

Combined Use of Thermomechanics and UV Spectroscopy To Rationalize the Kinetics of Bioactive Compound (Caffeine) Mobility in a High Solids Matrix

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An investigation of the diffusional mobility of a bioactive compound (caffeine) within a carbohydrate matrix (glucose syrup) at a glassy consistency is reported. The experimental temperature range was from 30 to -70 °C, and the techniques of modulated differential scanning calorimetry, small-deformation dynamic oscillation on shear, and UV spectrometry were employed. It is not a straightforward matter to identify the relaxation dynamics of such a glassy matrix. This makes suggestions of the relationship between the structural properties of the matrix and the diffusional mobility of bioactive compounds reported earlier in the literature rather tenuous. To address this issue, we recorded mechanical spectra over the aforementioned temperature range and utilized the combined framework of the Williams, Landel, and Ferry (WLF) equation with the time–temperature superposition principle to rationalize results. The protocol produced a fundamental definition of the glass transition temperature and free volume parameters of the glucose syrup sample within the glass transition region. Results were related to the kinetic rates of caffeine diffusion derived by UV spectroscopy leading to the conclusion that the diffusional mobility of the chemical substance is independent of the carbohydrate matrix. This conclusion was further supported by the high level of fractional free volume of caffeine, which is congruent with the predictions of the reaction rate theory (modified Arrhenius equation), as compared to the collapsing levels of free volume in the glucose–syrup matrix that make appropriate WLF considerations.

KEYWORDS: Glucose syrup; caffeine; MDSC; dynamic oscillation; UV spectroscopy

INTRODUCTION

The glass transition temperature, T_g , reflects a pseudo second-order phase transition in which a supercooled melt transforms upon cooling into an isotropic structure of extremely brittle consistency. Applications of the concept have been well documented in organic polymers forming amorphous solids, which are used as common glasses and plastics (1, 2). In biomaterials, the rubber-to-glass transition has been observed in native and modified starches, their hydrolysates, nonstarchy polysaccharides, and proteins. These have been used to deliver a range of properties such as texture, processability, storage stability, delivery control, etc. (3, 4). The market value of such biomaterials is significant since their low water content imparts a long shelf life, thus making them relatively inexpensive to handle and transport around the world. Some examples of commercial products that fit this category in the food and pharmaceutical industries include confections, the unfrozen phase of ice cream, dried fruit leathers, and the hard gelatin capsule as a pharmacotherapeutic (5, 6).

Manufacturing of high-solid food products was regarded as being craft-based, but eventually, a physicochemical method was embraced for the interpretation of vitrification phenomena,

which was inspired largely by the “sophisticated synthetic polymer approach” (7, 8). A notable example of this approach was the collection of hundreds of maximally freeze-concentrated solute glass transition temperatures (T_g') for starch hydrolysis products (SHP) and polyhydroxy compounds (sugars, polyols, and glycosides) using differential scanning calorimetry (DSC). This led to a relationship between the dextrose equivalent (DE) of SHP or the molecular weight (MW) of sugars and T_g' , with considerable utility in frozen produce, and to the prevention of structural collapse during freeze drying and storage (9, 10).

Technology transfer from the materials science of aqueous solid solutions to the functional attributes of ingredients used in the food-processing industry was further facilitated using the so-called “state diagram” (11). In its simplest form, a state diagram represents the pattern of change in the physical state of a material as a function of increasing levels of solids (12). Thus, basic understanding of physical properties in foodstuffs was extended by bringing together a combination of equilibrium (crystallization) and metastable (vitrification) processes as a function of time, pressure, and temperature of processing or preservation (13, 14). In a similar vein, the structural properties of sugar in the presence of gelatin and/or gelling polysaccharide were studied using thermomechanical analysis. These preparations are important for the confectionery industry and have other applications,

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for example, flavor encapsulation and preservation of bioactive molecules in glassy carbohydrate matrices (15). Using the combined framework of the Williams, Landel, and Ferry (WLF) equation with free volume theory and by refining the analysis further via the stress relaxation dynamics of “the coupling model”, the work was able to define on fundamental grounds a network or mechanical glass transition temperature (T_{gn}). The new concept was found to be distinct from the commonly reported calorimetric T_g of sugars and polyols at levels of solids between 70 and 90% in formulations, thus offering new insights into the structure and mechanical properties of this class of materials (16–18).

The desire to improve the stability and quality of processed foods at which chemical reaction pathways and enzymatic processes are critical considerations led to understanding of the molecular dynamics of carbohydrates and proteins at the vicinity of T_g discussed in the preceding paragraph. In this context, the enzymatic activity relates to the diffusion-controlled substrate/enzyme interaction, whereas chemical studies are mainly interested in the prevention of flavor and color degradation and control of the oxidative reactions leading to, for example, fat rancidity (19, 20). Studies on nonenzymatic browning (NEB) monitored the interactions of reducing sugars (fructose, glucose, and xylose) with lysine in several matrices made of trehalose, maltodextrin, or poly(vinylpyrrolidone) (21–26). Other examples include the degradation of aspartame, the acid-catalyzed hydrolysis of sucrose, the deterioration of saffron quality, the methyl-esterase-catalyzed de-esterification of pectin, and the alkaline phosphatase-catalyzed hydrolysis of disodium *p*-nitrophenyl phosphatase (27–35).

The interpretation of results paid special attention to the reaction progress in relation to the amplitude of the difference between the glass and the experimental temperature ($T - T_g$). Attempts were made to follow the rates of reactant consumption with the predictions of the reaction rate theory (Arrhenius equation) or the WLF equation. There have been reports, however, where neither the Arrhenius nor the WLF framework is able to follow convincingly the kinetics of molecular processes in the glass transition region. The present work argues that the so-called “universal” C_1 and C_2 values of the WLF equation should not be employed in fundamental research that probes molecular property, since they cannot adequately describe the structural complexity of biomacromolecules. Furthermore, it is doubtful that values of C_1 and C_2 , obtained by fitting to the WLF equation various kinetic rates recorded above the glass transition temperature, relate directly to the free volume concept. Problems encountered in the literature include negative estimates or physically unrealistic high values of free volume, etc.

The present investigation utilizes mechanical spectroscopy and the principle of time–temperature superposition (TTS), as advocated by synthetic polymer research, to derive fundamentally valid values of C_1 and C_2 for a carbohydrate matrix. These are then used to assess whether the concept of free volume is the overriding consideration in the kinetics of diffusional mobility of a bioactive compound in a glassy matrix.

MATERIALS AND METHODS

Chemical Substances. Caffeine [1,3,7-trimethyl-1*H*-purine-2,6(3*H*,7*H*)-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 (CH_2Cl_2) is widely used as a solvent since it is one of the less harmful 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 (-40 to 30 °C). It was purchased as an anhydrous colorless liquid from Sigma-Aldrich.

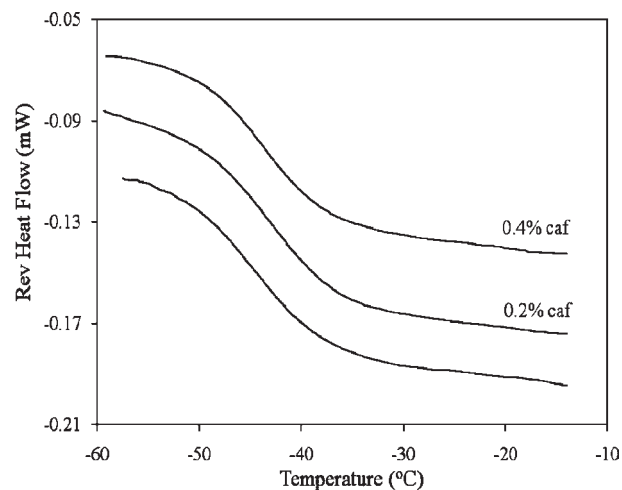


Figure 1. Variation of reversing heat flow as a function of temperature for a sample of 80.0% glucose syrup with or without small additions of caffeine (0.2 and 0.4%) obtained using MDSC at a heating rate of 1 °C/min.

Glucose Syrup. The glucose syrup (batch NX8472) used in this work was a gift from Cerestar (Mechelen, Belgium). Despite its name, the material is a nonsweet saccharide system. It is prepared by controlled α -amylase hydrolysis of wheat starch, with about 82% amylopectin content, down to glucose sequences linked by α -(1 \rightarrow 4) and α -(1 \rightarrow 6) glycosidic linkages. The material is a clear and stable solution at temperatures above 0 °C and becomes a transparent glass on further cooling. Amylose-like, that is, α -(1 \rightarrow 4) sequences having chain lengths of less than 110 residues, have been found to precipitate from solution, but the presence of α -(1 \rightarrow 6) glycosidic linkages keeps this material noninteracting with itself in the present formulations. This is further confirmed by the absence of any first-order thermodynamic transition in the DSC thermograms of glucose syrup, a sample of which is reproduced in **Figure 1**, and the viscoelastic glass transition obtained in the master curves of **Figures 2** and **4**.

The functionality of the product is determined by the DE, which gives the content of reducing end groups relative to glucose as 100. Thus, a DE of 25 corresponds to a (number average) degree of polymerization (DP) of 4, and the DE of our sample is 42. The high DE imparts solubility and bulking and bodying characteristics, which are desirable in confectionery products. The level of solids is 83.0%, and the water content of glucose syrup was taken into account in the calculation of the total level of solids for sample preparation. Gel permeation chromatography provided by the manufacturer indicates the following relationship between DP and peak area (%) of the glucose syrup spectrum:

DP1	17.54
DP2	12.99
DP3	10.55
DP4	8.79
DP5	7.29
DP6	5.28
DP7	4.78
DP8	4.21
DP9	3.19
DP10	1.98
> DP10	23.40
total	100.0

Modulated DSC (MDSC). Glucose syrup samples (15–25 mg) with or without small additions of caffeine were hermetically sealed in Alod-Al pans and subjected to MDSC measurements (Q1000 DSC, TA Instruments, New Castle, DE). Cooling was controlled via a refrigerated unit (RCS) that accompanies the calorimeter. The unit operates from -90 to 550 °C using a two-stage closed evaporative system. T_{zero} calibration was performed by heating the cells without pans in the temperature range of interest. Cell constant and temperature calibration were performed with

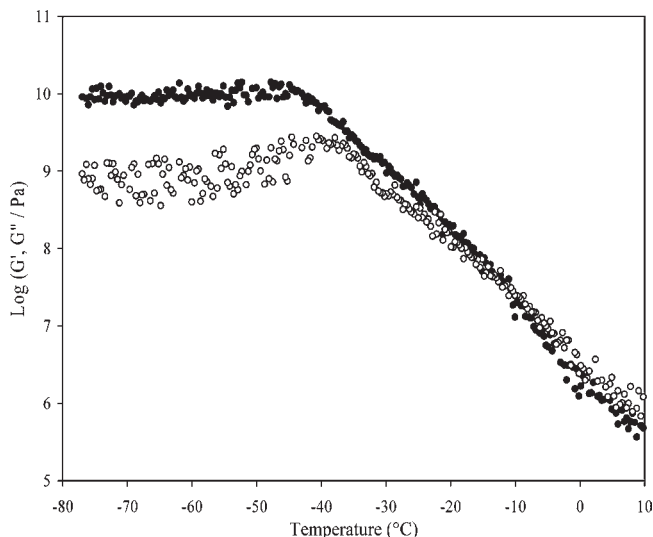


Figure 2. Storage (G' ; black octagon) and loss (G'' ; white circle) modulus variation as a function of temperature (heating or cooling scan) for a material of 80.0% glucose syrup cooled at a rate of 1 °C/min and a frequency of 1 rad s⁻¹.

indium and Milli-Q water at the heating rate of 1 °C/min, and the heat capacity was calibrated using sapphire. For all scans, an empty pan was used as a reference and nitrogen as a purge gas at the flow rate of 50 mL/min. The MDSC routine commenced by cooling the sample at the controlled rate of 1 °C/min to -80 °C. The pans were held at -80 °C for 30 min and scanned to 10 °C with an underlying heating rate of 1 °C/min, a period of 60 s, and an amplitude ± 0.53 °C described previously (36). Measurements were carried out at least in triplicate, yielding effectively overlapping traces.

Small Deformation Dynamic Oscillation. The analysis provides readings of the storage modulus on shear (G'), which is the elastic component of the network, loss modulus (G'' ; viscous component), and dynamic viscosity (η^*). Variations with time and temperature can further be assessed as a measure of the “phase lag” δ ($\tan \delta = G''/G'$) of the relative liquidlike and solidlike structure of the material (37).

Measurements were performed with the Advanced Rheometrics Expansion System (ARES), which is a controlled strain rheometer (TA Instruments). ARES has an air-lubricated and essentially noncompliant force rebalance transducer with the torque range between 0.02 and 2000 g cm. Nevertheless, particular care was taken to establish that any inherent machine compliance was insufficient to significantly offset measured values from the high modulus glass systems. This was achieved by progressive adjustment of geometry settings while measuring samples of known intermediate and high modulus, namely, polydimethylsiloxane (PDMS) at 30 °C ($G_c = 2.5 \times 10^4$ Pa) and ice at -5 °C ($G' = 10^9$ Pa). Thus, the maximum plate diameter (5 mm) and the minimum measuring gap (3 mm) between the two parallel plates consistent with accurate results could be established. During experimentation, the measuring geometry of parallel plates is enclosed within an oven, which supplies ultra clean and dry air at a controlled airflow through the ARES oven heaters.

Samples were loaded onto the plate of the rheometer, and the exposed edges were covered with a silicone fluid to minimize variation in the moisture content and cooled to subzero temperatures (down to -75 °C) at a rate of 1 °C/min. This was followed by a heating scan at 1 °C/min to 10 °C. In addition, isothermal mechanical spectra were obtained at fixed temperature intervals of 4 °C between 0.1 and 100 rad/s. In these, the applied strain varied from 0.00072% in the glassy state to 1% in the flow region to accommodate the huge changes in the measured stiffness of the sample. Overall, the experimental temperature accessed molecular motions that cover the glassy state, the softening dispersion (glass transition region), and the flow region of the “melt”. Two replicates were analyzed for each experimental preparation, with the melt-to-glass transition being readily reproducible within a 3% error margin as a function of temperature or time scale of measurement.

Kinetic Studies of Caffeine Diffusion in a High-Solids Matrix Using UV Spectroscopy.

An aqueous caffeine solution was added to the glucose syrup solution with continuous stirring to produce a final sample of 79.6% glucose syrup plus 0.4% caffeine. A solution of 80.0% glucose syrup solids served as the control. One milliliter of the prepared materials was transferred into a quartz cuvette using a syringe. A 2.5 mL amount of dichloromethane was then slowly added (as a separate phase on top of the glucose syrup) to the cuvette, promptly capped, and inserted into the measuring chamber without any delay. The spectrophotometer was set to take readings only from the dichloromethane phase. The cuvette was held at a constant temperature in the thermoelectric cell through the use of a water:ethylene glycol (2:3) circulator. Nitrogen gas was purged into the measuring chamber to prevent fogging of the cuvette due to condensation of water while operating at subzero temperatures.

Caffeine diffusion from the high-solids matrix to dichloromethane was monitored in the form of absorbance, which relates to caffeine's concentration, using a UV-vis spectrophotometer (model UV-1650PC, Shimadzu, Tokyo, Japan). The setting included Shimadzu S-1700 thermoelectric cell holder and PolyScience 9106 refrigerated/heated circulator. Maximum absorbance was read at λ_{\max} (275 nm) within an 8 h time period at time intervals of 60 s. Caffeine diffusion in dichloromethane was determined using Shimadzu's proprietary software (Tm Analysis System TMSPEC-8) and by considering the initial part of the absorbance curve as a function of the time scale of observation. Assessment of changes in caffeine levels at each experimental temperature were carried out in triplicate, and average values are reported.

RESULTS AND DISCUSSION

Estimation of the Glass Transition Temperature Using Thermal

Analysis. MDSC and small deformation mechanical spectroscopy are increasingly being used to elucidate the molecular mechanisms involved in various relaxations (38, 39). The two techniques are chosen presently as the tools to monitor the structural properties of glucose syrup matrices. **Figure 1** reproduces the shapes of typical MDSC curves obtained for samples of 80.0% glucose syrup or in the presence of small additions of caffeine (0.2 and 0.4%). Samples were cooled at a low scan rate (1 °C/min) from ambient temperature. Then, they were heated at the same scan rate from temperatures well below the glass transition temperature of the system, thus exhibiting a “pseudo-equilibrium” relaxation response to the changing thermal regime. The experimental protocol matched that of rheology to facilitate comparisons between the two techniques.

There is a well-defined change in heat flow (heat capacity), which can be associated with the “endothermic” glass transition region upon heating. The midpoint of this thermal event is readily detectable, and it is considered presently as the T_g . This is known as T_{g2} in the literature, with researchers also reporting values for the beginning (T_{g1}) and completion (T_{g3}) of the sigmoidal curve (40). These are empirical indicators of convenience since, in our view, there is no clear-cut (fundamental) relationship between local segmental motions, which dominate the onset of the glassy consistency, thus largely determining the T_g relaxation phenomena (41), and the pictorial thermal event in DSC experiments (42). DSC T_g at 80.0% glucose syrup solids with or without the small additions of caffeine was found to be -43.0 ± 1.0 °C. This value is comparable to those reported in the glass transition curve of the state diagram of sucrose and glucose preparations at 80.0% solids (40).

Qualitative Analysis of the Mechanical Data Obtained for the High-Solids Matrix. The MDSC results were augmented by considering the viscoelastic characteristics of the system, since typical relaxation processes associated with glass transitions are within time scales that can be accessed rheologically. **Figure 2** reproduces oscillatory data illustrated for 80.0% glucose syrup during heating or cooling at a scan rate of 1 °C/min. The passage from the flow region to the glassy state shows perfect thermal

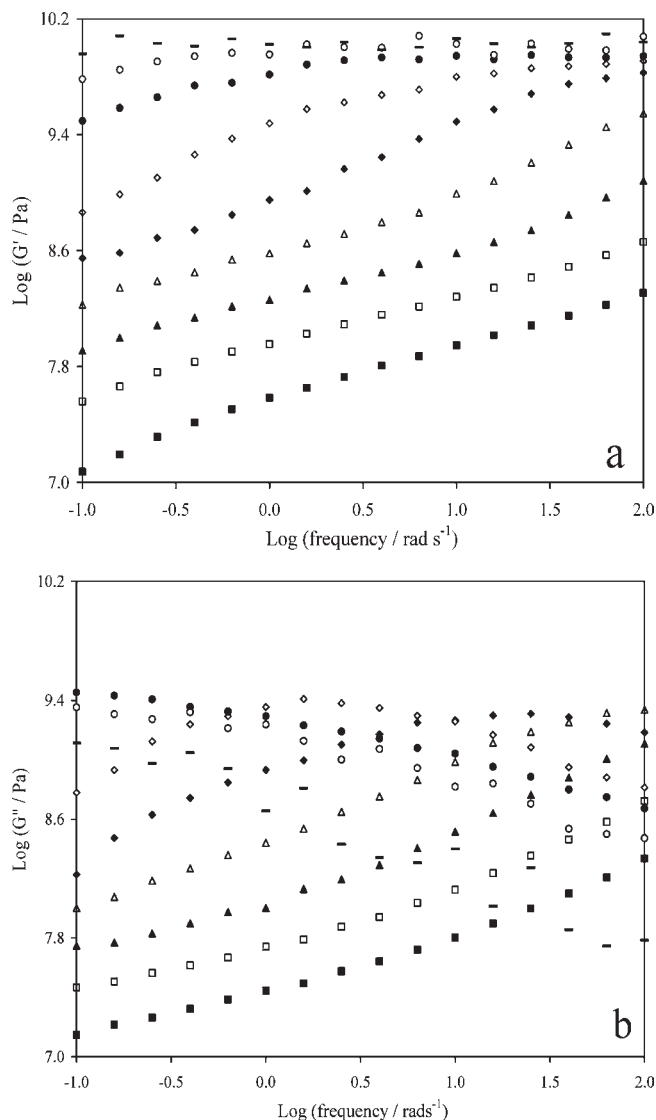


Figure 3. Frequency variation of (a) G' and (b) G'' for the 80.0% glucose syrup sample. The bottom curve is taken at -18 °C (black square); other curves were taken successively upward at -22 (white square), -26 (black triangle), -30 (white triangle), -34 (black diamond), -38 (white diamond), -42 (black octagon), -54 (white circle), and -66 °C (black line). Data at -46 , -50 , -58 , -62 , and -70 °C have not been plotted to avoid clutter.

reversibility, that is, the absence of thermal hysteresis. A reduction in temperature results in considerable reinforcement in mechanical properties (from $\approx 10^6$ to 10^9 Pa), which is a behavior identified in the research of amorphous synthetic materials as the glass transition region (43).

At the lowest range of experimental temperatures in **Figure 2** (i.e., below -45 °C), there is yet another development. The solidlike character is clearly the dominant contributor to the mechanical response, which reaches readings of 10^{10} Pa. This part of the master curve of viscoelasticity is known as the glassy state (44), where there is little variation of storage modulus with temperature and an increasing separation between the G' and the G'' traces. Clearly, our experimental results confirm that temperature-driven vitrification phenomena of the glucose syrup are recorded similarly using calorimetry and mechanical means. Further assessment of the relaxation processes of the carbohydrate matrix requires development of quantitative relations, which include parameters of the molecular free volume theory.

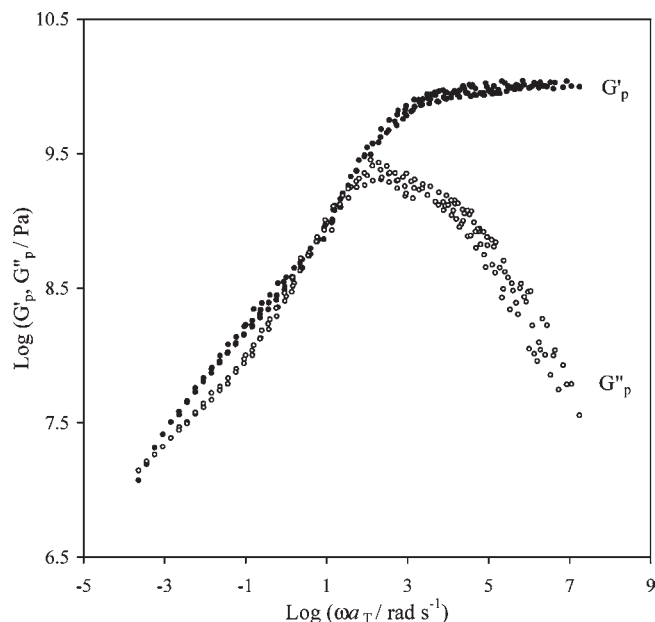


Figure 4. Master curve of reduced shear moduli (G'_p and G''_p) for the sample of 80.0% glucose syrup as a function of reduced frequency of oscillation (ωa_T) based on the frequency sweeps of **Figure 3** (the reference temperature is -30 °C).

Treatment of the Mechanical Data Using the Concept of Free Volume. In the absence of a phase transition as a function of changing temperature (or pressure), viscoelastic parameters can be related by implementing horizontal superpositions along the logarithmic axis of time or frequency of oscillation (45). This is known as the method of reduced variables, and it necessitates that changes in temperature affect all relaxation times in the same manner (46). Following this approach, standard mechanical spectra were taken at regular temperature intervals of 4 °C, with the outcome being illustrated in **Figure 3a,b**. Both moduli have relatively low values at high temperatures (e.g., -18 °C), whereas there is a substantial build up of viscoelasticity at the low temperature end (e.g., -70 °C). Data were processed by arbitrarily choosing a point within the glass transition region as the reference temperature ($T_o = -30$ °C) and shifting the remaining data along the log frequency axis until a uniform curve was obtained.

The master or composite curve obtained in this work for 80% glucose syrup is reproduced in **Figure 4**, and for both moduli (G'_p and G''_p), an 11-decade window of reduced frequency has emerged. Gratifyingly, the progress in viscoelasticity in **Figure 4** appears to be the time analogue of the temperature effect in **Figure 2**. There are 2.5 orders of magnitude rise in moduli over six decades of reduced frequency (from 10^{-4} to 10^2 rad s^{-1}), an outcome that is associated with the glass transition region. Higher frequencies of oscillation introduce the glassy state at which the values of storage modulus reach a plateau at $\approx 10^{10}$ Pa and those of the loss modulus diminish rapidly.

Identical superposition of both G' and G'' traces is a prerequisite for valid application of the method of reduced variables (47), thus yielding the shift factor (a_T) that integrates two sets of temperature data:

$$\log a_T = -\frac{(B/2.303f_o)(T-T_o)}{(f_o/\alpha_f) + T - T_o} \quad (1)$$

This is the equation proposed by WLF, which utilizes the concept of free volume to develop a mechanistic understanding of glass

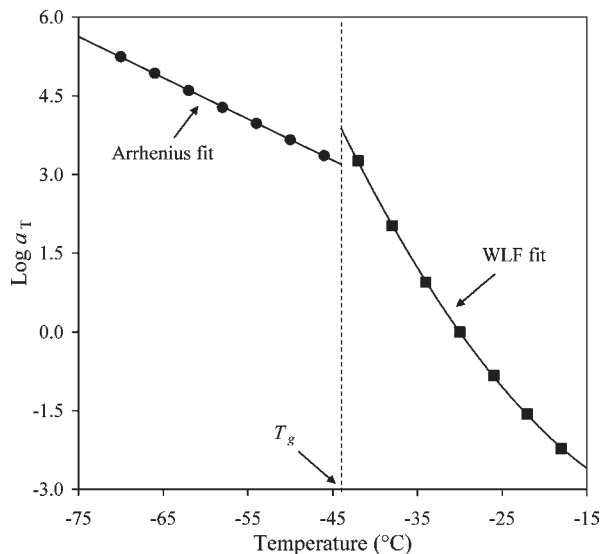


Figure 5. Temperature variation of the factor a_T within the glass transition region (black square) and the glassy state (black octagon) for the 80.0% glucose syrup, with the solid lines reflecting the WLF and modified Arrhenius fits of the shift factors in the glass transition region and the glassy state, respectively (the dashed line pinpoints the T_g prediction).

transitions (48). Thus, f_o is the fractional increase in free volume at T_o , α_f is the thermal expansion coefficient, and the value of B is set to be about one.

The pattern of structural relaxation, as documented in the factor a_T for the horizontal superposition of mechanical spectra in **Figure 3a,b**, was fitted with the WLF equation. The mathematical expression follows well the progress in viscoelasticity within the glass transition region, thus making free volume the molecular mechanism dictating diffusional mobility (**Figure 5**). However, the WLF/free volume approach does not hold for the temperature range of the glassy state (< -46 °C), at which the alternative plot (49),

$$\log a_T = \frac{E_a}{2.303R} \left(\frac{1}{T} - \frac{1}{T_o} \right) \quad (2)$$

that is, the modified Arrhenius equation, achieves a good linear relationship for the horizontal shift factors (R is the gas constant). The constant activation energy (E_a) argues that relaxation processes in the glassy state are heavily controlled by specific chemical features.

The appearance of an Arrhenius-related linear superposition delimits a discontinuity in the development of shift factors at the end of the WLF curvature upon cooling. This change in operational kinetics can be considered as a measure of the fundamental glass transition temperature at which free volume considerations become secondary to an energetic barrier of molecular rearrangements from one state to another. The above modeling produces a mechanical T_g value for 80.0% glucose syrup of -44 °C. Parameters of this framework of thought include the fractional free volume at T_o ($f_o = 0.031$), T_g ($f_g = 0.024$), and the thermal expansion coefficient, α_f , being equal to 4.84×10^{-4} deg.

Molecular Dynamics of Caffeine Diffusion in a Carbohydrate Matrix Using UV Spectroscopy. Many food materials like tea, coffee, sodas, and chocolate contain the mild stimulant caffeine in amounts that vary widely from 100 to over 1000 $\mu\text{g/mL}$ (50). In our work, diffusion patterns of caffeine were monitored using UV spectroscopy, which provides a sensitive method for quantification purposes. **Figure 6a** reproduces the UV behavior of 0.005% (w/w) caffeine as a function of wavelength of measurement for the

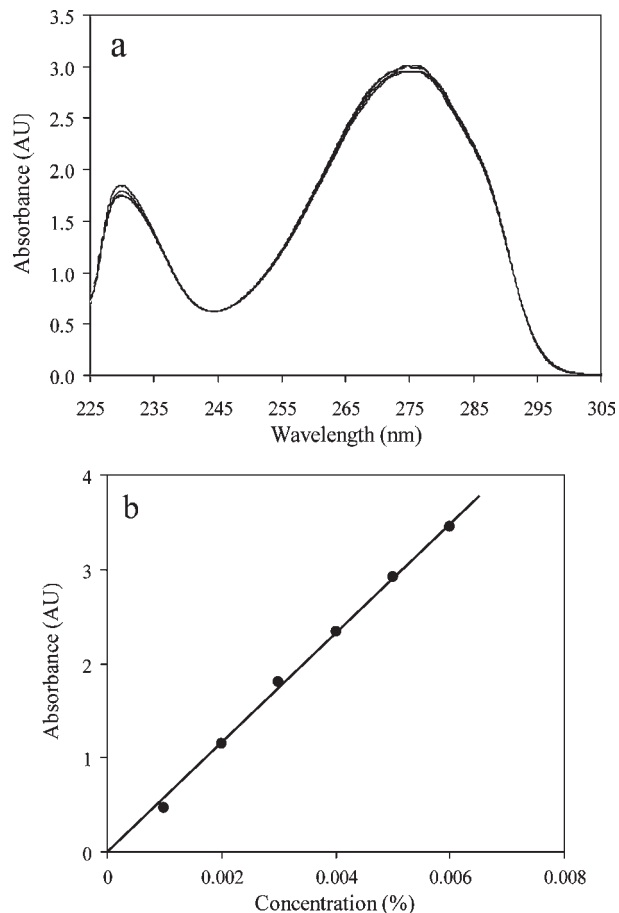


Figure 6. (a) Ultraviolet spectra of 0.005% caffeine in dichloromethane obtained at -20 , -10 , 0 , 10 , and 20 °C and (b) as a function of caffeine concentration in dichloromethane at 275 nm and 20 °C.

various temperatures of experimental interest. The UV absorption spectrum of caffeine in dichloromethane exhibits a pair of absorption bands peaking at about 230 and 275 nm. The intensity of the latter peak (λ_{max}) is considerably higher, and it was chosen for subsequent experimentation. Thus, it was first verified that instrumental readings at $\lambda_{\text{max}} = 275$ nm remained linear as a function of caffeine concentration (i.e., follow the Beer–Lambert law) within the experimental range of 0–3.0 AU (**Figure 6b**).

Figure 7 shows the variation in absorbance of 0.4% caffeine diffused from 79.6% glucose syrup to dichloromethane over 8 h of observation. An extensive temperature range of -40 to 30 °C was chosen to cover the glass transition region of the sample matrix (< -43 °C for 80.0% glucose syrup) as identified by DSC and theoretical modeling of mechanical data in **Figures 1** and **5**. The absorbance behavior is very evident with recording time, and spectra asymptotically approach a fixed value at the upper range of readings. Furthermore, spanning a temperature range of 70 °C results in a 60-fold increase in caffeine absorbance at the end of the 8 h experimental routine.

To further demonstrate the effect of matrix vitrification on the diffusional mobility of caffeine, data in **Figure 7** were recast as a function of temperature for selected recording times. This parallels the approach of plotting thermodynamic properties such as volume, enthalpy, and free energy of glass-forming liquids, thus looking for a reduction in the magnitude of these primary properties and a change in their derivatives with respect to temperature (51, 52). Clearly, the higher the amplitude of difference between T_g and experimental temperatures is, the more pronounced the diffusion of caffeine in the carbohydrate matrix

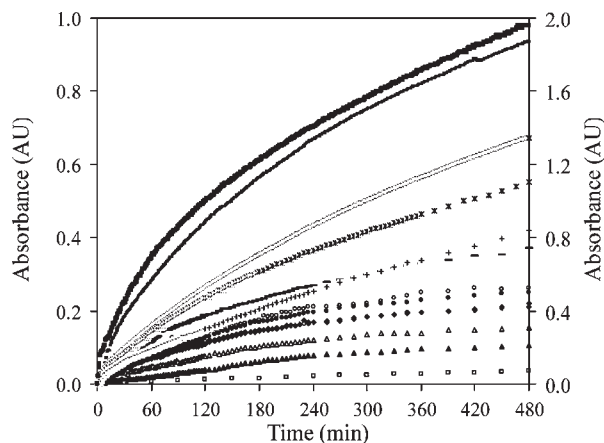


Figure 7. Absorbance of 0.4% caffeine diffused from 79.6% glucose syrup to dichloromethane as a function of time of observation (8 h) at -40 (white square), -35 (black triangle), -30 (white triangle), -25 (black diamond), -20 (white diamond), -15 (black octagon), -10 (white circle), 0 (plus), 5 (long black line), 10 (asterisk), 15 (crosses), 20 (short black line) (left y-axis), and 30 °C (black square; right y-axis) obtained at 275 nm (absorbance readings at -25 and -20 °C are partially overlapping on the graph).

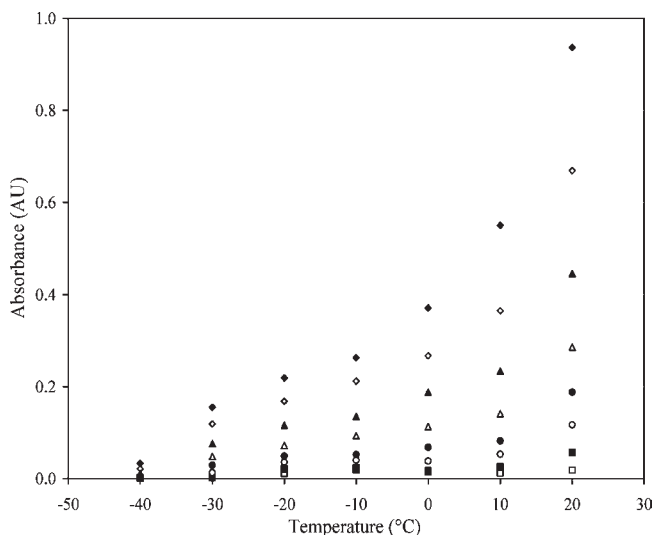


Figure 8. Absorbance of 0.4% caffeine diffused from 79.6% glucose syrup to dichloromethane as a function of temperature for the time periods of 3 (white square), 7 (black square), 15 (white circle), 30 (black octagon), 60 (white triangle), 120 (black triangle), 240 (white diamond), and 480 (black diamond) min obtained at 275 nm.

(**Figure 8**). This becomes minimal at -40 °C, a temperature that lies close to the DSC or mechanical T_g discussed in **Figures 1** and **5**.

The main issue dealt with in this section is that of assessing the control of a glassy matrix over the mobility of a bioactive compound, and the outcome of such an effort is illustrated in **Figure 9**. In doing so, the extent of glucose syrup/caffeine interplay was determined by considering the first part (20 min) of the spectra in **Figure 7**, which exhibited a very acceptable linearity between absorbance and recording time; R^2 ranged between 0.974 and 0.993. This is equivalent to an appropriate rate law of a zero-order reaction for this part of the spectrum where the gradient (i.e., rate) is constant at $dx/dt = k$. Gradient or rate constant values were found to develop from 2.0×10^{-4} and through 24×10^{-4} to 223×10^{-4} at -40 , 0 , and 30 °C, respectively. Finally, the spectroscopic shift factor ($\log k_o/k$) was calculated for each

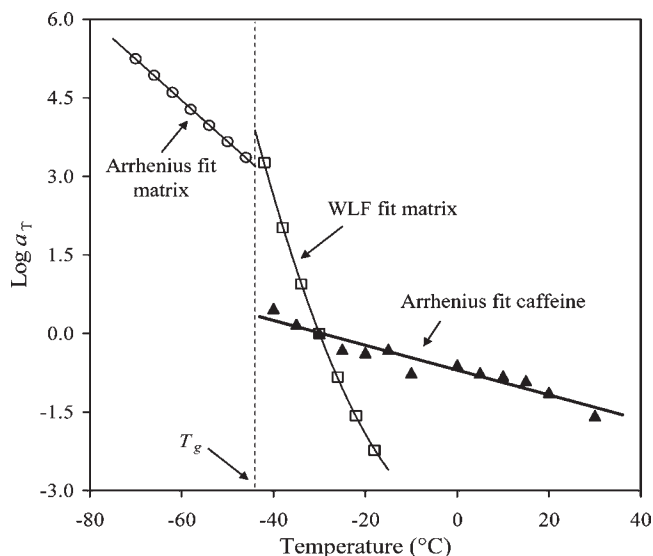


Figure 9. Temperature variation of the factor a_T within the glass transition region (white square) and the glassy state (white circle) for 80.0% glucose syrup (shown in **Figure 5**) and for kinetic data of 0.4% caffeine (black triangle) diffused from the carbohydrate matrix to dichloromethane (the reference temperature for both systems is -30 °C).

experimental temperature, with k_o being the rate constant at the reference temperature of -30 °C. This allowed direct comparison with the molecular dynamics of glucose syrup in the glass transition region derived earlier using mechanical spectroscopy.

It was felt that such a comparison was justified since we were able to properly derive the WLF parameters ($C_1^* = B/2.303f_o = 14.13$; $C_2^* = f_o/\alpha_f = 64.0$ deg) for 80% glucose syrup at the reference temperature of -30 °C using eq 1 and the principle of TTS on mechanical spectra. Thus, the use of inappropriate “universal constants” of 17.44 and 51.6 deg was avoided, since according to Ferry and our own data for biomaterials, “it is evident that the actual variation from one polymer (or biomaterial) to another is too great to permit use of these universal values” (48, 52). Furthermore, WLF parameters of the present work yield valid predictions of fractional free volume at T_o and T_g (quoted earlier) as compared to some kinetic data in the literature that correspond to physically unrealistic values of f_o or f_g within the glass transition region.

Figure 9 summarizes the molecular dynamics for an 80% glucose syrup and caffeine within the syrup, with data being normalized at the reference temperature of -30 °C ($\log a_T = 0$). The rates of molecular diffusion of caffeine were assessed against the predictions of the modified Arrhenius equation, which produces a good quality fit ($R^2 = 0.968$) and a constant value for the energy of activation, $E_a = 0.47$ kJ/mol. This is a relatively low estimate and reflects the freedom of mobility of the compound within the carbohydrate matrix. In contrast, the activation energy of the latter ranges from 98 to 140 kJ/mol, thus indicating a strong temperature effect on the rubber-to-glass transition of polysaccharides or the melt-to-glass transformation of glucose syrup (53).

Finally, it has been suggested in the literature that an operational Arrhenius function is a sign of a comparatively high level of free volume, but it has been proved difficult to assign values both for the matrix and the suspended material. As discussed in this work, the fractional free volume of 80.0% glucose syrup was found to vary from 0.031 at T_o to 0.024 at T_g . We have also performed a WLF fit on the kinetic data of caffeine ($R^2 = 0.847$), which yielded a fractional free volume of 0.209 at the reference

temperature of $-30\text{ }^{\circ}\text{C}$. Conditions of molecular relaxation and rapid diffusional mobility correspond to a free volume toward 20 and 30% of the total volume of the molecule (54); hence, the value of 20.9% indicates that this is indeed the case for caffeine within the glassy carbohydrate matrix. The latter, on the other hand, is characterized by collapsing free volume in the glass transition region (e.g., 3.1% at T_g) that makes volume-related restrictions or WLF considerations the overriding molecular mechanism of vitrification. The discrepancy in the values of activation energy and free volume derived from the shift factors in **Figure 9** argues that the relaxation kinetics of caffeine are distinct from those of the glucose syrup within the extended temperature range of this investigation.

LITERATURE CITED

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