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Non-Isothermal and Isothermal Crystallization of Sucrose from the Amorphous State

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The crystallization of a model compound, sucrose, from the amorphous solid state has been studied non-isothermally using differential scanning calorimetry to determine crystallization temperature, Tc, and isothermally at 30°C by subjecting samples to 32.4% relative humidity and gravimetrically monitoring water vapor uptake and subsequent loss with time due to crystallization. From the measurement of glass transition temperature, Tg, and melting temperature, Tm, for sucrose alone and in the presence of absorbed water it was possible to predict Tc and thus to directly relate the plasticizing effects of water to its tendency to promote crystallization. Colyophilization of sucrose with lactose, trehalose, and raffinose, all having Tg values greater than that of sucrose, increased Tc significantly, even at levels as low as 1–10% w/w. In the isothermal studies the time required for crystallization to commence, due to the plasticizing effects of water, i.e., the induction time, assumed to be mostly affected by rates of nucleation, was greatly increased by the presence of the additives at these low levels, with raffinose producing a greater effect than lactose and trehalose. Similarly, these additives reduced the rate of water loss, i.e., the rate of crystal growth, but now no significant differences were noted between the three additives. The possible relationships of nucleation and crystal growth and the effects of additives on molecular mobility are discussed.

KEY WORDS: crystallization; amorphous; glass transition temperature; sucrose, water absorption.

INTRODUCTION

An understanding of the mechanisms and kinetics of crystallization from solution has been shown to be critical in the development of drugs and drug products (1). It is also well recognized that crystallization can occur from the amorphous solid state with serious implications for the long-term stability of many types of drugs (2,3). However, relative to the large amount of work reported for crystallization from solution, very little fundamental research has been reported with amorphous pharmaceutical systems (4). The need for greater attention to such phenomena is made apparent when one recognizes that most crystalline pharmaceutical solids undergoing processing are likely to take on a certain degree of amorphous character, not often detectable by standard techniques (5). Such processing can include: lyophilization; spray drying; wet granulation; aqueous film coating; milling; and compaction. Problems arising because of unanticipated crystallization from such amorphous states include: collapse of lyophilized cake structure (2,6) and post-compaction hardening of tablets with a possible reduction in dissolution rate (7,8). A fundamental understanding of those factors would be helpful in either preventing crystallization in such systems during the shelf-life of the product, or by properly conditioning the solid initially to eliminate amorphous regions that might later recrystallize in an uncontrolled manner or give rise to sites of chemical degradation (9).

In this study, using amorphous sucrose as a model system, we have investigated some of the possible mechanistic factors involved in crystallization from the amorphous state under isothermal and non-isothermal conditions. Sucrose was chosen because some studies of its crystallization from solution and the amorphous state have been reported earlier by workers in the food science field (10–13), thus providing a basis for more in-depth probing. Sucrose can be obtained in a highly pure crystalline state and also can be easily formed into a completely amorphous state by lyophilization. Furthermore, it can be retained in the amorphous state under dry conditions and room temperature over the time course generally required for various experimental procedures, yet it can be induced to crystallize over short and convenient time periods when the temperature is raised above its glass transition temperature, Tg, or when the sample is subjected to moderate relative humidities and to absorption of water vapor that reduces Tg (11,13). The reason for this effect is that sufficient molecular mobility exists above Tg to promote tendencies for crystallization (13,14). Of particular interest in the current study was the estimation of the crystallization temperature, Tc, of amorphous sucrose obtained from non-isothermal DSC measurements and an assessment of possible quantitative relationships between Tg and Tc. It was also of interest to assess those factors that influence the induction time, τ, preceding the crystallization that normally occurs under isothermal conditions and which are presumed to be primarily affecting the rate of nucleation (15). Factors considered were: the presence of crystalline sucrose and its ability to induce crystallization in the system; the presence of absorbed water and its ability to act as a plasticizer and, hence, to lower Tg; and the colyophilization of sucrose with other sugars, i.e. lactose, trehalose and raffinose, all having Tg values greater than that of sucrose and, hence, the potential to inhibit crystallization through antiplasticization effects. The emphasis of this work was to see if any link exists between the tendencies for crystallization from the amorphous state under various conditions and the molecular motion produced at a particular temperature T by the morphological structure of the amorphous solid, reflected in the relationship of Tc and τ to Tg.

MATERIALS AND METHODS

Materials

Crystalline sucrose and raffinose pentahydrate were obtained from J. T. Baker Chemical Co. at 99+% reagent grade quality. Trehalose dihydrate (Sigma Chemical Co.) had a reported purity of 99.9%, while α-lactose monohydrate, Tablettose®, (Meggle, Co., Germany), had a purity of 99.8%.

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Preparation of Amorphous Samples

Amorphous samples of sucrose, lactose, trehalose and raffinose, as well as mixtures of sucrose with each of the three other sugars in varying amounts, were prepared by lyophilization in a Dura-Stop DC, FTS Systems (Stone Ridge, NY) tray dryer in combination with a Dura-Dry MP condenser module. For the four sugars individually, lyophilization was initiated from a 10% w/v aqueous solution. For pure sucrose and pure lactose, the solution temperature was reduced to −45°C in the dryer and held there for 72 hours, during which time the system was evacuated to a pressure of 50 mTorr or less. The shelf temperature was then raised successively to: −35°C for 24 hours; −30°C for 24 hours; −20°C for 24 hours; −10°C for 12 hours; and 0°C for 12 hours. Secondary drying was accomplished at 25°C for 24 hours and further drying of the lyophilized cake was carried out at 60°C for at least 48 hours. For trehalose a 10% solution was held at −45°C for two hours, followed by evacuation at this temperature over an eight hour period. The temperature was then increased to: −32°C for 24 hours; −20°C for 8 hours; −10°C for 2 hours; −5°C for 4 hours; 25°C for 24 hours and 60°C for 48 hours. Amorphous raffinose was obtained by holding a 10% aqueous solution at −45°C for two hours and then evacuating at this temperature for 8 more hours. The shelf temperature was increased to: −30°C for 24 hours; −20°C for 6 hours; 0°C for 24 hours; 25°C for 24 hours and 60°C for 48 hours. Colyophilized samples of sucrose with other sugars were prepared following the protocol developed for pure sucrose, as described above. To assure proper estimation of the mole fraction of each component present, the solids were first dried under vacuum at an appropriate temperature to remove any residual water and waters of hydration: raffinose at 100°C for 24 hours; trehalose at 130°C for 24 hours; lactose at 90°C for 24 hours and sucrose at 95°C for 2 hours. Any residual water present, as measured by Karl Fisher titration (Aquastar® C2000, EM Science, Cherry Hill, NJ), was reduced to below 0.1% w/w in all cases.

The samples were shown to be completely amorphous from x-ray powder diffraction measurements using a Pad V Scintag scanning x-ray powder diffractometer (Scintag, Inc., Santa Clara, CA) controlled by a model B010610 Tetronix computer (Tetronix Inc., Wilsonville, OR) as described in detail elsewhere (5).

Specific Surface Area Measurement

Specific surface area measurements were carried out with a Quantasorb surface area analyzer (Model IM0325, Quantachrome Co., Syosset, NY) using Krypton gas adsorption and applying the BET equation, as described elsewhere (5).

Estimation of Tg and Tc

The glass transition temperatures, Tg, and crystallization temperatures, Tc, for all individual amorphous sugars and their colyophilized mixtures were measured using a TCII/DSC 30 differential scanning calorimeter (Mettler, Highstown, NJ) attached to an IBM PS/2 computer, model 55SX. All samples were measured using hermetically sealed aluminum pans at a heating rate of 10°C/minute. Approximately 5–10 mg of each sample were weighed and transferred to pans under a controlled relative humidity environment using a glove box. Additional experiments of this type were carried out at a heating rate of 10°C/minute for various mixtures of crystalline and amorphous sucrose in the dry state to observe possible seeding effects on Tc, and for amorphous sucrose samples at water contents below that required to cause crystallization within the time frame of any experimental procedures. Water was introduced into the solid by storing samples at 30°C in desiccators containing saturated salt solutions at various relative humidities. Determination of the water vapor absorption isotherm for amorphous sucrose at 30°C at various relative humidities was carried out gravimetrically in desiccators, as well as in a vacuum assembly containing a Cahn electrobalance, described earlier (5). Water vapor absorption by amorphous lactose, trehalose, and raffinose at 30°C and 32.4% relative humidity also was determined.

Isothermal Studies of Induction Time and Crystal Growth Kinetics

Previous studies with sucrose (11,12) have established that exposure of amorphous sucrose to a relative humidity of 32.4% after equilibrium absorption of the expected amount of water, i.e. about 6%, will cause a spontaneous loss of this water due to crystal growth only after a lag or induction period where the water content remains constant (11,12). Although one cannot discount secondary nucleation events, this induction period is believed to reflect the overall rate at which primary nucleation can occur under the particular set of conditions used, while the rate of water loss is believed to primarily reflect the rate of crystal growth (11,12). Samples of purely amorphous sucrose, amorphous sucrose physically mixed with different proportions of crystalline sucrose, a milled sample of sucrose rendered partially amorphous (5) and various colyophilized mixtures were all placed into the vacuum assembly mentioned above and exposed to 32.4% relative humidity at 30°C. The weight of water taken up and then later lost because of crystallization was followed in order to ascertain the induction time and the apparent rate of crystal growth (11,12).

RESULTS

Non-isothermal Studies

Table I lists Tg, Tc and Tm for the various sugars obtained from DSC scans at 10°C/min, as described in detail in an earlier paper (5). Note that under the conditions of these experiments trehalose and raffinose, which exist as a dihydrate and pentahydrate, respectively, in the crystalline form, did not exhibit any crystallization up to a temperature of 200°C. Lactose exhibited a recrystallization of its anhydrous crystal form at 185°C, in agreement with previously reported results (16).

If, indeed, an important effect on Tc is manifested through the nucleation step, as will be discussed in more detail later, it might be expected that sucrose crystals mixed with the amorphous form, while not affecting Tg, would act as seeds for nucleation and, therefore, reduce Tc. Two types

Table I. Glass Transition Temperature, Crystallization Temperature and Melting Temperature for Amorphous Sucrose, Trehalose, Lactose and Raffinose

Sugar	Tg(°C)	Tc(°C)	Tm(°C)
Sucrose	74	130	188[b]
Trehalose	115	[a]	[a]
Lactose	108	185	213[b]
Raffinose	102	[a]	[a]

[a] Did not recrystallize.

[b] Melting with decomposition.

Table II. Glass Transition Temperature, Crystallization Temperature of Amorphous Sucrose and Melting Temperature in the Presence of Absorbed Water

% w/w Water	Tg(°C)	Tc(°C)	Tm(°C)
0	74	130	188
0.99	60	125	180
1.47	58	115	170
1.98	50	100	160
3.13	32	92	158

of mixtures were prepared and evaluated; one containing physically mixed amorphous (40%) and crystalline (60%) sucrose and the other a crystalline sample rendered partially amorphous (30%) by milling (see reference 5 for details on milling studies). Indeed, it was observed that such seeding apparently does occur with a reduction of Tc from 130°C to 115°C and 74°C, for the physically mixed and milled samples, respectively.

In Figure 1 is given the water absorption isotherm for amorphous sucrose at 30°C at relative humidities where crystallization does not take place before equilibration occurs. These results agree very well with previously reported values (11). Table II lists the Tg, Tc and Tm of samples of amorphous sucrose containing different amounts of equilibrated absorbed water. Given the low Tg of water itself (135K) and its ability to plasticize amorphous solids (9,13), it is not surprising that it has a very significant effect on Tg and, hence on Tc. (To be discussed more fully later.)

In Table III we present data to show how the colyophilization of sucrose with the three other sugars affects the Tg of sucrose. The fact that only one value of Tg is observed, rather than 2 values, indicates that the sugars are miscible with sucrose in all proportions in the amorphous state (17). This is in contrast to studies with macroscopically mixed samples of amorphous sucrose with the other sugars in the amorphous form where two distinct values of Tg, equal to those for each individual component, were observed at all proportions and no affect on Tc occurred (data not shown). As will be discussed in more detail later, the lack of significant reduction in Tg at the lower levels of each additive would be expected from theoretical considerations involving mixing of amorphous substances, and relative contributions to Tg from each component.

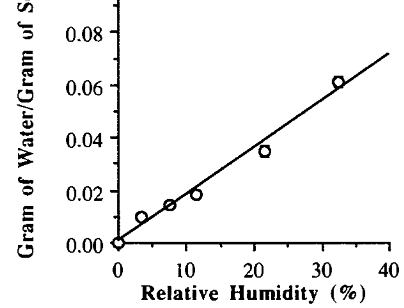

Figure 1. Water vapor absorption as a function of relative humidity at equilibrium for amorphous sucrose at 30°C.

In Table IV are given values of Tc of sucrose for various mixtures with the three sucrose-sugar combinations up to a concentration of about 10% w/w. As can be seen in all three cases, whereas no effects on Tc were observed up to 1% w/w of additive, significant increases occurred at 5% w/w. The small decrease in Tc at a 1% additive level for trehalose and raffinose relative to no additive most likely reflects experimental error in measuring crystallization exotherms. Above 10% w/w no crystallization exotherms could be detected. It should also be pointed out that the melting temperature of sucrose in the presence of the other sugars up to 10% was reduced from 188°C to no less than 185°C, as might be expected for a relatively dilute solid-solution. No evidence for any crystals other than sucrose could be obtained from x-ray powder diffraction measurements.

Isothermal Studies

As indicated in previous studies (11,12), subjecting sucrose to 32.4% relative humidity at 30°C results in water uptake to a constant value followed by a sudden and spontaneous loss of water. The time period over which the sample takes up water and remains constant is generally taken as the overall induction time, τ, required for nucleation to occur, while the initiation of a weight loss is assumed to be governed primarily by crystal growth (11,12). Since a certain period of time exists before the water content can reach its equilibrium values some uncertainty exists as to the actual steady-state induction period for nucleation. In all studies at 30°C and 32.4% relative humidity, except with the milled samples, equilibrium was attained at about 2 hours. One therefore could simply subtract 2 hours from the total time elapsed between initial exposure to 32.4% relative humidity

Table III. Glass Transition Temperature for Various Proportions of Colyophilized Mixtures of Sucrose and Additives

	Glass transition temperature (°C)		
% w/w Additive	Lactose	Trehalose	Raffinose
0	74	74	74
1.0	75	73	73
10.0	78	74	73
50.0	82	87	82
80.0	—	102	96
100.0	108	115	102

Table IV. Crystallization Temperatures for Sucrose with Various Proportions of Additives

% w/w Additive	Crystallization temperature (C°)		
	Lactose	Trehalose	Raffinose
0.0	130	130	130
1.0	131	128	128
5.0	137	145	148
10.0	156	161	160

and initial weight loss to obtain the induction period. However, this assumes that no nucleation begins to occur before such equilibrium is reached, a very unlikely situation. Consequently, all values of τ reported in this study should be taken to represent a steady-state time period dominated by the nucleation process at 6% water, as well as a short non-steady state period when water vapor is still being taken up. Given the similarity in structure of sucrose and all of the additives, this appears to be a reasonable manner in which to compare the various systems.

In Table V we compare the induction times for purely amorphous sucrose and three additional samples: 90% amorphous and 10% crystalline physically mixed; 12% amorphous and 88% crystalline physically mixed; and a milled sample with about 10% amorphous and 90% crystalline composition. Here, we can see how the presence of a crystalline phase significantly reduces the induction period, presumably by acting to seed the sample and to increase the rate of nucleation. One would expect that a critical factor in the "seeding" process would be the specific surface area of the various samples, the greater available surface area the greater the tendencies for nucleation to be induced. This seems to be consistent with the observation that the milled and physically-mixed samples, with specific surface areas of 2.12 m^2/g and 0.425 m^2/g, but at about the same fraction of crystalline/amorphous structure, have significantly different rates of apparent nucleation, i.e. milled much greater than physically mixed. The extent to which such factors affect the rate of water loss in pure and physically mixed samples might be less important as seen in Figure 2 where the weight loss versus time is shown for the purely amorphous sample and one containing only 12% amorphous material mixed with crystalline sucrose. Here, the total specific surface areas measured for purely amorphous sucrose and the 12% amorphous mixture are 0.713 m^2/gram and 0.425 m^2/gram, respectively. Despite the differences in the specific surface areas and the concentration of seed crystals present, the overall kinetics of weight loss for both systems are quite similar, indicating no measurable effect of seed crystals on growth kinetics. Furthermore, it may be suggested that most likely no significant secondary nucleation occurs during water loss, since both samples with different specific surface areas (thus different potential for secondary nucleation) showed a very similar rate of water loss. However, when the milled sample, initially containing 10% amorphous form, was studied in this way crystallization after the induction period was complete in the order of a few minutes (data not shown). Therefore, for the milled sample it is possible that some secondary nucleation may be playing a rate-determining role in promoting crystallization.

Table V. Induction Time, τ, for Crystallization from Amorphous Sucrose at 30°C and 32.4% Relative Humidity

Sample	Induction time (hours)
Lyophilized	17.5
10% crystals[a]	7.5
88% crystals[a]	3.0
Milled[b]	0.16

[a] Macroscopic mixture of crystalline and amorphous sucrose particles.

[b] Sample milled to give 90% crystalline and 10% amorphous structure (5).

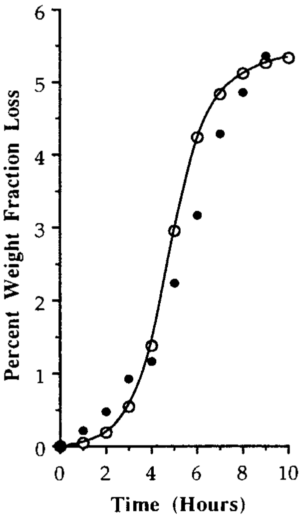

Figure 2. Percent weight fraction loss of absorbed water in amorphous sucrose at 30°C and 32.4% relative humidity after the induction time, τ, for: (○) purely amorphous sucrose; (●) mixture of 12% amorphous and 88% crystalline sucrose.

Similar studies of induction time and water loss kinetics at 30°C and 32.4% relative humidity are summarized in Figures 3 and 4, for colyophilized samples of sucrose and the various sugars. Beyond 10% w/w of additive all samples gave no indication of crystallization, for 14 days. In Figure 3 we see that the two disaccharides, lactose and trehalose, produced almost identical increases in the induction time, relative to 100% sucrose, while raffinose, a trisaccharide produced a significantly greater increase at any comparable percentage. Comparison of the specific surface areas for three colyophilized mixtures did not show a significant difference relative to purely amorphous sucrose at the concentrations of additives studied, suggesting a similar potential for any secondary nucleation for the three systems. Interestingly, (Figure 4) all three sugars at a 1% w/w level significantly inhibit the rate of water loss of amorphous sucrose, but in contrast to effects on induction time, raffinose, trehalose, and lactose all appear to produce about the same rate of decrease. Similar lack of difference between the three additives was observed at the 5 and 10% level as well, with much lower rates of water loss as the concentration of additive was increased. Independent measurements of equilibrium absorption of water before any crystallization occurred at 30°C

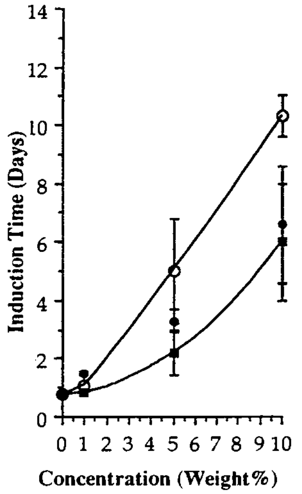

Figure 3. Induction time for crystallization of sucrose vs. weight percent of colyophilized additives at 30°C and 32.4% relative humidity: (○) raffinose; (■) lactose; (●) trehalose.

and 32.4% relative humidity for amorphous lactose, trehalose, and raffinose yielded values of 7.0%, 7.1% and 6.7%, respectively, as compared to 6.2% for amorphous sucrose alone (Figure 1). For all mixtures with sucrose, reported in Figures 3 and 4, the water content during the induction period and at the point that crystal growth was initiated ranged between 6.2% and 6.5%, and therefore, it can be considered to have been essentially the same for all isothermal studies carried out under these conditions. Attempts to measure the Tg of such samples equilibrated in a desiccator to 6% water, however, proved difficult because the time frame of such experiments led to complete crystallization. It was possible, however, to equilibrate the mixtures to water contents of about 2% and measure the Tg of such samples successfully. Consistently for all mixtures up to 10% additive, one Tg was observed, and whereas the Tg of "wet" pure sucrose under such conditions was 49.5° ± 0.1°C, the values for the various mixtures containing 2% water were all in the range of 56° ± 2°C. Consequently, when compared to values of Tg for "dry" samples up to 10% additive, as shown in Table III, a constant plasticizing effect of water for pure and mixed systems may be observed. It is concluded, therefore, that this constancy of effect of water on Tg most likely also exists at the 6% water level.

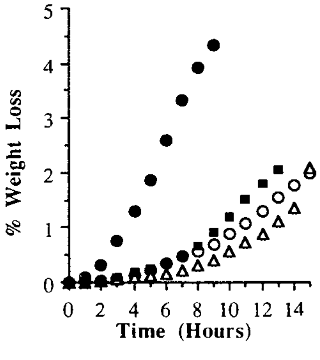

Figure 4. Percent weight loss of absorbed water in amorphous sucrose at 30°C and 32.4% relative humidity colyophilized with various additives as a function of time after the induction period: (●) sucrose alone; (■) trehalose; (○) raffinose; (△) lactose.

DISCUSSION

Factors Governing Crystallization of Sucrose from the Amorphous State

In order to understand the critical factors underlying crystallization from the amorphous state it is important to recognize that the two major steps in any crystallization process are nucleation and crystal growth. From earlier studies with amorphous polymer crystallization (14,18,19) we can assume that crystallization from the amorphous state is primarily controlled by the rate of nucleation, although secondary nucleation can occur. In the present case with sucrose, this is qualitatively shown by the sensitivity of the crystallization temperature, Tc, and the induction time, τ, to the presence of crystalline sucrose.

Starting from homogeneous nucleation theory (15) it can be shown that the rate of nucleation is governed by two major factors: (1) the Gibbs free energy change, ΔG_K, for the formation of a nucleus with a critical size, and (2) the Gibbs free energy associated with the transport of molecules across the nucleus/amorphous matrix interface, ΔG_D. An equation describing the rate of nucleation in condensed systems that takes these two terms into account was developed by Turnbull and Fisher (20):

$$V^* = -\left(\frac{NkT}{h}\right) \exp - \left(\frac{\Delta G_D + \Delta G_K}{kT}\right) \qquad (1)$$

where V^* is the nucleation rate per unit volume, N is the number of molecules per unit volume, k is the Boltzman Constant, h is Plank's constant, and T is temperature. The term ΔG_D is the activational free energy for the diffusion of molecules across the interface that reflects the effect of viscosity on molecular diffusion. The term ΔG_K can be shown to be dependent on the interfacial energy between the nucleus and the homogeneous medium, as well as on the extent of supersaturation, or in the case of condensed matter, supercooling relative to the melting point (18).

From equation 1 it can be seen that nucleation will be affected by two opposing factors. As temperature is reduced below the melting temperature, Tm, and hence supercooling is increased, we would expect the nucleation rate to increase significantly. However, with such a decrease in temperature we also can expect significant increases in viscosity, and hence a marked reduction in diffusion and molecular motion in general. This has been summarized very well by Jolley (19) for the crystallization from amorphous gelatin, as shown schematically in Figure 5. Here we see how the effect of changing the nucleation rate due to supercooling below Tm is balanced by the loss in molecular motion as the temperature is brought closer to Tg, where such motion is assumed to approach very small values, to give a crystallization temperature, Tc, that is intermediate to Tg and Tm. In Figure 5 the rates of change for nucleation and molecular motion are shown in somewhat symmetrical manner, which if true, would lead to a crystallization temperature at the midpoint between Tg and Tm. Since the two exponential terms in

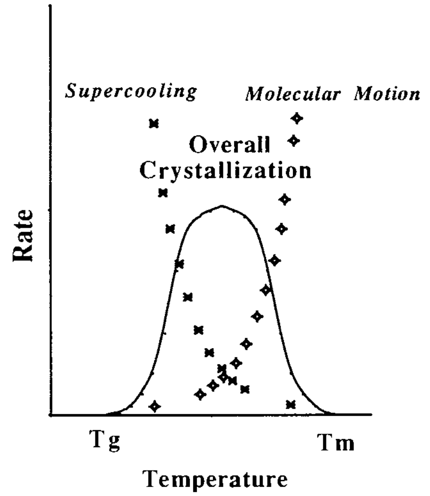

Figure 5. Schematic representation of the rates of various processes associated with crystallization from the amorphous state above Tg and below Tm (see text for details).

equation 1 containing ΔG_K and ΔG_D very likely have somewhat different temperature dependencies, this assumption is not exactly correct. For example, above Tg we would expect viscosity to change with temperature relative to Tg in a non-Arrhenius manner as determined by free volume considerations and reflected in William, Landel Ferry kinetics (21). However, despite this uncertainty, it is interesting to note in Table VI for sucrose alone and in the presence of absorbed water that Tc values determined from the non-isothermal experiments described above are in excellent agreement with values calculated at the midpoint between the Tg and Tm values given in Table II. Minimally this tells us that free volume considerations, as reflected in Tg, presumed to control the molecular motion of the sucrose molecules in the amorphous state are dominant in these systems. Further support for the importance of Tg in affecting nucleation is seen in the isothermal studies at 32.4% relative humidity and 30°C, wherein at this point a very significant reduction in the induction time occurs relative to that observed at just slightly lower relative humidities (11,12). From the data showing Tg as a function of water content (see Table II), it is clear that the amount of water absorbed at 32.4% relative humidity and 30°C, about 6%, is about the amount that reduces the Tg of sucrose to below 30°C, hence placing the sucrose sample at this temperature above its Tg.

Table VI. Comparison of Measured and Predicted[a] Values of Crystallization Temperature for Sucrose at Different Water Content

	Tc(°C)	
% w/w Water	Predicted[a]	Measured
0	131	130
0.99	120	125
1.47	114	115
1.98	105	100
3.13	95	92

[a] The predicted value is the midpoint between the glass transition and melting temperatures.

Effects of Colyophilized Additives

It is well known that relatively small amounts of a second component in solution can have profound inhibitory effects on the crystallization of a substance. Such effects can be due to a change in solubility, a reduction in mass transfer rates, or competitive adsorption at the nucleating or crystal growth sites (1). For crystallization from the amorphous state any additive that causes an increase in Tg would be expected to produce a corresponding increase in viscosity and reduction in molecular motion. In the case of sucrose many studies of crystallization from solution and the amorphous state have shown that other sugars, such as fructose, lactose and raffinose, or more complex systems such as dextran, corn syrup, gelatine and microcrystalline cellulose, at relatively low levels, significantly retard nucleation and crystal growth (10,22,23). How such inhibition might be mechanistically related to effects on Tg vs. more specific inhibitory effects, however, has not been fully addressed.

In the present study we chose to examine the effects of lactose, trehalose and raffinose for a number of reasons. First, preliminary studies showed that colyophilized mixtures of these sugars with sucrose in all proportions produce only one Tg (see Table III), a clear indication that the binary mixtures are miscible in all proportions (17). Secondly, as seen in Table I, the values of Tg for the three sugars are all about the same, i.e. in the range of 102°–115°C, and all are greater than the value for sucrose, 74°C. Consequently, if the effects of these sugars on crystallization of sucrose are due to their antiplasticization properties alone (an increase in Tg) the extent of any inhibition should be very similar for all three. Thirdly, preliminary experiments using x-ray powder diffraction demonstrated that all three sugars, individually and mixed with sucrose in all proportions, did not crystallize under the non-isothermal and isothermal conditions used in these experiments. Finally, it was of particular interest to study raffinose because in earlier studies of sucrose crystallization from solution (10,22) and from the amorphous state (23) raffinose appeared to inhibit crystallization at relatively low concentration levels. In solution this apparently occurs via insertion of raffinose into the crystal growth site of sucrose so as to inhibit particular patterns of crystal growth (10,22).

From the results of the experiments described above with the three sugars we can first check to see if the Tc values reported in Table IV fall out at about midpoint of Tg and Tm, as they did for sucrose alone and in the presence of water (see Table VI). As seen in Table VII, the effects of each sugar at the 10% level on the Tc are much greater than would be predicted by choosing the midpoint between Tg and Tm. Also, note that on a weight basis the values of Tc are all very close, but that 10% w/w raffinose really represents 6.8 mol%, as compared to 10 mol% for lactose and trehalose. Hence, raffinose is more effective than the other sugars on an equimolar basis in reducing the Tc of sucrose. Such behavior for raffinose is also seen in Table IV and Figure 5 for the effect of various concentrations of additives on Tc and τ, indicating that some additional effect of raffinose might be operating at the nucleation site. Interestingly, when we compare the rates of water loss in the isothermal experiments, as in Figure VI, we see significant inhibition of crystal growth

Table VII. Effects of 10% w/w Additives on the Crystallization Temperature of Sucrose Predicted[a] and Experimentally Determined

Additive	Tc(°C) Predicted[a]	Tc(°C) Experimental
Trehalose	131	161
Lactose	133	156
Raffinose[b]	131	160

[a] The predicted value is the midpoint between the glass transition and melting temperatures.

[b] At 10% w/w raffinose is at 6.8 mol% in sucrose, whereas lactose and trehalose are at 10 mol%.

kinetics relative to sucrose alone in all cases, but now we do not see any unique effects due to raffinose. If we accept the proposal that the rate of water loss in these isothermal experiments primarily is reflecting crystal growth kinetics, as opposed to nucleation, it would appear that the differences between raffinose and the other two disaccharides, lactose, and trehalose, is associated with the nucleation step.

At this point a number of questions concerning the effects of colyophilized additives on sucrose may be raised. Why do these additives have such significant effects, at levels as low as 1% w/w, when even at 10% w/w the reduction in Tg is quite small? Why does raffinose on a molar basis have a much greater effect on the nucleation of sucrose from the amorphous state and no apparent difference in its effect on subsequent crystal growth? Are there possibilities for specific interactions involving raffinose, as shown under solution conditions (10,22), or is it a matter of the larger molecular size of raffinose causing a greater steric hinderance to mass transport than might be expected on the basis of Tg alone? To start to address these questions, it is worthwhile to probe any possible tendencies for specific interactions between sucrose and other sugars in the amorphous state. All of the experiments reported above appear to indicate that the sugars are all miscible in the colyophilized systems at all proportions, since as mentioned above, any significant phase separation would reflect itself in the appearance of two Tg values, as seen with macroscopic mixtures. It is possible to further probe the extent to which any nonidealities might occur due to specific interactions by quantitatively examining the Tg values in these colyophilized systems (see Table III) as a function of weight fraction using a model developed by Gordon and Taylor for polymer systems from free volume theory (24). If we have volume additivity and no specific interactions it should be possible to estimate the glass transition temperature of the mixture, Tgm, from the weight fraction of each component, W_1 and W_2 and the individual glass transition temperatures, Tg_1 and Tg_2 using the following expression:

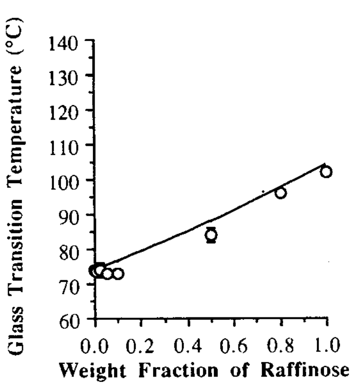

Figure 6. Glass transition temperature vs. weight fraction of raffinose colyophilized with sucrose: (○) experimental points; (—) predicted values from the Fox equation (eqn. 4 in text).

$$\mathrm{Tgm} = \frac{W_1 Tg_1 + KW_2 Tg_2}{W_1 + KW_2} \tag{2}$$

where K is a constant that can be estimated from the following equation:

$$K = \frac{\rho_1 Tg_1}{\rho_2 Tg_2} \tag{3}$$

and where ρ_1 and ρ_2 represent the densities of the two amorphous solids. We can take this analysis one step further and show that when $\rho_1 \approx \rho_2$, which would be the case for these sugars, equation 2 becomes the Fox equation (25):

$$\frac{1}{\mathrm{Tgm}} = \frac{W_1}{Tg_1} + \frac{W_2}{Tg_2} \tag{4}$$

As can be seen in Figure 8, the values of Tgm vs. composition of raffinose in sucrose are reasonably well-described by equation 4, indicating that volume additivity is occurring with this system and that no strong interactions are occurring between the two components. The tendency for no change, and even a minimum, at and below a weight fraction of 0.1 most likely reflects the limited precision associated with Tg measurements, although really small negative deviations from ideality cannot be totally discounted. Similar results were observed with the other sugars (data not shown). What this analysis cannot address is the possibility that the additives can accumulate at the solid-particle interface as an adsorbed layer during colyophilization in an amount sufficient to inhibit any nucleation that might be initiated on the surface of the particle, or that steric hinderance for mass transport is playing a role. Differences in molecular size between the di- and trisaccharides might be enough to produce such an effect. To address this further it will be necessary to carry out similar studies with additives that are chemically identical, but variable in molecular weight.

CONCLUSIONS

A model for nucleation-controlled crystallization from the amorphous state, that relates Tc to Tg and Tm, has been shown to be applicable for amorphous sucrose under non-isothermal crystallization conditions in the absence and presence of residual water. The effects of water appear to be directly linked to its ability to lower the glass transition temperature of sucrose. Nucleation control is further confirmed by the significant effect of sucrose crystals, mixed with the amorphous sucrose, on isothermal nucleation-controlled induction times.

From non-isothermal and isothermal crystallization studies of sucrose colyophilized with lactose, trehalose, and raffinose, all having very close values of Tg, it has been possible to separate inhibition due to general effects on molecular mobility and effects specific to the additive. Nonisothermal crystallization studies indicate that the model described above (19) is quantitatively not applicable when additives are present and that some contribution to mass transport, beyond a general effect on molecular mobility through an effect on Tg, is operating. In particular, the consistently greater inhibiting effect of the trisaccharide raffinose on a molar basis, and the agreement between results with the disaccharides, trehalose and lactose, argue for some interference with mass transport to the nucleation site due to larger molecular size of the additive and/or its possible adsorption on the particle surface.

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