High pressure study on molecular mobility of leucrose

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Broadband dielectric measurements on leucrose were performed under ambient and high pressure. We showed that in this disaccharide, there are two secondary relaxation modes, a slower one sensitive to pressure and a faster one that is not. This finding clearly indicates that the faster secondary relaxation originates from the intramolecular motion. This conclusion contradicted previous interpretations of this mode observed for trehalose and maltitol, systems very closely related to leucrose. In addition, pressure sensitivity of the slower relaxation confirms our recent interpretation about the character of this process. Furthermore, we discovered that unlike the faster relaxation, the slower secondary relaxation is sensitive to the thermodynamic history of measurements. Finally, monitoring the changes in maximum loss of the slower secondary relaxation measured at the same pressure and temperature conditions for glasses obtained via different thermodynamic routes enabled us to draw a conclusion about the density of the formed glasses. Our observations may be helpful in establishing a new method of suppressing crystallization of amorphous drugs. © 2008 American Institute of Physics. [DOI: 10.1063/1.2969816]

INTRODUCTION

During the past decade, there has been growing interest in glassy materials because they possess properties that are attractive to various industries, particularly the preservation of food1–3 and pharmaceuticals.4–7 Preparation of medicines in the amorphous form gives the obvious advantages of enhanced bioavailability and chemical reactivity of drugs. Since, however, the amorphous state is metastable, pharmaceuticals stored in this form may revert to the crystalline state and consequently lose their properties. Levine and Slade8 claimed that the key parameter for safe storage of medicines in the amorphous form is their glass transition temperature, or Tg. However, recent studies performed on indomethacin showed that even below Tg, crystallization from the amorphous phase is possible;9,10 therefore, preparation of such systems is risky and can be met with exceptional difficulty.

A very promising method for resolving the problem of preparation of drugs in the amorphous state is by combining pharmaceuticals with carbohydrates. The choice of saccharides seems reasonable for several reasons. First, they are very good glass formers and in the series of mono-, di-, oligo-, and polysaccharides, the value of Tg strongly increases with the molecular weight of the carbohydrate. Thus, the Tg of the mixture may be designed to be much higher than room temperature, which is crucial for medicinal storage. Next, saccharides are polar, as are nearly all pharmaceuticals, and therefore there is a real chance that they will be very good solvents. Furthermore, they may form hydrogen bonds with target biomolecules, improving their stability and capability to form glasses in which diffusion is reduced, thereby increasing the stability of the solved drugs and preventing their crystallization. However, it was shown both experimentally and theoretically that the translational mobility of small molecules such as water or fluorescein in sugar glassy matrix is still significant.11,12 It can be combined with the fact that several relaxation processes (secondary relaxations) can be still active in the glassy state.12

In many previous dielectric investigations dealing with carbohydrates, one secondary relaxation is revealed. This process has a very similar characteristic in all mono-, di-, and polysaccharides; thus, it can be concluded that in all these cases the same molecular motion governs this process. In literature, there are various interpretations of the origin of this relaxation process.13–17 The question about the nature of this phenomenon is still unanswered. Recently, we have performed broadband dielectric measurements on sucrose,18 and showed that it has two secondary relaxation modes: the faster one (γ relaxation) with similar characteristics as previously observed and the slower one (β relaxation), which has henceforth been unseen. We have concluded that the β process is probably a Johari–Goldstein (JG) relaxation19 coupled with the rotation of the D-glucopyranose and D-fructofuranose rings around the glycosidic bondage.

In this paper we present dielectric studies in leucrose using different thermodynamic conditions. We shall discuss possible scenarios responsible for the secondary relaxations. Moreover, we will show how to produce a denser glass, as well as what may result in significantly reducing the translational mobility and the diffusion coefficient of the captured active components.

EXPERIMENTAL

The sample of leucrose (99% purity) was supplied by Sigma Aldrich. This compound is one of the disaccharides and is composed of D-glucopyranose and D-fructopyranose linked by the glycosidic bondage α (1–5) where 1 and 5 are the first and the fifth carbon atoms of the D-glucose and the

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**RESULT AND DISCUSSION**

In the upper panel of Fig. 1 we present dielectric loss spectra of the leucrose measured at ambient pressure and varying temperatures (from 383 down to 133 K). One can observe the structural α relaxation and the dc conductivity moving towards lower frequencies with decreasing temperature. A characteristic feature of the sugars is that the α relaxation is usually far more sensitive to temperature than the dc conductivity. Consequently, the maximum loss of the α relaxation is somehow related to the dynamics of the H bonds.

Below $T_g$, two secondary relaxation modes were readily observed. The faster one, visible at higher frequencies, is characterized by a large dielectric strength, which decreases significantly with lowered temperature. Alternatively, the amplitude of the slower one increases when temperature is decreased. It is also evident that separation between these secondary relaxations becomes greater with a reduction in temperature, indicating that they have different activation energies.

In order to determine the relaxation times of the α-relaxation process close to $T_g$ (i.e., in the region where the dc conductivity has the dominant contribution in the loss spectra) we used a derivative method of analysis [Eq. (1)] based on the Kramers–Kronig relation.

$$\varepsilon'' \propto -\frac{2}{\pi d} \frac{d\varepsilon'}{d \log \nu}. \quad (1)$$

This equation links imaginary $\varepsilon''$ and real $\varepsilon'$, which are parts of the complex dielectric permittivity $\varepsilon^*$. If there is an overlap between the relaxation peak and the dc conductivity (as in the case of leucrose) analysis of the $\varepsilon'$ is more adequate because conductivity does not influence the real part of $\varepsilon^*$. Thus, application of the above relationship enables us to estimate the relaxation times with greater precision.

To analyze the γ- and β-relaxation peaks the Havriliak–Negami and the Cole–Cole functions were used, respectively. In view of the common procedure of analyzing secondary relaxation with the use of the Cole–Cole function, it may be very interesting that the γ-relaxation process cannot be satisfactorily described by this function. The asymmetric shape of this mode may indicate that this relaxation is somehow related to the dynamics of the H bonds.

In the lower panel of Fig. 1, the relaxation times of various relaxation processes versus the inverse temperature are plotted. The temperature dependence of $\tau$ was described by means of the Vogel–Fulcher–Tamman (VFT) equation.

![Scheme 1. Scheme 1. The chemical structure of leucrose.](image)

![FIG. 1. (Color online) Upper panel: Dielectric loss curves obtained for leucrose on cooling at $P=0.1$ MPa. Lower panel: Relaxation map of log $\tau$ vs $1/T$ of the α relaxation (filled squares), β relaxation (open circles), and γ relaxation (open squares). The solid lines represent the Arrhenius law.](image)
\[
\tau = \tau_{\text{VFT}} \exp(D_T/T - T_0).
\] (2)

From the VFT fit, the glass transition temperature, defined as the temperature at which \( \tau = 100 \text{ s} \), was determined as \( T_g = 341 \text{ K} \). It is interesting to note that the value of \( T_g \) of disaccharides may be related to the \( T_v \) values of monosaccharides of which they consist. For example, disaccharides that have fructose (\( T_g = 288 \) K) in their structure [sucrose \( T_g = 340.5 \) K], leucrose have considerably lower \( T_g \) than disaccharides that are not built with this monosugar [lactose \( T_g = 387 \) K, Ref. 26], trehalose \( T_g = 378 \) K, Ref. 27]. However, it should be noted that leucrose is a special case among disaccharides built with glucose and fructose as the glycosidic bond \( \alpha (1 \rightarrow 5) \) forces fructose to form a pyranose ring.

The temperature dependencies of two secondary relaxation processes (\( \beta \) and \( \gamma \)) were fitted by the Arrhenius equations:

\[
\tau_x = \tau_{\alpha x} \exp(E_x/RT),
\] (3)

where \( x \) stands for either \( \beta \) or \( \gamma \). The following values of the activation energies were determined: \( E_\beta = 76 \pm 0.5 \text{ kJ/mol} \) and \( E_\gamma = 45 \pm 0.1 \text{ kJ/mol} \).

Taking into account the value of \( E_\gamma = 45 \text{ kJ/mol} \) and the dielectric characteristic of the \( \gamma \) relaxation, it may be inferred that this process in leucrose likely has the same origin as that in glucose, fructose, maltose, sucrose, etc. Recently, we have shown that \( \gamma \) relaxation in two monosaccharides is nearly insensitive to pressure. We therefore, based on our observations and literature review, believe that the most probable mechanism of the \( \gamma \) relaxation is an internal motion of the monosugar unit. In turn, the \( \beta \) relaxation has very similar properties to those observed in sucrose. We have classified this secondary process as a JG relaxation coupled with the rotation of the D-glucopyranose and D-fructofuranose rings around the glycosidic bond.

In Fig. 2, the dielectric spectra obtained at \( T = 295 \text{ K} \) and varying pressures are presented. The figure illustrates that \( \beta \) relaxation is, in fact, sensitive to pressure. The maximum loss in this secondary relaxation shifts by more than 1.5 decades towards lower frequencies with an increase in pressure of 450 MPa. It is a remarkable effect, especially when the compressing procedure was performed well below the \( T_g \). It is therefore clear that this process is sensitive to structural changes occurring in the glassy state and may be regarded as an indicator of the volume changes of the glass. We determined the activation volume for the \( \beta \)-relaxation process as \( \Delta V_\beta = 21.5 \pm 0.3 \text{ ml/mol} \) derived from the equation \( V = RT/\log(e)(d \log \tau/dP) \) for pressures in the range \( P = 0.1 \) to 440 MPa. The estimated value of the activation volume is quite large and it should be stressed that this value cannot be directly compared to the molar volume necessary to make a full rotation of the unit possible. We determined \( \Delta V_\gamma \) difference in molar volumes of activated \( (V_1) \) and nonactivated \( (V_0) \) species at \( T \) and \( P \). This observation confirms the thesis that the JG \( \beta \) relaxation may be responsible for this relaxation. However, as it was previously noted, we found that for sucrose the JG relaxation is likely coupled with the twisting motion of the two monosugar rings via a glycosidic bond. Consequently, we can expect that the same situation occurs for leucrose.

The activation volume determined for the \( \gamma \) relaxation is equal to \( \Delta V_\gamma = 4 \pm 1 \text{ ml/mol} \), much less than the value estimated for the \( \beta \) relaxation. These results are very interesting in view of the work done up to now. Carpenter and Descamps investigated two systems, maltitol (composed of glucose connected by the glycosidic bond with sorbitol) and trehalose (consisting of two glucose units), respectively. They reported that two secondary relaxation modes can be observed in the studied systems. The faster one was classified as a true JG relaxation while the origin of the slower secondary relaxation, was not discussed. Moreover in Ref. the authors pointed out that the \( \gamma \) relaxation (they called it \( \beta_1 \)) observed in trehalose has a different origin than the one observed in monosugars. Analysis of the high pressure measurements performed on leucrose, which turns out to be much more informative than the ambient pressure data, leads to quite opposite conclusions. The insensitivity of the \( \gamma \) process to the applied pressure indicates unquestionably that this relaxation cannot be an intermolecular process. In such case, it probably originates from the intramolecular motion within the glucopyranosyl or fructopyranosyl moiety. Furthermore, the behavior of the \( \gamma \)}
relaxation of leucrose is very similar to that observed previously in monosugars.\textsuperscript{17} It can therefore be concluded that the same kind of motion governs this relaxation in mono-, di-, and even polysaccharides.

Very recently it was reported in Ref.\textsuperscript{31} that the \(\gamma\)-relaxation can be sensitive to thermodynamic routes through which a glassy state was formed. Therefore, we decided to check if this phenomenon can also be observed in our case. For this purpose further pressure measurements were carried out. The position of the maximum loss of the \(\gamma\)-relaxation was established as an indicator of the changes in volume and, consequently, in density of the glass. In the experiment we have chosen two thermodynamic pathways, see inset in Fig.\ 4, i.e., (i) leucrose was compressed in the glassy state from \(P=0.1\text{ MPa}\) up to \(450\text{ MPa}\) at \(T=295\text{ K}\); (ii) leucrose was compressed in the liquid state to \(P=450\text{ MPa}\) at \(T=379\text{ K}\) and the temperature was decreased to \(T=279\text{ K}\). For both pathways the measurements of dielectric spectra at the same ther-

modynamic conditions \(T=279\text{ K}\) and \(P=450\text{ MPa}\) were performed. The result of our study is presented in Fig.\ 3. In the upper panel of this figure we show the spectra obtained after application of paths (i) (open circles) and (ii) (filled squares). The difference in the position of the two \(\gamma\)-relaxation peaks is well visible. It is clear evidence that the structure of the glass depends on the thermodynamic pathway by which the glass is formed. It is also noteworthy that the position of the maximum loss of the faster relaxations is the same in both cases. It is in fact intuitive due to the fact that we previously recognized this process as intramolecular in origin.

In the lower panel of Fig.\ 3, the temperature dependencies of the \(\gamma\)-relaxation times obtained after compression of the leucrose from 0.1 MPa up to 450 MPa at \(T=295\text{ K}\) (filled squares and triangles respectively) and the dependence of \(\log r_\gamma \) vs \(1/T\) after compression of the leucrose at \(T=379\text{ K}\) (open circles).
In the lower panel of Fig. 3, we also depicted relaxation times determined for the faster secondary relaxation from isobaric measurements performed at \( P = 450 \) MPa (leucrose was compressed at \( T = 295 \) K). One can see that the activation energy of this relaxation (\( E_a = 46.6 \pm 1.2 \) kJ/mol) is not much different than what was obtained for this relaxation from measurements carried out at ambient pressure during cooling of the molten leucrose (\( E_a = 45 \) kJ/mol).

The next step in our investigations was to check if changes in the position of the peak of the \( \beta \) process will be maintained after decompression of leucrose to atmospheric pressure. In Fig. 4, two loss spectra collected at \( T = 295 \) K are presented. The first one (squares) was measured after supercooling the molten sample at ambient pressure, while the second (circles) was obtained in the following way: the sample of leucrose was isothermally compressed from \( P = 0.1 \) MPa up to \( P = 450 \) MPa at \( T = 379 \) K, then isobarically cooled down to \( T = 297 \) K, and finally at this temperature the pressure was decreased to \( P = 0.1 \) MPa. Our analysis showed that the difference between the maxima of loss of the \( \beta \) relaxations achieved on different thermodynamic pathways is about 0.3 decade. To better visualize this difference we performed the following procedure: The loss spectrum obtained after decompression of leucrose (circles) was described by two fits. The first fit was with free \( \tau_\beta \) (solid line) and the second fit was with a fixed \( \tau_\beta \) (equal to the \( \beta \)-relaxation time obtained for leucrose supercooled at ambient pressure; dotted line). It is clearly visible that the fit with fixed \( \beta \)-relaxation time is much worse than the free fit. It should be noted that the difference between the \( \beta \)-relaxation times for both fits is also about 0.3 decade.

CONCLUSIONS

In this paper we presented a high pressure study on the dynamics of secondary relaxations observed in leucrose. The conclusions drawn from our experiments are in contrast with the common view on the origin of these relaxations. We showed that \( \gamma \)-relaxation is the intramolecular motion within the gluco- or fructopyranosyl rings. Moreover, similarity in behavior of the \( \gamma \)-relaxation in di- and monosaccharides indicates that the same kind of motions are responsible for the occurrence of these relaxations. Alternatively, pressure sensitivity of the \( \beta \) relaxation shows that, in fact, JG \( \beta \) relaxation coupled with the rotation of the monosugar rings around glycosidic bond may be the origin of this relaxation, as it was shown in sucrose.\(^{18}\) Another important result of our investigation was the observation that by application of high pressure, it is possible to form a glass with higher density than by standard supercooling at ambient pressure. This observation seems to be very important since it is well known that glassy sugars may be used as matrices for pharmaceuticals and stabilizers of their amorphous form. In such cases, one can expect that in denser glass, the diffusion coefficient of the entrapped pharmaceuticals may be significantly reduced which, in turn, may be attributed to the drug remaining stable in the amorphous form and not crystallizing during storage.

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