii, F.; MacNaughtan, W.; Farhat, I. A. Enthalpy relaxation of gelatin in the glassy state. *Int. J. Biol. Macromol.* **2005**, *36*, 263–269.
- (2) Liu, Y.; Bhandari, B.; Zhou, W. Glass transition and enthalpy relaxation of amorphous food saccharides: A review. *J. Agric. Food Chem.* **2006**, *54*, 5701–5717.
- (3) Li, D. X.; Liu, B. L.; Liu, Y. S.; Cheng, C. L. Predict the glass transition temperature of glycerol-water binary cryoprotectant by molecular dynamic simulation. *Cryobiology* **2008**, *56*, 114–119.
- (4) Hogan, S. A.; Famelart, M. H.; O'Callaghan, D. J.; Schuck, P. A novel technique for determining glass-rubber transition in dairy powders. *J. Food Eng.* **2010**, *99*, 76–82.
- (5) Bengoechea, C.; Arrachid, A.; Guerrero, A.; Hill, S. E.; Mitchell, J. R. Relationship between the glass transition temperature and the melt flow behaviour for gluten, casein and soya. *J. Cereal Sci.* **2007**, *45*, 275–284.
- (6) Liu, P.; Yu, L.; Liu, H. S.; Chen, L.; Li, L. Glass transition temperature of starch studied by a high-speed DSC. *Carbohydr. Polym.* **2009**, *77*, 250–253.
- (7) Kasapis, S.; Sablani, S. S.; Rahman, M. S.; Al-Marhoobi, I. M.; Al-Amri, I. S. Porosity and the effect of structural changes on the mechanical glass transition temperature. *J. Agric. Food Chem.* **2007**, *55*, 2459–2466.
- (8) Douglas, J. F.; Leporini, D. Obstruction model of the fractional Stokes-Einstein relation in glass-forming liquids. *J. Non-Cryst. Solids* **1998**, *235–237*, 137–141.
- (9) Champion, D.; Hervet, H.; Blond, G.; Meste, M. L.; Simatos, D. Translational diffusion in sucrose solutions in the vicinity of their glass transition temperature. *J. Phys. Chem. B* **1997**, *101*, 10674–10679.
- (10) Parker, R.; Ring, S. G. Diffusion in maltose-water mixtures at temperatures close to the glass transition. *Carbohydr. Res.* **1995**, *273*, 147–155.
- (11) Miao, S.; Roos, Y. H. Nonenzymatic browning kinetics of a carbohydrate-based low-moisture food system at temperatures applicable to spray drying. *J. Agric. Food Chem.* **2004**, *52*, 5250–5257.
- (12) Thomsen, M. K.; Lauridsen, L.; Skibsted, L. H.; Risbo, J. Temperature effect on lactose crystallization, Maillard reactions, and lipid oxidation in whole milk powder. *J. Agric. Food Chem.* **2005**, *53*, 7082–7090.
- (13) Bell, L. N.; Hageman, M. J. Differentiating between the effect of water activity and glass transition dependent mobility on a solid state chemical reaction: Aspartame degradation. *J. Agric. Food Chem.* **1994**, *42*, 2398–2401.
- (14) Terefe, N. S.; Nhan, M. T.; Vallejo, D.; Loey, A. V.; Hendrickx, M. Modeling the kinetics of the pectin methyltransferase catalyzed de-esterification of pectin in frozen systems. *Biotechnol. Prog.* **2004**, *20*, 480–490.
- (15) Terefe, N. S.; Delele, M. A.; Loey, A. V.; Hendrickx, M. Effects of cryostabilizers, low temperature, and freezing on the kinetics of the pectin methyltransferase-catalyzed de-esterification of pectin. *J. Agric. Food Chem.* **2005**, *53*, 2282–2288.
- (16) Kasapis, S.; Shrinivas, P. Combined use of thermodynamics and UV spectroscopy to rationalize the kinetics of bioactive-compound (caffeine) mobility in a high solids matrix. *J. Agric. Food Chem.* **2010**, *58*, 3825–3832.
- (17) Nunez-Santiago, M. C.; Tecante, A.; Garnier, C.; Doublier, J. L. Rheology and microstructure of κ -carrageenan under different conformations induced by several concentrations of potassium ion. *Food Hydrocolloids* **2011**, *25*, 32–41.
- (18) Kasapis, S. The morphology of the gellan network in a high-sugar environment. *Food Hydrocolloids* **2006**, *20*, 132–136.
- (19) Liu, Y.; Bhandari, B.; Zhou, W. Study of glass transition and enthalpy relaxation of mixtures of amorphous sucrose and amorphous tapioca starch syrup solid by differential scanning calorimetry (DSC). *J. Food Eng.* **2007**, *81*, 599–610.
- (20) Roos, Y. H. Food components and polymers (Chapter 5) and Prediction of the physical state (Chapter 6). *Phase Transitions in Foods*; Academic Press: San Diego, 1995; pp 109–192.
- (21) Roos, Y. H. Melting and glass transitions of low molecular weight carbohydrates. *Carbohydr. Res.* **1993**, *238*, 39–48.
- (22) Fischer, P.; Windhab, E. J. Rheology of food materials. *Curr. Opin. Colloid Interface Sci.* **2010** in press.
- (23) Nickerson, M. T.; Paulson, A. T.; Speers, R. A. Time-temperature studies of gellan polysaccharide gelation in the presence of low, intermediate and high levels of co-solutes. *Food Hydrocolloids* **2004**, *18*, 783–794.
- (24) Kasapis, S. Recent advances and future challenges in the explanation and exploitation of the network glass transition of high sugar/biopolymer mixtures. *Crit. Rev. Food Sci. Nutr.* **2008**, *48*, 185–203.
- (25) Hrma, P. Arrhenius model for high-temperature glass-viscosity with a constant pre-exponential factor. *J. Non-Cryst. Solids* **2008**, *354*, 1962–1968.
- (26) Meinders, M. B. J.; van Vliet, T. Modeling water sorption dynamics of cellular solid food systems using free volume theory. *Food Hydrocolloids* **2009**, *23*, 2234–2242.
- (27) Ferry, J. D. Dependence of viscoelastic behavior on temperature and pressure. *Viscoelastic Properties of Polymers*; John Wiley: New York, 1980; pp 264–320.
- (28) Tsui, N. T.; Paraskos, A. J.; Torun, L.; Swager, T. M.; Thomas, E. L. Minimization of internal molecular free volume: A Mechanism for the simultaneous enhancement of polymer stiffness, strength, and ductility. *Macromolecules* **2006**, *39*, 3350–3358.
- (29) Kouassi, K.; Roos, Y. H. Glass transition and water effects on sucrose inversion in noncrystalline carbohydrate food systems. *Food Res. Int.* **2001**, *34*, 895–901.
- (30) Shrivastava, K.; Wu, H.-F. Rapid determination of caffeine in one drop of beverages and foods using drop-to-drop solvent microextraction with gas chromatography/mass spectroscopy. *J. Chromatogr., A* **2007**, *1170*, 9–14.
- (31) Odagaki, T.; Yoshidome, T.; Koyama, A.; Yoshimori, A. Free energy landscape approach to glass transition. *J. Non-Cryst. Solids* **2006**, *352*, 4843–4846.
- (32) Rahman, M. S.; Al-Marhubi, I. M.; Al-Mahroqi, A. Measurements of glass transition temperature by mechanical (DMTA), thermal (DSC and MDSC), water diffusion and density methods: A comparison study. *Chem. Phys. Lett.* **2007**, *440* (372), 377.
- (33) Pottier, N. Out of equilibrium generalized Stokes-Einstein relation: Determination of the effective temperature of an aging medium. *Phys. A* **2005**, *345*, 472–484.