Investigating the Moisture Sorption Behavior of Amorphous Sucrose Using a Dynamic Humidity Generating Instrument

X. Yu, S.M. Kappes, L.A. Bello-Perez, and S.J. Schmidt

ABSTRACT: The moisture sorption behavior of freeze-dried amorphous sucrose was investigated using a dynamic humidity generating instrument, the Dynamic Vapor Sorption (DVS) instrument. The kinetic moisture sorption profiles of freeze-dried amorphous sucrose samples with 29% crystalline content were obtained using the DVS instrument at 9 relative humidity (RH) values, ranging from 10% to 90%, at 25 °C. Moisture-induced crystallization was observed for %RH values between 40% and 80%, where the crystallization onset time decreased as %RH increased. The moisture sorption behavior of freeze-dried amorphous sucrose with 3 crystalline contents, 23%, 29%, and 80%, was also compared, revealing that the crystalline content had a significant impact on the pseudo-sorption isotherm of freeze-dried amorphous sucrose. In general, for %RH values below 90%, samples that had a lower percent crystalline content had a higher pseudo-equilibrium moisture content, with the difference becoming most pronounced for the 60% to 80% RH values. The moisture-induced crystallization results as a function of %RH obtained in this study were compared to those previously reported in the literature, leading to an extensive discussion of both the experimental protocols used and the hypothesized mechanisms governing the long-term stability of amorphous materials. The hypothesized mechanisms discussed included the glass transition temperature boundary, the zero mobility temperature, and the hydration limit. Based on the dissimilarity in these hypothesized mechanisms, additional theoretical and experimental exploration is still merited in order to adequately predict the conditions (for example, moisture content, %RH, and temperature) required to ensure long-term stability of amorphous solids.

Keywords: amorphous sucrose, crystallization onset time, dynamic vapor sorption, moisture-induced crystallization, pseudo-moisture sorption isotherms, stability

Introduction

Sucrose, a disaccharide composed of 1 α-D-glucopyranosyl unit and 1 β-D-fructofuranosyl unit joined by a glycosidic linkage (Flink 1983), is one of the most commonly used food ingredients. Sucrose can exist in both the crystalline and amorphous states. The crystalline state is an equilibrium, lowest energy and entropy, solid state that exhibits an orderly molecular arrangement of molecules, with a repeating pattern extending in all 3 spatial dimensions. When temperature is increased, the crystalline solid state experiences a 1st-order phase transition to the liquid state, called melting, which occurs at a characteristic temperature (Tm) for each crystalline material. The melting temperature of crystalline sucrose, generally reported in the literature, ranges from 185 to 190 °C (Hurtta and others 2004).

On the other hand, the amorphous (or noncrystalline) state is a nonequilibrium, excess free energy and entropy, solid state that retains the disorder of the liquid state, exhibiting some short-range molecular order (Yu 2001) but no long-range order. Amorphous food materials, also called vitreous or glassy materials, are commonly formed when crystalline materials are melted and cooled quickly or when crystalline materials are dissolved in water and then the water is rapidly removed (Roos 1995, 2007). The widespread application of the glass transition concept for the investigation of the amorphous solid state in food materials is attributed to the pioneering work of Slade and Levine (Levine and Slade 1986; Slade and Levine 1991, for example).

When temperature is increased, the amorphous solid state (glassy state) experiences a pseudo-2nd-order phase transition (Schmidt 2004) to the supercooled liquid state (rubbery state). This transition is called the glass transition and occurs over a characteristic temperature range for each amorphous material. A typical amorphous material undergoes a glass transition at 100 to 150 °C below its equilibrium Tm (Sperling 1992; Roos 2007). The glass transition temperature (Tg) of pure, dry amorphous sucrose has been measured by a number of researchers and has been reported to range from 52.0 to 75.9 °C (Roe and Labuza 2005). The variation in Tg reported in the literature is dependent upon a number of sample, processing, and measurement method factors (discussed in Schmidt 2004), such as residual moisture content (Roos 1993), melting conditions (Vanhal and Blond 1999), and physical aging (Wungtanagorn and Schmidt 2001), to name a few.

The distinctions between crystalline and amorphous solid sucrose described previously underlie the differences observed in their water sorption behavior. In the case of crystalline sucrose, a very small amount of water adsorbs to the surface of the sucrose crystals at low a_w values, then at a characteristic a_w, for a given

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temperature (called the deliquescence point), the sucrose crystals begin to adsorb water and dissolve (Type III or "J" shaped isotherm; Bell and Labuza 2000).

In the case of amorphous sucrose, water is adsorbed into the amorphous sucrose at both low and high a_w values. However, at high enough a_w values the amorphous sucrose molecules adsorb enough water to transition to their more stable crystalline solid state (called moisture-induced crystallization), with subsequent release of the previously adsorbed water (Karel 1973). Crystallization can occur as a result of an increase in molecular mobility of the amorphous solid acquired by plasticization via water adsorption, promoting rapid nucleation and crystal growth. At even higher a_w values (above the %RH of a saturated sucrose solution at the experimental temperature), the amorphous sucrose adsorbs water and dissolves, forming a sucrose solution with virtually the same equilibrium moisture content as that obtained by the adsorption of water by crystalline sucrose at the same a_w and temperature values. It is interesting to note that the a_w (or %RH) of a saturated sucrose solution made with amorphous or crystalline sucrose is in effect the same as the deliquescence point for crystalline sucrose at the same temperature.

When attempting to measure sorption isotherms of amorphous sugars, it is important to note that true equilibrium moisture content values are not achieved for a_w values at which moisture-induced crystallization occurs, because, over time, the amorphous sugars change state and release the water they originally adsorbed. Thus, for a_w values at which moisture-induced crystallization occurs, pseudo-equilibrium moisture contents are often used to construct a pseudo-moisture sorption isotherm for an amorphous sugar. The pseudo-equilibrium moisture content is defined as the moisture content obtained by a sample at a specified relative humidity (or water activity) just prior to when the amorphous sugar begins to crystallize and lose the moisture it had previously gained. An important parameter for quantifying the moisture-induced crystallization process is the onset time, which is defined as the time needed for an amorphous sugar sample, when placed at a specified relative humidity and temperature, to begin releasing water and crystallizing.

The moisture-induced crystallization feature of the sorption isotherm behavior of amorphous sugars has been studied by several researchers and is influenced by a number of factors, which are summarized in Table 1, using example research studies from the literature. It has been reported that increasing relative humidity, increasing temperature, and increasing crystalline content (decreasing amorphous content) promotes the occurrence of moisture-induced crystallization. The type of drying method was also found to impact moisture-induced crystallization of amorphous sugars. Freeze-dried amorphous lactose was found to exhibit a higher pseudo-equilibrium moisture content than spray-dried amorphous lactose when exposed to the same relative humidity and temperature (Haque and Roos 2006; Miao and Roos 2006). In addition, the spray-dried amorphous lactose crystallized slightly faster than the freeze-dried amorphous lactose (Haque and Roos 2006; Miao and Roos 2006). These results were attributed to the different physical structures developed in the amorphous samples due to the different drying methods (Miao and Roos 2006).

Most studies that have investigated the moisture sorption behavior of amorphous sugars have used methods where the amorphous sugar samples are stored in chambers (for example, desiccators) over saturated salt or sulfuric acid solutions and are removed and exposed to environmental laboratory conditions for discrete weight measurements over time. Recently, humidity generating instruments have been developed where the sample is exposed to the desired relative humidity values at a select, constant temperature while sample weight changes are recorded by an ultrasensitive microbalance on a nearly continuous basis (for example, every 60 s) without needing to remove the sample from the chamber. The humidity generating instrument used in this study was the Dynamic Vapor Sorption (DVS) instrument by Surface Management Systems (London, U.K.).

Four studies were identified that used the DVS to investigate water sorption properties of amorphous or partially amorphous materials; however, no studies were found that specifically investigated the water sorption properties of amorphous sucrose using the DVS. However, 1 study was found that coupled a moisture sorption gravimetric analyzer (SGA-100, VTI Corp., Hialeah, Fla., U.S.A.) with a Raman spectrometer to better understand the various modes of water–solid interactions important in pharmaceutical systems, including freeze-dried amorphous sucrose (Gift and Taylor 2007). The amorphous sucrose sample was ramped to 60% RH during which Raman spectra were collected. The Raman spectra confirmed the moisture-induced amorphous to crystalline transformation of sucrose.

Buckton and Darcy (1995), studying mixtures of amorphous lactose prepared by spray-drying and crystalline alpha-lactose monohydrate, and Mackin and others (2002), studying amorphous and crystalline benzyl ether derivative, both reported the successful use of the DVS instrument for quantifying low levels of amorphous content in predominantly crystalline materials. Burnett and others (2004) used the DVS instrument to determine the onset relative humidity for glass transition and crystallization processes in spray-dried lactose samples at 25 °C, while Burnett and others (2006) used the DVS instrument to investigate moisture-induced crystallization kinetics in spray-dried lactose samples. Burnett and others (2006) performed 2 sets of experiments: (1) varying %RH at constant temperature and (2) varying temperature at constant %RH. Experiments performed between 48% and 60% RH at 25 °C showed that onset time to crystallization decreased dramatically

Table 1—Factors affecting the sorption properties of amorphous sugars illustrated using example research studies from the literature.

Factors	Materials studied	Experimental conditions	Literature reference
Relative humidity	Sucrose	% RH: 4.6 to 33.6 (25 °C)	Makower and Dye (1956)
		% RH: 32 to 70 (23 °C)	Carstensen and Van Scoik (1990)
	Milk powders	% RH: 33 to 76.4 (24 °C)	Jouppilla and Roos (1994)
Temperature	Sucrose	35 and 47 °C	Iglesias and others (1975)
		Approximately 20 and 40 °C	Wang and Langrish (2007)
	Lactose	12, 20, 30, 38 °C	Bronlund and Paterson (2003)
		Approximately 20 and 40 °C	Wang and Langrish (2007)
Drying method	Lactose, trehalose	Spray-dried compared with freeze-dried	Miao and Roos (2006)
	Lactose, lactose/protein mixtures	Spray-dried compared with freeze-dried	Haque and Roos (2006)
Amorphous content	Lactose	Ranged from 0.05% to 0.5%	Buckton and Darcy (1995)

with increasing humidity. At 50% and 51% RH, crystallization occurred rather slowly (approximately 600 to 700 min) and exhibited a bumpy decline (referred to as a 2-step crystallization process) in the amorphous fraction, while at 53%, 55%, 57%, and 60% RH, crystallization occurred relatively quickly (approximately 200 to 250 min) and exhibited a smooth (referred to as a 1-step crystallization process) decline in the amorphous fraction. Experiments performed at 51% RH between 22 and 32 °C indicated that onset time to crystallization decreased with increasing temperature. Above 25 °C at 51% RH, crystallization occurred in 1 step, while below crystallization occurred in 2 steps.

There are 4 main differences between experiments carried out using the saturated salt or sulfuric acid solution method compared to the new humidity generating instruments: (1) the air around the sample is static compared with dynamic (method difference), (2) the sample size is usually in grams compared with micrograms (method difference; smaller sample size, faster equilibration time), (3) data point collection is discrete compared with continuous (new humidity generating instruments greatly increase the time resolution of the sorption data), and (4) changes in sample weight are measured outside compared with inside the %RH and temperature controlled environment (a data collection advantage for the new humidity generating instruments). In light of these aforementioned differences and advantages of using a new humidity generating instrument and the limited number of studies that have used these instruments to investigate amorphous sugar materials, a reexamination of the dynamic moisture sorption behavior of amorphous sucrose is merited. Thus, the objective of our study was to investigate the moisture sorption behavior of freeze-dried amorphous sucrose using a dynamic humidity generating instrument.

Materials and Methods

Materials

Food-grade crystalline sucrose, donated by Tate & Lyle (Decatur, Ill., U.S.A.), was used "as is" without further purification and transformed to the amorphous, glassy state by freeze-drying. A General Purpose Freeze Dryer (The VirTis Co. Inc., Gardiner, N.Y., U.S.A.) was used to freeze-dry a 20% (w/w) solution of sucrose and distilled water. The 20% (w/w) solution was frozen in 8″ × 10″ × 2″ metal trays filled to 1″ in a −20 °C freezer for 24 h. The frozen sucrose solution was bathed in liquid nitrogen for approximately 5 min to achieve −45 °C conditions. The metal trays were then placed in the freeze-dryer at −45 °C and 30 mtorr for 24 h. The temperature was increased in 7 steps over the course of 6 d. After the initial freezing at −45 °C for 24 h, the temperature was increased to −35 °C for 24 h, −30 °C for 24 h, −20 °C for 24 h, −10 °C for 24 h, 0 °C for 24 h, and 25 °C for 24 h. The metal trays containing the freeze-dried sucrose were immediately transferred to a vacuum oven where they were dried under full vacuum at 60 °C for 48 h.

After vacuum oven drying, the freeze-dried sucrose was immediately transferred to moisture impermeable bags and vacuum-sealed using a Food Saver Compact II (Tilia Inc., San Francisco, Calif., U.S.A.). The vacuum-sealed bags were stored in a 25 °C incubator until use. Freeze-dried amorphous sucrose samples used for each DVS experimental run were fresh, obtained from newly opened bags, because it was observed that samples from bags that were reopened repeatedly slowly increased in crystalline content over storage time, presumably due to adsorption of moisture from the air and subsequent slow moisture-induced crystallization. The 80% crystalline content amorphous sucrose samples (freeze-dried, vacuum oven dried, then equilibrated in the DVS to the initial sample a_w value, and then exposed to 0% RH prior to exposed to the target %RH) used for comparison from Yu (2007) were obtained as a result of this slow (during storage time) crystallization process. Whereas the 23% crystalline content amorphous sucrose samples used for comparison from Kappes (2001) were obtained from fresh samples (freeze-dried, vacuum oven dried, then exposed in the DVS to the target %RH).

The initial water activity of the freeze-dried amorphous sucrose sample in this study was determined in triplicate using an AquaLab 3TE chilled-mirror water activity instrument (Decagon Devices, Pullman, Wash., U.S.A.) and found to be 0.130 a_w at 25 °C. The initial moisture content of the freeze-dried amorphous sucrose was determined in triplicate using the DVS instrument and found to be 1.81% (wet basis). The freeze-dried amorphous sucrose sample was loaded into the DVS chamber and the balance was stabilized at the initial a_w of the sample (0.130 a_w or 13% RH). Then the sample was exposed to 0% RH until no additional weight change was observed using a 0.0005% dm/dt weight change criterion for 5 consecutive min.

DVS Profiles

Moisture sorption profiles were obtained for freeze-dried amorphous sucrose using a DVS Series 1000 instrument (Surface Measurements Systems, London, U.K.). The DVS instrument is composed of 2 sealed chambers, the reference chamber and the sample chamber. The relative humidity inside these chambers is controlled by using electronic mass flow controllers to proportionally mix dry air with moisture-saturated air, which then flows passed the sample at a set flow rate. A freeze-dried sucrose sample weighing approximately 10 mg was placed on a quartz DVS round bottom sample pan. Each new sample was placed into the DVS instrument at 13% RH, corresponding to the initial a_w value of the sample (0.130 a_w) to help stabilize the sample and the balance before the sample was ramped to the desired experimental %RH. The relative humidity values used ranged from 10% RH to 90% RH in 10% increments. For %RH values from 10% to 80%, a 1-step special automatic operation (SAO) method was created for each sample using the dm/dt mode. The dm/dt criterion was set at 0.0005% for 5 consecutive min and the maximum amount of time at each %RH was set at 2000 min. For the 90% RH value, since water sorption was expected to be quite large, the step time mode was used with a maximum time of 9000 min; however, equilibrium was reached at 2800 min and the run was terminated at that time. Since each sample started at 13% RH only the 10% RH condition resulted in desorption of water from the sample, all other %RH conditions resulted in adsorption.

After each experiment, the data were exported to Microsoft Excel using a DVS Macro. The change in mass of the sample in the DVS was converted to moisture content on a dry basis (g water/g solid) by determining the moisture in grams of the initial sample and adding or subtracting (depending on the relative humidity) it from the weight change measured in the DVS. The total amount of water in grams was then divided by the grams of solid freeze-dried amorphous sucrose in the initial sample to calculate the moisture content on a dry basis (g water/g solid). The air flow in the DVS was set at 500 standard cubic centimeters for all experiments. The balance was calibrated at 25 °C with a 100 mg weight and the probe and target %RH values were verified using standard salts (Levoguer, Application Note 1).

Crystallization onset (also called induction) times were also determined from the moisture sorption profiles. Determination of crystallization onset times does not appear to be strictly defined in the literature. The approach used in this study and also applied to the cited literature sources herein was as follows. The crystallization

onset times for samples that exhibited moisture-induced crystallization were taken as the point in time when the moisture content decreased by an amount approximately greater than the precision of the moisture content measurement method employed. For example, if the precision of the moisture content measurement method was given as 0.05%, then the onset time was taken as the point in time when the equilibrium moisture content decreased by an amount greater than 0.05%.

Percent crystalline content

The percent crystalline content of the freeze-dried sucrose samples was determined using a TA Instruments 2920 Differential Scanning Calorimeter (DSC) (New Castle, Del., U.S.A.), equipped with a refrigerated cooling accessory. Thermal Solutions Instrument Control software was used for instrument operation and Universal Analysis software (New Castle) was used for data analysis. The samples were equilibrated at 10 °C and then ramped to 220 °C at 10 °C/min. Duplicate samples were run for each sample. Indium was used for DSC baseline and for temperature calibration. Hermetically sealed DSC pans were used for all samples. All DSC pans were cleaned before use to remove any residual machine oil by sonicating in ethanol for 30 min followed by air drying at room temperature. The crystalline content of the freeze-dried amorphous sucrose in percent was calculated by dividing the difference in enthalpy between the melting peak and the spontaneous crystallization peak by the enthalpy of the melting peak and multiplying by 100. The DSC determined percent crystalline content was 29% for the freeze-dried amorphous sucrose samples used in this study.

Statistical analysis

Variation in experimental run time (average standard deviation ± 630 min), pseudo-equilibrium moisture content (average standard deviation ± 0.0071 g water/g solid), and crystallization onset time (average standard deviation ± 18.4 min) was calculated between duplicate runs at different %RH values using Microsoft Excel. The greatest variation was observed in the run time and smaller variation for the pseudo-equilibrium moisture content and crystallization onset time. Variation in the data is not surprising, since amorphous solid materials are by nature highly variable and innately unstable and are, in these experiments, being subjected to sorption processes, which are dynamic and kinetic in nature.

The SAS 9.1 "PROC REG" program was used to estimate the 3rd-order polynomial prediction equation and upper and lower 95% confidence limits for the Sun and others (1996) sucrose data, which were used in the construction of Figure 8.

Results and Discussion

The moisture sorption profiles of freeze-dried amorphous sucrose obtained using the DVS instrument at each %RH are shown in Figure 1 (10% to 90% RH profiles with full *x*- and *y*-axis scales) and 2 (10% to 80% RH profiles with modified *x*- and *y*-axis scales to show profile details). From these moisture sorption profiles, we can assess the effect of %RH on the moisture sorption behavior of freeze-dried amorphous sucrose, obtaining both the pseudo-sorption isotherm and crystallization onset times. In addition, prediction of the occurrence of the moisture-induced crystallization behavior of amorphous sucrose will be discussed.

Effect of %RH on moisture sorption behavior

The freeze-dried amorphous sucrose samples exposed to 10% RH desorbed a very small amount of moisture, then maintained that equilibrium moisture content for the 2000-min duration of the experiment. Freeze-dried amorphous sucrose samples exposed to 20% and 30% RH values adsorbed a small amount of moisture and also maintained the resultant equilibrium moisture content for the 2000-min duration of the experiment.

Freeze-dried amorphous sucrose samples exposed at 40% to 80% RH values also adsorbed moisture initial; however, over time moisture loss began to occur. Samples at these %RH values exhibited the characteristic water adsorption-release behavior associated with the well-documented moisture-induced crystallization behavior of amorphous sugars (amorphous sucrose examples, Makower and Dye 1956; Carstensen and Van Scoik 1990; Saleki-Gerhardt and Zografi 1994; Hancock and Dalton 1999; Roe and Labuza 2005). As can be seen from the profiles shown in Figure 1 and 2, the experimental runs between 40% and 80% usually met the 0.0005% dm/dt for 5 consecutive min criterion before the maximum amount of time allowed at each %RH (2000 min) was reached. The 40% to 80% RH individual experimental run times ranged from a low of 300 min to the maximum of 2000 min.

It is interesting to note differences in shape between the 40% and 80% moisture sorption profiles in Figure 2. The middle 3 profiles (50%, 60%, and 70%) exhibited a similar pattern—a quick water

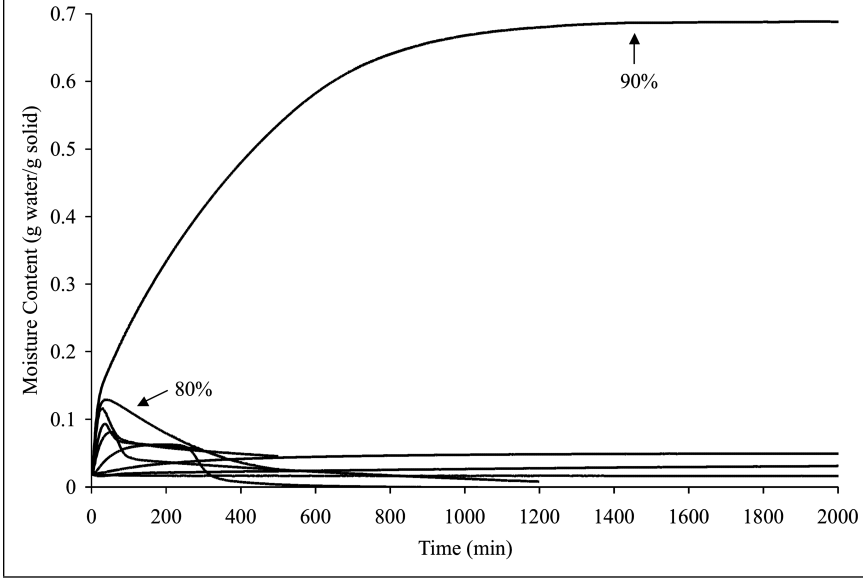

Figure 1—Moisture sorption profiles of freeze-dried amorphous sucrose exposed at 10% to 90% RH at 25 °C using the DVS instrument. Details of the moisture sorption profiles for the other %RH values can be seen in Figure 2.

adsorption peak followed by an initial rapid water loss stage and then a slower second water loss stage. Whereas the 80% RH moisture sorption profile showed the quick water adsorption peak, but was followed by a much less rapid water loss stage. This may be due to dilution effects that slow down crystallization at this high relative humidity value. On the other hand, the 40% RH moisture sorption profile did not exhibit the quick water adsorption peak, but rather adsorbed water more slowly with almost a plateau in moisture content before subsequent rapid water loss. This may be due to the smaller difference between 40% RH and the "as is" amorphous sucrose a_w (0.130 at 25 °C), which translates into a decrease in the moisture sorption driving force.

Freeze-dried amorphous sucrose samples at 90% RH continually adsorbed moisture, forming a solution with an average equilibrium moisture content of 0.688 g water/g solid at 25 °C, which was much higher than the moisture contents reached at the other relative humidity values. This 90% RH equilibrium moisture content for amorphous sucrose is similar to that obtained by others for crystalline sucrose at 90% RH. For example, the sucrose isotherm in Kou and others (1999) reveals an equilibrium moisture content value of approximately 0.66 g water/g solid for crystalline sucrose at 90% RH at 20 °C (note the temperature difference between the 2 isotherms). The 90% RH experimental run, using the step time mode, reached equilibrium in approximately 2800 min. No decrease in moisture content was observed at 90% RH, indicating that no moisture-induced crystallization occurred at that %RH; rather the freeze-dried amorphous sucrose simply dissolved in the water being adsorbed.

Pseudo-sorption isotherm

The maximum equilibrium moisture contents achieved by the freeze-dried amorphous sucrose samples at each relative humidity, termed the pseudo-equilibrium moisture content for the samples exposed to %RH values that exhibit moisture-induced crystallization, were plotted against relative humidity in Figure 3 to yield the pseudo-sorption isotherm of freeze-dried amorphous sucrose

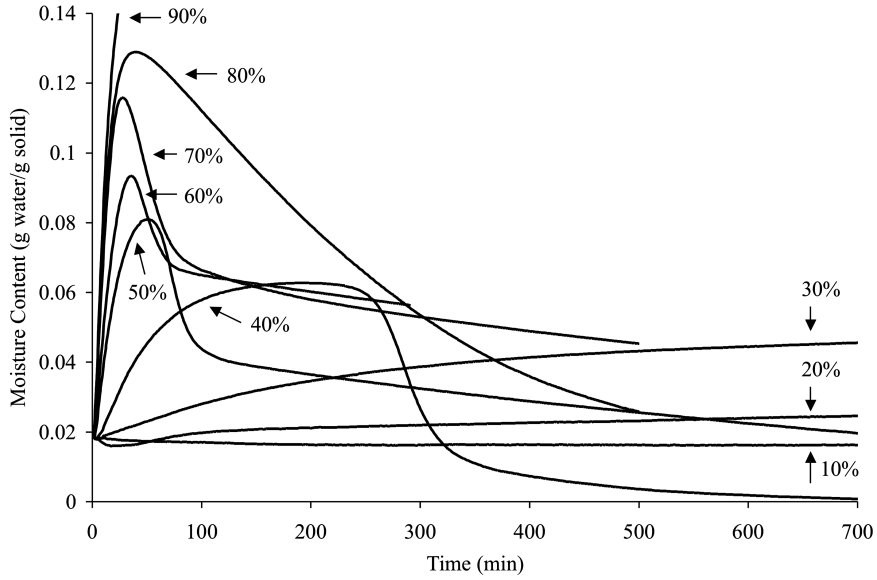

Figure 2—Close-up of the moisture sorption profiles of freeze-dried amorphous sucrose exposed at 10% to 80% RH at 25 °C using the DVS instrument (full scale plot is shown in Figure 1).

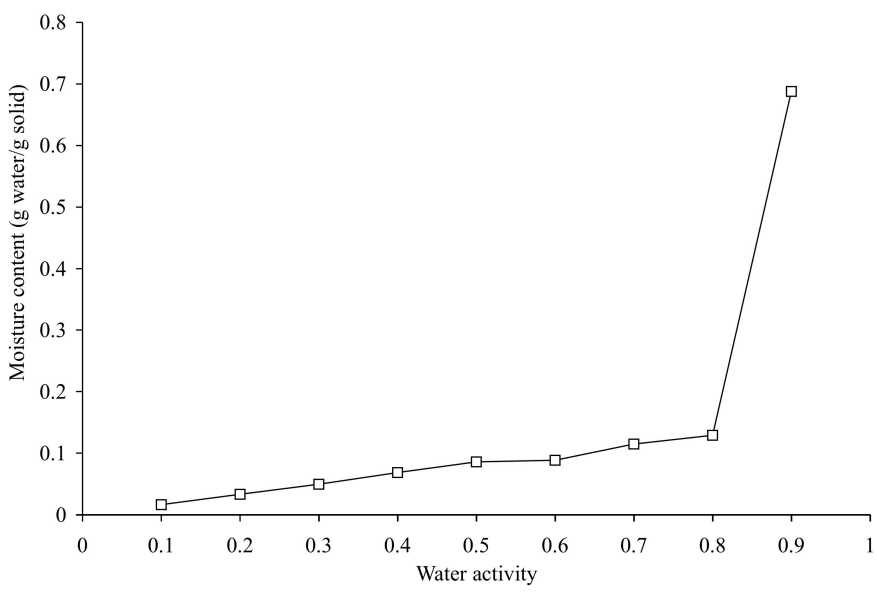

Figure 3—Pseudo-moisture sorption isotherm of freeze-dried amorphous sucrose at 25 °C obtained using the DVS. Note that the line in the figure is to guide the eye and is not a fit to the data.

at 25 °C. As %RH increased, the moisture content of the freeze-dried amorphous sucrose samples increased.

Figure 4 shows the comparison of the pseudo-moisture sorption isotherms of freeze-dried amorphous sucrose obtained with different percent crystalline contents of 25% (Kappes 2001), 29% (this study), and 80% (Yu 2007). The different percent crystalline content amorphous sucrose samples were prepared using the same freeze-drying technique. Additional sample details are mentioned in the Materials and Methods section. Figure 4 shows that, in general, for %RH values below 90%, samples that had a lower percent crystalline content had a higher pseudo-equilibrium moisture content. This difference became most pronounced at the 60% to 80% RH values. At 90% RH, all of the samples reached a similar equilibrium moisture content, since at 90% RH the freeze-dried amorphous sucrose completely dissolves in the water. The impact of crystalline content on the equilibrium moisture contents observed in Figure 4 is consistent with the findings for amorphous lactose reported by Buckton and Darcy (1995).

Several other researchers have also reported pseudo-moisture sorption isotherms of amorphous sucrose (Iglesias and others 1975; Sloan and Labuza 1975; Van Scoik 1987; Saleki-Gerhardt 1993; Saleki-Gerhardt and Zografi 1994). All of the available amorphous sucrose literature isotherms produced by both freeze-drying and spray-drying were plotted along with the isotherm from this study (Figure 5). Variation between the moisture content and %RH for the studies plotted in Figure 5 can be examined by dividing the figure into 3 regions. At low %RH values (0% to 40% RH), the relationship between the moisture content and %RH appears relatively consistent between studies. However, between 50% and 80% RH, moisture contents vary between studies. Variations in this %RH range can be attributed to the differences between the amorphous sucrose samples studied, such as initial concentration of the sucrose solution, drying method used to produce the amorphous sucrose, percent crystalline content of the sample, experimental temperature, and sorption technique employed. Above the %RH at which the amorphous sucrose is dissolved by water (the saturated

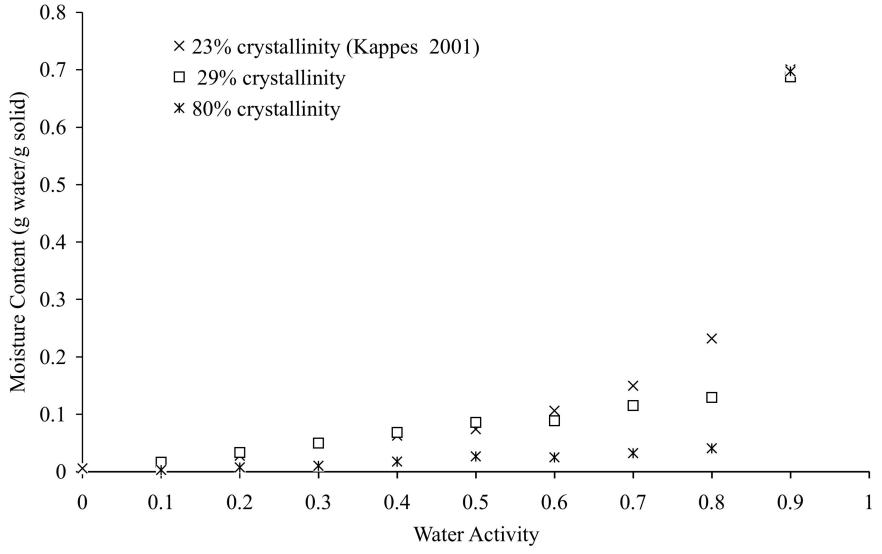

Figure 4—Pseudo- moisture sorption isotherms for freeze-dried amorphous sucrose with three different percent crystalline contents obtained at 25 °C using a DVS instrument.

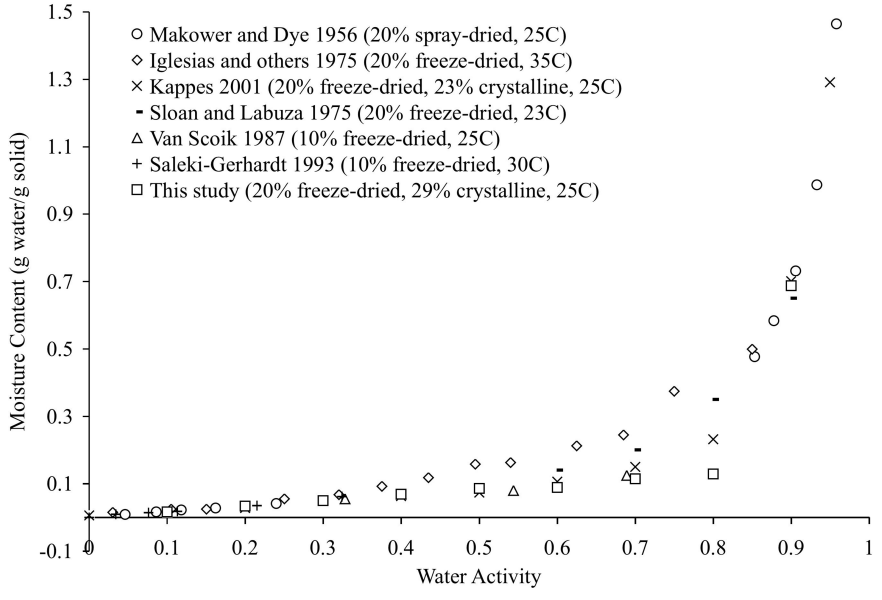

Figure 5—Pseudo-sorption isotherms of amorphous sucrose from this study and literature sources, with specific drying methods used and isotherm temperatures given in the legend.

solution concentration at the experimental temperature) the moisture contents once again are consistent between studies. The large variation in the 50% and 80% RH region of Figure 5 is thus caused by the combination of experimental differences, as well as the innate instability and variability of amorphous materials and the dynamic and kinetic (for example, ramping the %RH from 13% to say 50% while collecting weight data over time) nature of sorption measurements.

Crystallization onset times

The time required for the onset of moisture-induced crystallization to occur for freeze-dried amorphous sucrose was obtained for %RH values at 40% to 80% for the 29% crystalline content samples reported here compared to the 23% (Kappes (2001) and 80% (Yu 2007) crystalline content samples are shown in Figure 6. As can be observed, in general, as %RH increased, the onset time for crystallization decreased; and as the percent crystalline content of the sample increased, the onset time decreased. This is because when %RH increases, more water is available to facilitate sucrose mobility (that is, as water content increases *T*g decreases and sucrose mobility increases) and thus speed crystallization; and when percent crystalline content increases, more nucleation sites (already present crystals) are available to speed the crystallization process.

The time required for the onset of moisture-induced crystallization is also compared in Figure 7 among the techniques, DVS (this study), the sulfuric acid method (Makower and Dye 1956), and the saturated salt solution method (Iglesias and others 1975; Van Scoik 1987). Where comparable, at equivalent %RH values, amorphous sucrose crystallization onset times were shorter when obtained using the DVS instrument compared to the other static techniques. For example, at 70% RH, it took a total of 660 min for the onset of crystallization to occur for the saturated salt solution method (Van

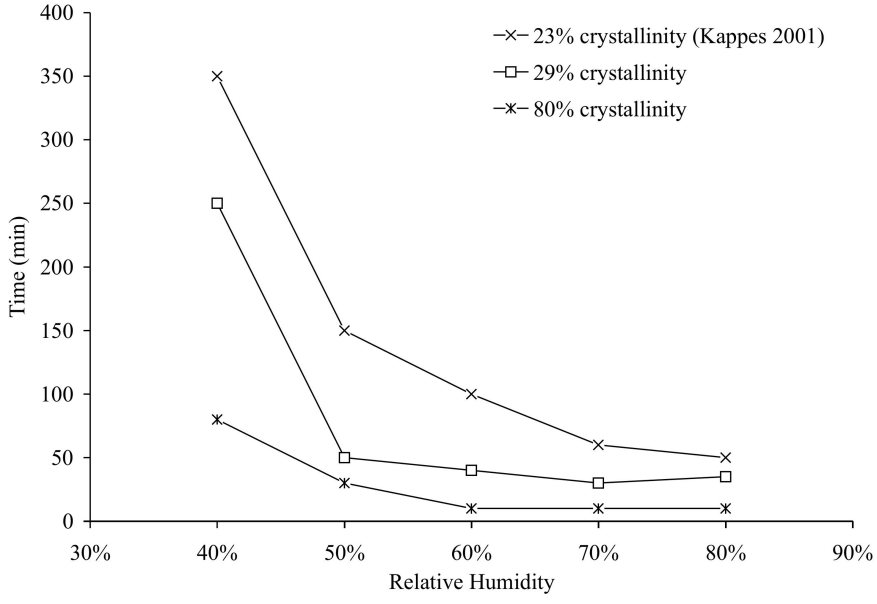

Figure 6 — Onset times for moisture-induced crystallization of freeze-dried amorphous sucrose with varying percent crystalline contents obtained using the DVS instrument at 25 °C. Note that the line in the figure is to guide the eye and is not a fit to the data.

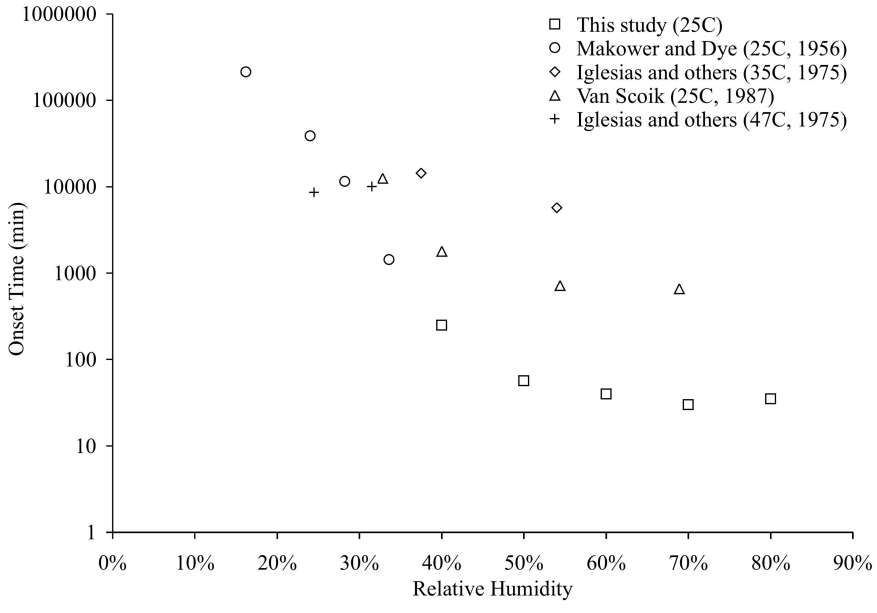

Figure 7 — The onset time required for moisture-induced crystallization to occur at different relative humidity values from this study and from the literature. Note that the *y*-axis is plotted on a log scale.

Scoik 1987) compared to 30 min for the DVS instrument. These differences in crystallization onset time may be explained by both the dynamic nature of the DVS instrument compared with the static nature of the traditional salt or sulfuric acid solution method and the small sample sizes (approximately 10 mg used here) used in the DVS instrument. Yu (2007) reported a marked decrease in sorption isotherm equilibration time from weeks or months using the traditional saturated salt solution method to hours or days using the DVS instrument. In the DVS instrument, the desired %RH air continuously flows past the sample, enhancing sample equilibration; whereas in the traditional salt or sulfuric acid solution method there is usually minimal air circulation in the chamber (for example, desiccator). A motorized fan can be placed in the desiccator or a magnetic stir bar and plate used to help circulate the air in the chamber, but these techniques were not mentioned in the research methods for the literature values plotted in Figure 7. However, Makower and Dye (1956) did mention using air-free (vacuum) desiccators, because they reduce the time of approach to equilibrium (Makower and Dehority 1943). From these data, it appears that dynamic compared with static air flow (a sorption technique difference) as well as small sample size are additional factors that can impact the onset time required for moisture-induced crystallization to occur.

It is interesting to note that moisture-induced crystallization occurred faster in this study at 25 °C compared to Iglesias and others (1975) done at 35 °C. Since there was no direct overlap in %RH values that resulted in moisture-induced crystallization between this study and Makower and Dye (1956), no direct onset time comparison can be made.

It is important to mention that a number of researchers have referred to the Makower and Dye (1956) study and the length of time required for crystallization to occur, especially at 16.2% RH (usually reported as 200 d). The approach used to determine the crystallization onset times in this study (detailed in the Materials and Methods section) was also applied to data from the literature for comparison purposes. Applying this approach to the Makower and Dye (1956) data resulted in the following crystallization onset times (plotted in Figure 7): 149 d (16.2% RH), 27 d (24.0% RH), 8 d (28.2% RH), and 1 d (33.6% RH). These values are somewhat shorter than those reported by Lechuga-Ballesteros and Miller (2006; approach not specified), 200 d (16.2% RH), 42 d (24.0% RH), 12 d (28.2% RH), and < 2 d (33.6% RH). The induction period specifically noted in the text of the Makower and Dye (1956) paper for the 16.2% RH sample was "approximately 150 days" (probably rounded compared to the value we selected of 149 d given in Table I) and "several days" at 28.2% RH. Later on, however, during the discussion of the crystallization calculations Makower and Dye (1956) mentioned assuming a value of 3 d for the onset of crystallization at 28.2% RH.

Can the occurrence of moisture-induced crystallization be predicted?

A general hypothesis exists that moisture-induced crystallization from the amorphous state is prevented if the storage (Ts) or experimental temperature is lower than the glass transition temperature (Tg) of the material, referred to here as the *T*g boundary hypothesis. Based on this premise, prediction of the occurrence of moisture-induced crystallization of amorphous sucrose can be done by plotting the moisture contents from the pseudo-moisture sorption isotherm (Figure 3) at the temperature of the experiment (25 °C) on the state diagram for amorphous sucrose. Such a plot is given in Figure 8 and was constructed using the amorphous sucrose state diagram data from Sun and others (1996; Figure 1 sucrose data only) and the moisture content data plotted at 25 °C from this study. The solid line shows the best-fit 3rd-order polynomial equation to all of the Sun and others (1996) amorphous sucrose *T*g data and the dotted lines are the 95% confidence limits to the best-fit equation. Even though the *T*g data from Sun and others (1996) extend to higher moisture contents, only the moisture content range of interest here was plotted in Figure 8, but all of the sucrose data were used for the polynomial fit. A solid (or filled-in) symbol indicates that the sample crystallized and an open circle indicates that the sample did not crystallize over the duration of the experiment.

Theoretically, only amorphous sucrose samples with moisture contents at or above the *T*g curve (at the experimental temperature) should undergo moisture induced crystallization (Labuza and Labuza 2004; Roe and Labuza 2005). The 95% confidence limits

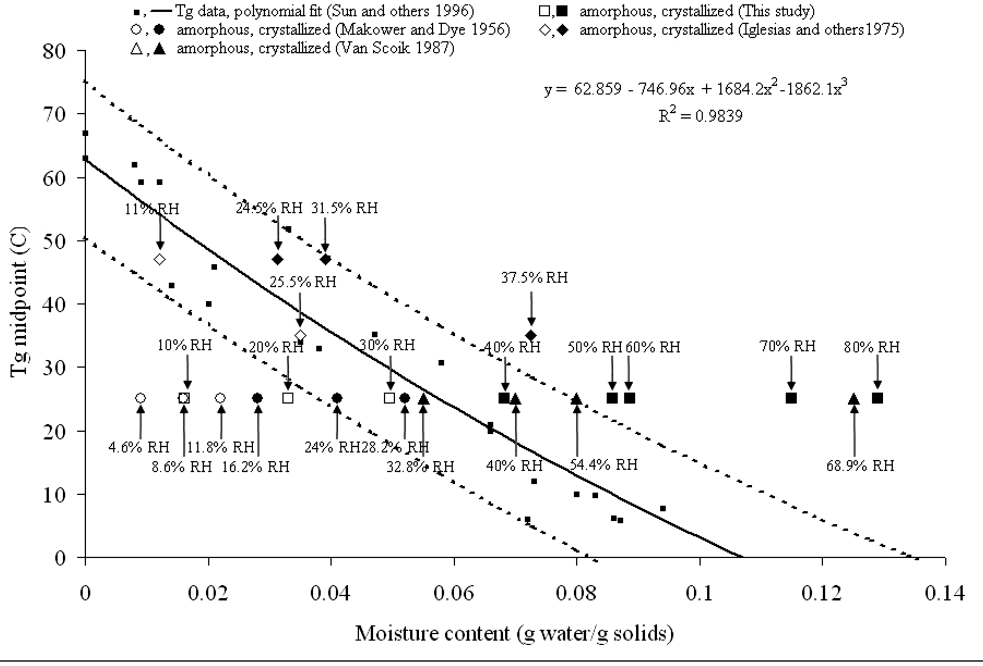

Figure 8—The amorphous sucrose state diagram was constructed using the sucrose data in Sun and others (1996; Figure 1). The dotted lines are the 95% confidence limits to the best-fit 3rd-order polynomial equation (solid line). Also plotted are the amorphous sucrose moisture content data from this study and the literature at the respective experimental temperatures, where a solid (or filled-in) symbol indicates that the sample crystallized and an open circle indicates that the sample did not crystallize, and thus remained amorphous, over the duration of the experiment.

were added to acknowledge the large standard deviation associated with *T*g values in the literature (Roe and Labuza 2005), expanding the lower moisture content limit (and thus %RH) for crystallization. In this study, moisture induce crystallization was observed for 40% RH, but did not occur at 30% RH during the 2000-min duration of the DVS experimental run at 25 °C, perhaps a longer duration experiment would result in crystallization at 30%, since this %RH value falls within the lower 95% confidence limit of *T*g as shown in Figure 8. Additional moisture-induced crystallization experiments with amorphous sucrose at low %RH values (> 40%) for longer duration times are currently under way. The moisture-induced crystallization results for this study (crystallization occurred in samples at %RH values ≥ 40%) correspond to that prediction by the *T*g boundary hypothesis illustrated in Figure 8. In regard to the rate of crystallization, in general, the lower *T*g is below Ts the faster the rate of crystallization. This general relationship also held for this study, where the rate of crystallization increased as %RH increased (increasing %RH corresponds to decreasing Tg, while Ts was held constant at 25 °C) for the amorphous sucrose samples held at 40% to 80% RH (Figure 7).

Also plotted in Figure 8 are the amorphous sucrose moisture content data from the literature at their respective experimental temperatures, where, again, a solid (or filled-in) symbol indicates that the sample crystallized and an open circle indicates that the sample did not crystallize, and was thus still amorphous, over the duration of the experiment. In general, amorphous sucrose samples from the literature followed the *T*g boundary hypothesis, exhibiting crystallization in samples near (below the *T*g curve, but within the lower 95% confidence limit), at, and above the amorphous sucrose *T*g curve (Figure 8). The only exception to the *T*g boundary hypothesis plotted in Figure 8 is the Makower and Dye (1956) data for 16.2% RH. In Makower and Dye (1956), the moisture sorption properties of 20% spray-dried amorphous sucrose were investigated using sulfuric acid solutions at 4.6%, 8.6%, 11.8%, 16.2%, 24.0%, 28.2%, and 33.6% RH at 25 °C. They reported that crystallization occurred at %RH values as low as 16.2% RH, albeit after an induction period of about 150 d. As can be seen in Figure 8, the moisture content associated with 16.2% RH is below the 95% confidence limit for the best fit 3rd-order polynomial equation to the amorphous *T*g curve, which is contrary to the *T*g boundary hypothesis.

Possible explanations for this discrepancy between the reported crystallization of amorphous sucrose at 16.2% RH (Makower and Dye 1956) and that predicted by the *T*g boundary hypothesis can be partitioned into 2 main categories. First, the *T*g boundary hypothesis is correct, but the *T*g data used to construct the state diagram were not representative enough or errors in the Makower and Dye (1956) experiment were responsible for the observed result. An example of an explanation related to the *T*g data used to construct the state diagram is that the Makower and Dye (1956) samples, produced via spray-drying, would actual fall within the confidence limits if a broader subset of literature obtained amorphous sucrose *T*g data were used or if only spray-dried amorphous sucrose *T*g data were used to construct the *T*g curve and confidence limits in Figure 8. An example of an explanation related to possible errors in the *T*g data is that in the DVS instrument used here, a sample is weighed continually without being removed from the humidity and temperature controlled chamber, while in the technique available to Makower and Dye, the samples had to be removed from the humidity and temperature controlled chamber to be weighed, therefore samples were exposed to ambient humidity and temperature conditions each time the samples were weighed. Over the long experiment time (846 d, as noted in table I in Makower and Dye 1956), these short exposures to ambient conditions may have facilitated crystallization in the samples stored at low %RH values. In a previous paper (Makower and Dehority 1943), interrupting the experiment to weigh the samples was noted as a disadvantage of the method. Prevention of this disadvantage was also mentioned in Makower and Dehority (1943), via the use of a McBain-Bakr (1926) quartz spiral balance. However, no mention of using this balance to collect their data was made in Makower and Dye (1956). Others have previously noted the unexpected crystallization of amorphous sucrose observed at 16.2% in the Makower and Dye (1956) study and mentioned that it could be due to an anomaly, an error in measurement, or a small inhomogeneous region in which random crystallization was induced (Labuza and Labuza 2004).

Second, the *T*g boundary hypothesis is incorrect and another hypothesis governs the stability of amorphous materials. Other researchers have also reported observing nucleation and crystallization in samples stored at %RH values that place them below the *T*g curve of the material at the experimental temperature and have proposed other hypotheses regarding the stability of amorphous materials (Abe and others 1976; Chryssikos and others 1991; Yoshioka and others 1994; Okamoto and Oguni 1996; Andronis and others 1997). For example, Andronis and others (1997) reported that crystallization of indomethacin from the amorphous state occurred over practical time scales (a few weeks) below the corresponding *T*g (50 °C) when stored at low %RH values (at 30 °C), most likely due to surface-initiated crystallization being able to occur under such conditions. These researchers have hypothesized (Handcock and others 1995; Handcock and Zografi 1997; Zografi and Byrn 1999) that to prevent amorphous materials from undergoing moisture-induced crystallization over very long time frames (that is, the 3 to 5 y of shelf life required for a pharmaceutical product), they need to be stored at a temperature approximately 50 °C below their *T*g value. This approximate stable storage temperature (approximately 50 °C below *T*g), referred to as the zero mobility temperature, T_0, can be calculated from the Vogel–Tammann–Fulcher (VTF) equation and is the temperature at which, essentially, all important molecular motions would cease (Handcock and Zografi 1997). The value of T_0 in the VTF equation is believed to correspond to the theoretical Kauzmann temperature (T_K), where T_K is thought to mark the lower limit of the experimental glass transition temperature and to be the point at which the configurational entropy of the system reaches zero. It should be noted that, unlike sucrose, which is a very hydrophilic molecule in both the crystalline and amorphous states, crystalline indomethacin is very hydrophobic with no known deliquescence point and amorphous indomethacin shows small, but measurable, water sorption with a subsequent decrease in *T*g (Andronis and others 1997). The hydrophobic nature of the indomethacin molecule may be an important factor in explaining indomethacin's moisture-induced crystallization behavior below *T*g when in the amorphous state. Additional research attempting to correlation the calorimetric *T*g with molecular mobility (related to the zero mobility temperature hypothesis), via relaxation times measured by both isothermal calorimetry and dielectric spectroscopy, for both spray-dried and freeze-dried amorphous sucrose was recently report by Bhugra and others (2007). They reported that molecular mobility in amorphous materials at temperatures both above and below *T*g was correlated to macroscopic physical changes such as crystallization, but predication of crystallization onset from relaxation time was only qualitatively correct at temperatures well below *T*g.

Another hypothesis to predict amorphous material stability is the concept of the hydration limit proposed by Lechuga-Ballesteros and Miller (2006). These researchers have found, using RH-perfusion microcalorimetry (Lechuga-Ballesteros and others

2003), that at and above a certain relative humidity value, water molecules in the vapor state interact with the hydrated amorphous samples as they would liquid water. The water content at this identified relative humidify is referred to as the hydration limit, Wm. For amorphous sucrose, they determined the hydration limit to be beyond 15% RH,[1] consistent with the %RH demarcation observed in the Makower and Dye (1956) study. The researchers suggest that there may be a possible relationship between hydration limit and the zero mobility temperature.

Both the zero mobility temperature and hydration limit hypotheses suggest that the freeze-dried amorphous sucrose samples at the low %RH values (at least at 20% and 30% RH) in this study would eventually undergo moisture-induced crystallization given a longer experimental duration time than 2000 min. As previously mentioned, additional experiments at longer duration times for the low percent relative humidity values are currently under way to further explore these stability prediction hypotheses.

From the aforementioned discussion, it is clear that additional, long duration time experiments using the new dynamic humidity generating instruments are needed, in concert with experiments that advance our understanding of the mechanism(s) governing the long-term stability of amorphous materials.

Conclusions

Sorption properties of freeze-dried amorphous sucrose samples were studied using the DVS instrument. Compared to the traditional relative humidity controlling techniques, such as saturated salt or sulfuric acid solutions in desiccators, the DVS instrument provides a fast and accurate means of investigating the moisture sorption behavior of amorphous hydroscopic materials such as freeze-dried amorphous sucrose, with the DVS allowing for continuous weight change monitoring while the sample remains under constant humidity and temperature control. It was found that the minimum relative humidity for crystallization to occur, with a maximum possible experimental duration time of 2000 min, was 40% with an onset time of 250 min. Crystalline content of the amorphous sucrose was found to have a significant impact on the pseudo-sorption isotherm. As crystalline content increases, the pseudo-equilibrium moisture content at the same relative humidity decreases. The mechanism governing moisture-induced crystallization, and therefore the ability to predict the conditions (that is, %RH and temperature) to ensure long-term stability of amorphous solids, remains as stated by Lechuga-Ballesteros and Miller (2006) "an unresolved challenge."

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[1]In Lechuga-Ballesteros and others (2003), the term threshold RH (RH_m) was used. Based on the moisture-induced thermal activity trace method, they reported a value of 13% RH_m for spray-dried amorphous sucrose at 25 °C.

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