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Determination of the relaxation characteristics of sugar glasses embedded in microfiber substrates

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Abstract

Recently there has been considerable interest in developing sugar glasses that enable storage of biologics without refrigeration. Microfiber filter papers are good substrates for drying biologics in the presence of sugar glass-formers, providing for an even distribution of samples and an enhanced surface area for drying, but the opaqueness prevents macroscopic observation of the sample and can introduce complexities that impede physical characterization. Because drying kinetics and processing conditions can impact the relaxation dynamics (e.g., α - and β -relaxation), which can influence the efficacy of the glass as a stabilizer, methods are needed that can enable a determination of relaxation phenomena of sugar glasses in such complex environments. In this study we present a method which provides verification of the absence of crystallinity following drying on glass fiber filter paper and also enables the determination of relaxation characteristics of amorphous sugar compositions embedded within these filter substrates. Using material pockets to contain the sugar glass-embedded microfiber paper, the α -relaxation temperature, T_α , was determined as a function of the water content in trehalose and sucrose samples using Dynamic Mechanical Analysis (DMA). Results were verified by comparison with previous calorimetric and spectroscopic studies. The data also demonstrated the plasticizing effects of water, as T_α was shown to correlate with water content via a Gordon-Taylor-like relationship. Our findings validate a new approach for determining the relaxation characteristics of microfiber embedded sugar glasses, and offer new insights into the relaxation characteristics of glasses prepared by microwave-assisted drying on filter papers.

Keywords

Glass stability; relaxation; trehalose; microwave drying; anhydrous preservation

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1. Introduction

Preservation in a glassy state has been widely recognized to confer considerable benefits for food production and storage, pharmaceutical formulating, and bio-banking of living cells and tissues. The attainment of a glassy state can effectively retard molecular motions that can induce food deterioration, pharmaceutical instability and biological degradation. Generally compositions and processing conditions are chosen so that the desired storage temperature is significantly below the glass transition temperature, T_g . Although the T_g , which delineates the glassy state from the more mobile molten state, is a good indicator for this purpose, it does not always result in optimized storage conditions, nor does it provide the comprehensive information about molecular mobility that can enable the rational design of superior preservation formulations [1]. Indicators of molecular mobility at various scales can provide better insights for this purpose.

The relaxation process of amorphous solids is characterized by the glass relaxing toward the quasi-equilibrium super-cooled liquid state (also known as the equilibrium glassy state), and is associated with a decrease in its energy, entropy, and free volume, and an increase in the structural order [2, 3]. The two main representative relaxation modes are α - and β -relaxations, which are also known as primary or structural relaxation and secondary or Johari-Goldstein relaxation, respectively, in the literature [2–5]. The α -relaxation refers to the diffusional motion and viscous flow of whole molecules in small molecule glass-formers, such as the sugar compositions reported here. It reflects the global and cooperative molecular translations above T_g associated with the glass-to-liquid transition and the subsequent changes in the matrix viscosity [6]. Specifically, the long-range molecular motions (over atomic distances) become increasingly significant at the α -relaxation temperature, T_α , correlating with liquid-like behavior and viscous flow [7]. The β -relaxation usually refers to intramolecular motions below T_g , such as the rotation or vibration of side chains in a polymer or the rearrangement or reorientation of a small group of atoms on a macromolecule [8]. These motions are not coupled with the motion of the whole molecule, thereby being local and non-cooperative. For example, in saccharide molecules, it is thought that the β -relaxation is related to the motions of the -OH groups, which are sensitive to the molecular structure of the saccharide [4].

The relaxation behavior of sugar glasses can significantly influence the molecular mobility of molecules embedded within this matrix. For example, the molecular motions of enzymes can be reduced, and the shelf life of formulations extended, by utilizing a trehalose/glycerol (95 wt%:5 wt%) mixture as the preservative composition, compared to 100 wt% trehalose, even though the latter has the higher T_g [9]. It was suggested by Cicerone and Soles that enzyme stability in a room-temperature glass depends on suppressing the short-range, high-frequency dynamics within the glass [10]. Based on molecular modeling results, Dirama et al. [11] suggested that the fast dynamics (i.e., the local, high frequency dynamics) of the glassy trehalose was suppressed by the presence of 5 wt% glycerol. The dielectric study by Anopchenko et al. [1] also demonstrated that the addition of glycerol could increase the secondary dielectric relaxation time (which is inversely proportional to the local, non-cooperative molecular mobility) of glassy trehalose. These studies indicate that the bio-protective efficacy of a glass does not necessarily scale with the difference between the

storage temperature and T_g [9]. The degradation or viability loss of preserved specimens should be evaluated in the context of the molecular mobility remaining in the glassy state, which can be related to relaxation processes that arise from thermal, dielectric or mechanical perturbations, on the experimental time scale.

While it is clear that an understanding of relaxation phenomena in sugar glasses is important for developing appropriate preservation strategies for various biologics, oftentimes the processing conditions, sample size, or sample containment device used to prepare samples for measurement are not synchronous with the manner in which samples are processed for storage studies. This, paradoxically, becomes a hurdle to better understanding the effect of processing on the glassy state behavior.

Glass microfiber filter papers have been employed as a drying substrate for many biopreservation applications. Alcock et al. [12] prepared two viral vectors (MVA and AdHu5) for dry storage by air-drying samples into a glassy film made of sugars embedded in glass microfiber membranes. The viruses retained infectivity and were immunogenic for 6 months at temperatures up to 45 °C. Similarly, by using amorphous sugar-embedded glass microfiber filter papers as the drying matrix, cat germinal vesicles [13] and vesicular stomatitis virus and Sendai virus (both serve as vaccine vectors) [14] were successfully preserved by a microwave-assisted drying process. Glass microfiber filter papers (typically as thin as several hundred micrometers) can produce uniformly dispersed samples while enlarging the drying surface, which can improve the drying efficiency and facilitate formation of the amorphous sugar matrix for preserving various biologics. Therefore, validating the glassy state after drying on filter papers is essential, but this can be challenging with current methods. A dynamic mechanical analysis (DMA) method is presented that utilizes a filter paper-steel pocket composite to contain the sample for analysis. This approach allows samples to be processed on the microfiber matrix using a range of different drying approaches.

In the current study sucrose and trehalose samples were dehydrated using microwave-assisted processing. This drying approach is recognized as a fast, uniform and reproducible method to dehydrate biomaterials into the glassy state [15, 16]. The microwave-assisted technique has been successfully applied to dehydrate viruses and cat germinal vesicles [13, 14]. Since the drying kinetics and processing conditions can impact the ultimate efficacy of the glass as a stabilizer, it is necessary to clarify the effect of the filter substrate on the relaxation characteristics of embedded glasses. The plasticizing effects of water on sugar glasses were also examined by analyzing the compositional dependence of the T_α and the modulus change associated with the α -relaxation. The difference of relaxation dynamics between amorphous trehalose/water and sucrose/water were compared. Results were also verified by comparison with previous calorimetric and spectroscopic studies on similar formulations. Aspects of β -relaxation were also evaluated as a function of composition.

2. Materials and methods

A DMA (Q800, TA Instrument, USA) was used to determine the viscoelastic properties of dehydrated sugar samples by measuring their storage modulus E' , loss modulus E'' and

mechanical damping parameter $\tan\delta$ in the temperature sweep at a fixed frequency. The instrument employed cold nitrogen gas as the cooling source which was generated from controlled heating of liquid nitrogen. The DMA was calibrated in the single-cantilever mode at 1 Hz using a standard polycarbonate bar. Relaxation behavior is revealed by precise features in the temperature dependence of the viscoelastic properties, such as a significant decrease of E' that indicates the softening and loss of stiffness of the amorphous solids and a noticeable peak of E'' in the vicinity of the glass-to-liquid transition. The combination of these two changes results in a peak of $\tan\delta(=E'/E'')$. Therefore, the onset temperature of the step change of and the temperatures corresponding to the peak of E'' and $\tan\delta$ were identified as the α -relaxation temperature T_α .

To elucidate the effects of Standard 14 (S-14) borosilicate glass micro ber paper substrates (Whatman, Maidstone, UK) on the T_α determination, anhydrous D-sorbitol (Sigma-Aldrich, USA) was used as a standard. Samples were prepared by loading 30 mg sorbitol powders into a material pocket with or without filter paper. The single-cantilever mode was used at a frequency of 1 and 10 Hz, respectively. The composite specimen containing sorbitol powders was heated to 150 °C at 10 °C/min for complete melting and removing residual water in the sample. Then, it was quenched to -80 °C at 20 °C/min to vitrify the sorbitol melt. Finally, the sample was heated to 100 °C at 5 °C/min to determine the T_α of the amorphous sorbitol.

Two disaccharides of relevance to biopreservation were then evaluated. High-purity α - α trehalose dihydrate and sucrose were purchased from Ferro Pfanstiehl Laboratories and Sigma-Aldrich, respectively. Both were dissolved in 18.2 M Ω -cm water in order to achieve a 40.5 wt% (gH₂O/gdw=1.47) sucrose solution and a 41.2 wt% (gH₂O/gdw=1.43) trehalose solution. Both compositions were within solubility limits at room temperature. A 50 μ L droplet of each stock solution was pipetted onto a filter substrate of dimensions 30 mm in length $\times$ 5.5 mm in width. The solution/filter specimen was then subjected to a microwave-assisted drying process (SAM 255, CEM Corp., USA) for 5, 10, 15, 20 or 30 minutes in an 11.0 $\pm$ 2.5% Relative Humidity (RH) environment to achieve a range of end moisture contents that are relevant for biopreservation. Two replicates were prepared and dried for each duration. At each time point one of the two samples was inserted immediately into a stainless steel material pocket (Perkin-Elmer, USA), which is specifically fabricated for the DMA single-cantilever mode. The moisture content was determined by placing the other sample into a Karl Fisher titrator (V20, Mettler-Toledo, Germany). The water content in the sample was presented on a gH₂O/gdw basis (mass of water/mass of dry solids). Both the drying and titration were completed in a chamber with a controlled RH (11%) at an ambient temperature (24.5 $\pm$ 1.5 °C). The steel-sample-steel sandwich complex was pressed with pliers to minimize moisture change during preparation for analysis. The single-cantilever mode was used at a frequency of 1 Hz. Samples were initially quenched to -100 °C at 20 °C/min and then held at that temperature for 5 min to stabilize the DMA signal. Samples were then heated to 150 °C at 5 °C/min to determine the relaxation properties of amorphous sugar/water mixture. Multiple heating rates (mainly, 1–5 °C/min) have been reported by others [7, 17, 18] but a rate of 5 °C/min was used for the majority of the current study in

order to avoid significant moisture changes in the sample during a relatively long heating scan.

In order to determine the T_g of amorphous sucrose and trehalose (in anhydrous form), sucrose and trehalose dihydrate crystals were first heated up to 210 and 230 °C, respectively, at 20 °C/min and then immediately quenched to −10 and 0 °C, respectively, at 10 °C/min. The temperatures and heating rate were carefully selected to avoid decomposition. Finally, the sugar glasses were heated to 200 and 220 °C, respectively, at 5 °C/min to examine the α -relaxation behavior. All quenching or cooling steps in this study were achieved by controlled delivery of cold nitrogen gas (produced from evaporating liquid nitrogen in a Dewar) into the DMA chamber.

3. Results and discussion

3.1. Effect of filter substrates on the viscoelastic behavior of sorbitol

The mechanical response of pure sorbitol as a function of temperature, for a 1 Hz frequency sweep in the single-cantilever mode, is shown in Figures 1(a) and 1(b). Sorbitol was chosen for methodology validation purposes because it has been widely investigated in the literature [19, 20] and does not caramelize or decompose with moderate thermal excursions above its melting point. Scans of the material pocket and the pocket/filter substrate complex without sugar are also displayed in this figure. The featured changes of E' , E'' , and $\tan\delta$ can be attributed to the primary relaxation of sorbitol associated with its glass-to-liquid transition. Additionally, $\tan\delta$ fluctuated between 0.004 and 0.007 for both control pockets with and without the filter substrate (data not shown), very close to the expected value of 0, reflecting the near-ideal solid nature of the steel pocket.

Table 1 shows the results of T_g determined by DMA at 1 and 10 Hz with and without the filter substrate in the material pocket. All measurements determined with the filter paper present were marginally lower than without the filter paper. As expected, the T_g was observed to shift to a higher value with increasing frequency. This trend was not altered by the presence of filter substrates. The difference in T_g determined at 1 Hz (based on E' , E'' or $\tan\delta$) was considered to be not statistically significant ($P=0.0719$, 0.0927 , 0.1300 , respectively). The difference in T_g determined at 10 Hz was found to be significant at the $P=0.05$ level with $P=0.0172$, 0.0365 , 0.0396 , respectively. Therefore, the filter substrate contributes insignificantly to the T_g determination of sorbitol at 1 Hz but may result in statistically significant differences at an elevated frequency. Since the filter paper is used to intentionally disperse the sample to improve drying kinetics, the molecular packing of amorphous sorbitol embedded in the filter paper is slightly sparser than without the filter, resulting in increased mobility of sorbitol molecules. Therefore, at a given oscillation frequency, less energy input is needed by sorbitol molecules scattered in the filter to catch up with the frequency of the applied oscillation and to reach the maximum loss modulus, producing a slightly lower T_g compared to the sorbitol without filter as demonstrated in Table 1. As the oscillation frequency is elevated, the difference in the molecular mobility between sparsely and highly packed sorbitol becomes more pronounced. As a result, the differences between T_g of sorbitol with and without filter paper at the elevated frequency will be more pronounced.

In a previous DMA study by Talja and Roos, the T_{α} of sorbitol (measured at 2 °C/min) was reported to be -1.9 °C [20], which is considerably lower than the values determined in the current study. The slower heating rate in the previous study can provide greater sensitivity for detecting changes in the α -relaxation and thus shift the observed relaxation peak to a lower temperature [20]. The difference in scanning rate is thought to be the main reason for the differences in values obtained by different authors. The effect of scanning rate was therefore investigated in this study, as shown in Figures 1(c) and 1(d). It is evident that a higher scanning rate (e.g., 10 °C/min) can shift the α -relaxation peak of sorbitol to a significantly higher temperature (i.e., 34.5 °C), compared to 10.9 °C for samples scanned at 2 °C/min. The value we obtained at 2 °C/min (10.9 ± 0.4 °C) is still higher than the literature value (-1.9 ± 0.4 °C). Such discrepancy may be caused by differences in the material thermal histories and annealing treatments between various studies [20] as well as effects caused by different DMA operational configurations. Talja and Roos [20] firstly quenched the sorbitol melt (obtained by heating it above its melting point) to room temperature and then transferred the sample into the sample holder where it was cooled down to -120 °C to reach the glassy state. We obtained the amorphous sorbitol in a controlled manner (i.e., 10 °C/min heating to 150 °C and 20 °C/min quenching to -80 °C). It is inferred that the undefined cooling rate used in their study is slower than that in our study, which could result in a glass transition happening at a lower temperature and therefore produce a lower T_{α} . Moreover, Talja and Roos [20] used a specific disk-bending sample holder consisting of stainless steel sheets and PET plastic lms which is different from the simple stainless steel pocket in our study. The amplitude of the deformation used in their study was 15 μm while we used an amplitude of 30 μm to maximize the signal to noise ratio on our instrument.

3.2. The state of sugar solutions dehydrated on glass fiber filter paper

In order to investigate the effect of processing conditions on the relaxation characteristics of amorphous glasses formed by drying dilute sugar solutions, we used a microwave-assisted drying approach [16]. When using this approach to process samples, the moisture content in the sample can be decreased, within 30 minutes, to an end-point moisture level that enables the formation of a glassy state at room temperature. Such glasses are desirable for storage of biologics at supra-zero temperatures. Macroscopic visualization to verify the absence of sugar crystals is possible when the sample is dehydrated in droplets on non-porous surfaces [21], but when drying samples on opaque glass fiber papers even this most basic level of sample inspection is challenging. Validation of the glassy state after microwave-assisted drying is an important goal that is enabled by the current DMA technique.

In order to investigate the nature of sugar samples dried by microwave-assisted drying, both trehalose and sucrose solutions were dried on glass fiber papers and then analyzed by DMA. Figure 2(a) shows the drying kinetics of a 50 μL droplet of sugar solution, represented on a $\text{gH}_2\text{O/gdw}$ basis. An exponential decay function, as given in Eq. (1), was used to quantify the water loss w ($=w_0-w_t$, in mg, w_0 is the initial water mass and w_t is the water mass at t) as a function of the drying time, t , in minutes as shown in Figure 2(c).

$$\Delta w = w_c \left[1 - \exp \left(-\frac{t}{t_c} \right) \right] \quad (1)$$

In this equation the fitting parameter, w_c , represents the maximum water loss for the given conditions and t_c is the characteristic time for the drying process, namely, the time needed to reach $(e-1)/e \times 100\%$ of the maximum water loss. For sucrose/water we obtained $w_c = 32.98$ mg and $t_c = 3.02$ min while for trehalose/water the values obtained were $w_c = 31.58$ mg and $t_c = 2.56$ min. Both solutions dried very similarly with only a moderate difference in the final moisture level achieved after 30 minutes. Iglesias et al. [22] reported that the equilibrium moisture content of amorphous trehalose at 11% RH and 25 °C was ~ 0.039 gH₂O/gdw and that the formation of trehalose dihydrate crystals only occurred at 44% RH and above, which resulted in the amorphous trehalose to acquire an end moisture content of ~ 0.105 gH₂O/gdw upon crystallization. Therefore, it was hypothesized that the trehalose/water sample microwave dried for 20 and 30 min at 11% RH (gH₂O/gdw = 0.096 and 0.085, both less than 0.105) should be in the glassy state, with predicted T_g values of 30.7 °C and 37.5 °C, respectively [23, 24].

In order to provide insight into the state of sugar samples during various stages of processing, we examined changes in the moduli E' and E'' of samples during the initial cooling scan. If after microwave processing the sample is still liquid (which we would expect for short drying times), one should observe a step-increase of E' in the cooling scan which represents an abrupt increase in the stiffness of the sample as it transitions from liquid to glass. Furthermore the starting value of E' would be very close to the value at the end of the heating scan when the sample returns to liquid. Meanwhile, a pronounced peak of E'' should also accompany the increase of E' , a feature that is associated with the liquid-to-glass transition. If the sample after the microwave drying is already glassy, the starting value of E' prior to cooling would be significantly higher than the value at the end of the heating scan, and a peak in the cooling profile of E'' would not be expected. Figure 3 illustrates the E' and E'' variations of the trehalose/water samples after 5 and 30 mins of microwave-assisted drying, as a function of the scanning time. As seen in Figure 3(a), a sharp step-increase of E' can be observed at $t \sim 9$ min, as well as a peak in E'' . This first peak of E'' is associated with the liquid-to-glass transition while the second peak, at $t = 30.6$ min during the heating scan, represents the α -relaxation, affirming the expected liquid state after drying for just 5 min. The predicted T_g for this moisture level is -36.1 °C. It can also be observed that the value of E' at $t = 0$ min (19,836.7 MPa) is only $\sim 30\%$ higher than at $t = 60$ min (15,059.4 MPa), consistent with the expected liquid state. The features are quite different for samples dried for 30 min, which is predicted to have a T_g of 38.2 °C. In Figure 3(b), one can observe that for the sample dried for 30 min the value of E' at $t = 0$ min is almost 85% higher than at $t = 60$ min, which is the end of the heating scan, and the value of E' increases in a linear fashion during the initial cooling scan, with a slope that is much smaller than the slope for the sample which had been dried for 5 minutes. Additionally, only one remarkable peak of E'' can be observed at $t = 48.5$ min (82.1 °C) which represents the α -relaxation. In our preliminary experiments, multiple peaks of E'' were detected in the range of 100–220 °C when we heated trehalose dihydrate crystals. These peaks were assumed to correspond with the melting of crystals of trehalose dihydrate (~ 100 °C), anhydrous trehalose in α form

(~120 °C), and in β form (~200 °C) [25]. Therefore, the absence of peaks at 100°C and above for the dehydrated trehalose/water samples was further evidence that no trehalose dihydrate crystals were formed during microwave-assisted drying.

Using these criteria we then evaluated the samples dried to various end-points to identify the state of the sugar solution at the end of drying. We found that the trehalose/water mixtures dried to 0.096 and below (>20 min processing) were in the glassy state at the end of drying, which is consistent with the predicted T_g as a function of moisture content based on data collected from numerous sources [23, 24, 26]. In all cases, by examining the events that occurred during cooling and heating scans, it was concluded that all samples were either glassy at the onset of the DMA study, or were vitrified as a result of the quenching step. Evidence of crystal melting was not apparent in any of the scans. Therefore, we believe that the primary characteristic temperatures (corresponding to the onset of the step change of E' and the peaks of E'' and $\tan\delta$, respectively) determined by DMA during the heating scan were associated with α -relaxation.

3.3. Relaxation characteristics of sugar glasses

Figure 4 shows the mechanical response of the samples to the temperature sweep determined by the DMA during the heating scan. It is evident that the onset of the decrease in E' shifts to a higher temperature as the water content is reduced in the sample during processing, as shown in Figures 4(a) and (c). The peak temperature of E'' also increases in value as water content is reduced as seen in Figures 4(b) and (d). The plasticization effect of water on glassy sucrose or trehalose, evidenced by a decrease in the T_α , is similar to that observed by Chan et al. [8] when they studied the relaxation dynamics of the glucose/water mixtures by dielectric spectroscopy. These authors suggested that the shift in the temperature of α -relaxation peak in terms of E'' with added water could have the same origin as the depression of the T_g [27]. It has been emphasized by Champion et al. [4] that T_g and T_α should not be considered fully equivalent since the sample is subjected to stresses of a different physical nature (e.g., temperature change in DSC and shearing or compression in DMA). A different coupling of the imposed perturbations with the structural units may be responsible for discrepancies in the data obtained with different techniques.

Relaxation dynamics are strongly dependent on the temperature, making it possible to resolve different modes of relaxation within different temperature ranges. Other than the primary peaks, which characterize the α -relaxations, we also observed small broad peaks for some of the samples under investigation, within a specific temperature range. These peaks, lying below the T_α , provide evidence of β -relaxation behavior as seen in the inset graphs of Figure 4. The $E''(T)$ profiles of amorphous sucrose/water mixtures, which were dried for 15, 20 and 30 min, yield secondary relaxation peaks at -15.1 ± 2.7 °C ($T_g/T_\beta = 1.10$), -9.3 ± 3.4 °C ($T_g/T_\beta = 1.09$) and 11.9 ± 5.0 °C ($T_g/T_\beta = 1.04$), respectively. The T_g value computed from the Gordon-Taylor equation (discussed in the next section) was used for the determination these ratios. Amorphous trehalose/water mixtures dried for 10 and 15 min have a β -relaxation peak temperature T_β of -14.5 ± 2.7 °C ($T_g/T_\beta = 1.09$) and -11.5 ± 3.1 °C ($T_g/T_\beta = 1.15$), respectively. Given the similar structures of trehalose and sucrose and their close T_g/T_β values, we believe that the rotation of the two rings in the trehalose molecule could be the

source of the β -relaxation observed here [28]. Consistent with our results, other studies have shown that T_β increases with the addition of 10 wt% water to glucose, fructose, mannose and rhamnose (four monosaccharides) but decreases when it is added to maltose (a disaccharide) [27]. The opposing effects of water on the β -relaxation for mono- and disaccharides is possible because the β -relaxation in saccharide molecules is shown to be highly sensitive to the molecular structure [4]. A monosaccharide molecule has a cyclic, planar ring structure and its β -relaxation is mainly related to the localized motions of –OH groups. We suggest that the smaller water molecules surrounding monosaccharide molecules may fill the gaps between the hydroxyl groups and form a stronger hydrogen-bonding network that might restrict overall –OH motion, thereby contributing to a higher β -relaxation temperature with added water. On the other hand, the two rings in a disaccharide molecule can rotate around each other, which is considered to be the main source of β -relaxation in these samples. They may be packed together tightly in the absence of water, which contributes to the higher β -relaxation temperature as moisture content decreases. The insertion of water molecules between the disaccharide molecules would alter this packing arrangement and any associated stabilizing ring-ring interactions, thus enabling rotation. The β -relaxation would thus be detected at a lower temperature. Since adding water can increase the magnitude (e.g., E'' peak height and $\tan\delta$) of β -relaxation of the amorphous mixture, likely related to the mobility of water molecules [27], it is believed that sucrose and trehalose samples dried less than 15 min and 10 min, respectively, would have a noticeable β -relaxation but at a lower temperature than our experimental range. On the other end of the scale, the secondary relaxation peak would be too broad to be distinguished from the primary relaxation of trehalose-containing samples that were dried for more than 15 min.

The scanning frequency f in this study is 1 Hz which can be converted to a β -relaxation time, τ_β equivalent to $1/2\pi f = 0.159$ s. In future work, by conducting the temperature sweep at multiple frequencies, we can generate a relaxation map that will present τ_β (which can be related to the anticipated storage time) as a function of T_β/T_g , the β -relaxation temperature scaled to T_g . If the dynamics of preserved biosamples is coupled with that of the preservative matrix, such a map could enable an estimation of the expected shelf life at a given sub- T_g temperature or conversely enable a prediction of the storage T that would be necessary to achieve the desired shelf life.

The composition dependence of T_α for both sucrose/water and trehalose/water mixtures were plotted in Figure 5. For comparison T_g curves were included, as modeled by the Gordon-Taylor equation:

$$T_g = \frac{(1-x)T_{g,w} + kxT_{g,s}}{(1-x) + kx} \quad (2)$$

where, in the case of sucrose/water, the fitting parameter is given by $k=0.196$ and the glass transition temperature of pure sucrose, $T_{g,s}=347$ K (74 °C). For trehalose/water, $k=0.188$ and $T_{g,s}=390$ K (117 °C) [23, 24]. The glass transition temperature of pure water was set at 135 K (–138 °C) [29]. Because the trend of the T_α - x curve is very similar to that of T_g - x curve, the same formalism was used here to model the T_α data, as given by Eq. (3):

$$T_{\alpha} = \frac{(1-x)T_{\alpha,w} + kxT_{\alpha,s}}{(1-x) + kx} \quad (3)$$

where the α -relaxation temperatures of pure trehalose and sucrose were determined based on the three moduli described previously. The parameters k and $T_{\alpha,w}$ are fitting parameters, with the latter assumed to be the T_{α} of glassy water. The values of these parameters are further articulated in Table 2. The k values listed in Table 2 actually are very close to those used to describe the glass transition temperature, pointing to a similar mechanism for the composition dependence of T_{α} and T_g . The theoretical T_{α} of water is estimated to be 148.7 ± 6.8 K. This would be a useful estimate for future studies since such a value has been difficult to determine experimentally.

The T_{α} of the trehalose/water compositions was observed to be higher than that of the sucrose/water compositions at the same moisture content. Moreover, it was observed that for both the sucrose/water and trehalose/water mixtures, the ratio of T_g/T_{α} is slightly above 0.9 based on the loss modulus which is close to previous results obtained by others for aqueous sucrose solutions (90–98 wt% sucrose) (0.94–0.99, determined by mechanical spectroscopy) [6], glucose, fructose and mannose solutions containing 10 wt% water (~0.92, determined by dielectric spectroscopy) [27] and glucose solutions containing 10–30 wt% water (0.89–0.92, determined by mechanical spectroscopy) [8].

In Figure 5, we also indicated the water content that corresponds to a T_g equal to the ambient temperature (24.5 °C). For sucrose/water solutions, no samples under investigation were expected to be in the glassy state since the final moisture content was higher than 0.058 even after 30 min drying. In the case of trehalose/water solutions, the samples dried for 15 min or longer could theoretically be glassy at ambient temperature if the processing conditions were adequate, which was consistent with our above findings.

3.4. Stiffness of trehalose and sucrose compositions

The water fraction in the sample has a pronounced effect on the temperature of the α -relaxation, but it also affects the magnitude of the modulus changes during the heating scan, as seen in Figure 6. E' was calculated as the difference in the storage modulus between the glassy state (the higher level) and in the rubbery state (the lower level), and this value was normalized to the glassy state level, to explore relative changes between different sugars. For example, the decrease of the storage modulus, E' , accounts for 55.9% of the initial E' (the beginning of the heating scan) for sucrose/water samples dried for 5 min but decreases to 34.5% when the sample processed for 30 min. Similarly, E'/E' is 56.6% for trehalose/water samples dried for 5 minutes but the proportion decreases to only 30.2% after 20 min drying. Since the rubbery plateau for the 30 min-dried trehalose cannot be identified unambiguously we did not include this data point in Figure 6. It is the same case for the relative peak height of E'' which was calculated in terms of E''/E'' . E'' was determined as the difference between the peak height and the value in the glassy state. It is interesting that the change of the moduli of trehalose/water mixtures tends to be smaller than that of sucrose/water mixtures while their actual water contents are almost the same at the same drying time point. We suggest that amorphous trehalose would be expected to have a

reduced mobility compared to sucrose even in the rubbery or liquid state since it has a higher tendency to self-associate via hydrogen-bonding. This would increase the stiffness of the sample in the non-glassy state and therefore decrease the relative change of the moduli. Or, in other words, amorphous sucrose/water samples tend to soften more than their trehalose counterparts during the glass-to-liquid transition. There has been evidence supporting the stronger self-association behavior in trehalose compared to sucrose. For example, Lerbret et al. [30] reported that sucrose formed self-clusters of smaller sizes than trehalose or maltose in aqueous solutions, which was probably related to the higher number of intramolecular hydrogen bonds of sucrose molecules compared to maltose and trehalose, since hydroxyl groups involved in these intramolecular interactions did not remain available for other intermolecular interactions. It was also found that poly(vinylpyrrolidone) was more able to disrupt the self-association of amorphous sucrose than other sugars such as trehalose and raffinose [31], mainly due to the weaker self-clustering ability of sucrose. With regard to the plasticizing effect of water, it was similarly observed that the storage modulus showed a smaller decrease and a higher level for the rubbery plateau in terms of E' for the milk solids with higher maltodextrin content [7]. In their case, the milk solids act as the plasticizer as does water in our case and the polymer maltodextrin functions as the glass former as does sucrose or trehalose here.

4. Conclusion

Drying biomolecules and biologics on porous surfaces is recognized as an important strategy for ambient preservation of small volumes, such as viral titers for vaccines, liquid samples for diagnostics, and gametes for *in vitro* fertilization. However, establishing the physical state of such formulations while embedded on this support matrix can be challenging. A dynamic mechanical analysis was performed on various dried sugar compositions on filter papers, using material pockets to contain the samples. The filter substrate was found to have insignificant effects on the visco-elastic behavior of samples, implying that the filter papers were not significantly changing the glassy characteristics of these stabilizers. By studying the mechanical response, and the compositional dependence of T_α , we established the physical state of samples at various stages of processing, and differences between trehalose and sucrose with regard to relaxation dynamics were resolved. It was observed that the change of the moduli during the α -relaxation of trehalose/water was smaller than sucrose/water, which can be attributed to a reduced mobility in the rubbery state of trehalose due to its stronger self-association. A Gordon-Taylor like correlation between T_α and the composition was observed. The plasticizing effect of water on the α -relaxation dynamics was mainly two-fold: depressing the T_α and increasing the magnitude of changes of E' and E'' . Amorphous sucrose/water mixtures dried by microwave processing for 15, 20 and 30 min and trehalose/water mixtures dried for 10 and 15 min exhibited characteristics of secondary β -relaxation, possibly due to ring rotation, a characteristic of disaccharide molecules. The validation of known sugar glass characteristics, coupled with new insights into relaxation behavior that were attained with this approach provides evidence for the utility of this methodology for fiber matrix embedded materials.

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Highlights

- A method to evaluate relaxation of glassy sugar within glass fiber paper is proposed.
- Absence of crystallinity following drying within fiber paper is verified.
- A Gordon-Taylor like correlation between T_{α} and the composition is observed.
- Water plasticizes α -relaxation dynamics by increasing magnitude of modulus change.

FIGURE 1.

The α -relaxation process of pure sorbitol with and without filter paper, respectively, determined by DMA at 1 Hz: (a) the variation of the storage modulus E' ; (b) the variation of the loss modulus E'' . The effect of heating rates on the determination of T_g (Mean $\pm$ SD) of amorphous sorbitol without filter paper (c) based on the $E''(T)$ profile (d).

FIGURE 2.

The microwave-assisted drying kinetics of a 50 µL droplet of sugar solution on a filter paper: (a) $gH_2O/gdw-t$, the inset graph (b) is a zoom enhanced region from 5 to 30 min; (c) $w-t$ and the solid and dashed lines are the fitting curves given by Eq. (1) and the inset graph (d) is the drying rate as a function of t given by the derivative of Eq. (1).

FIGURE 3.

The mechanical responses of (a) trehalose/water with $g_{H_2O}/gdw=0.282$ (after 5 min drying) and (b) trehalose/water with $g_{H_2O}/gdw=0.085$ (after 30 min drying) as a function of scanning time during the temperature sweep.

FIGURE 4.

Variations of the storage and loss moduli of sucrose/water (a and b) and trehalose/water (c and d) mixtures as a function of T (heating scan). The inset graphs are enlargements corresponding to the β -relaxation shown in the graphs (b) and (d).

FIGURE 5.

The α -relaxation temperature T_α of microwave-dried sucrose (left panel) and trehalose (right panel) solutions based on storage (a), loss (b) and $\tan\delta$ (c) moduli and their corresponding T_g calculated with the Gordon-Taylor equation reported in the literature[23, 24, 29].

FIGURE 6.

The magnitude of modulus change through the α -relaxation as a function of the water content ($\text{gH}_2\text{O/gdw}$). (a) the relative storage modulus change E'/E' and (b) the relative loss modulus change E''/E'' . The data points correspond to 5–30 min from right to left.

Table 1

The α -relaxation temperature T_α (Mean $\pm$ SD) of D-sorbitol measured by DMA with or without the filter substrate at 1 and 10 Hz

Modulus	T_α (1 Hz)/°C		T_α (10 Hz)/°C	
	Without filter	With filter	Without filter	With filter
E'	20.9 $\pm$ 0.8	19.7 $\pm$ 0.3	25.7 $\pm$ 0.1	23.4 $\pm$ 0.4
E''	25.7 $\pm$ 0.5	25.1 $\pm$ 0.4	32.3 $\pm$ 0.3	30.9 $\pm$ 0.1
$\tan\delta$	26.8 $\pm$ 0.4	26.4 $\pm$ 0.5	33.8 $\pm$ 0.2	32.5 $\pm$ 0.1

Table 2

The values of the parameters k , $T_{a,s}$ and $T_{a,w}$ in Eq. (3)

Composition	Modulus	k	$T_{a,s}/K$	$T_{a,w}/K$	R^2
sucrose/water	E'	0.21	358.1	153.5	0.9901
	E''	0.27	369.3	136.3	0.9636
	$\tan\delta$	0.25	371.0	150.5	0.9643
trehalose/water	E'	0.17	411.1	146.6	0.9756
	E''	0.21	417.9	155.2	0.9687
	$\tan\delta$	0.22	418.3	150.6	0.9679