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Assessment of disorder in crystalline solids

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Summary

In the processing of pharmaceutical solids, disruption or activation of the crystalline structure often leads to varying degrees of disorder through the formation of defects and amorphous regions. Since percent disorder of processed samples must be assessed quantitatively using premixed standard samples of highly crystalline and amorphous solids, in the past, a number of questions about the validity of such an approach have been raised. Using sucrose as a model solid, a comprehensive assessment of such disorder has been carried out on predetermined mixtures of crystalline and amorphous samples, as well as on crystalline samples mechanically milled for various periods of time. Particular emphasis was placed on determining low levels of disorder in highly crystalline samples, since most techniques can detect no less than about 10% disorder. With predetermined mixtures, measurements of X-ray powder diffraction, density and heats of crystallization revealed good linearity with the percent disorder and acceptable detectability down to about 10%, as expected. However, using water vapor sorption measurements under very carefully controlled conditions proved effective in being able to detect disorder as low as 1%. A comparison of these four methods for estimating the percent disorder of milled samples of sucrose gave very consistent results, once the underlying factors that make these techniques sensitive to the concentration of amorphous structure present were recognized and taken into account.

Introduction

In the processing of pharmaceutical solids (York, 1983), as in milling (Dialer and Küessner, 1973; Nakai et al., 1977; Hüttenrauch, 1978), heat drying (Hüttenrauch and Keiner, 1979), spray drying (Mumenthaler and Leuenberger, 1991) and lyophilization (Pikal et al., 1978), disruption or activation of the crystal structure often occurs, leading to various degrees of disorder in the form of crystal defects and/or amorphous regions. Such changes also have been reported to occur during the tablet compaction process (Down and McMullen, 1985; Hüttenrauch, 1988; Ahlneck and Alderborn, 1989), and they are very likely to occur during other pharmaceutical processes involving water and drying such as during wet granulation and polymer film coating. Low levels of

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disorder or disruption in a crystalline solid also can be brought about by the presence of small amounts of impurities or additives (Pikal and Grant, 1987).

The formation of disorder in a solid produces regions that are in a higher energy state than that of the crystal. This can result in more advantageous pharmaceutical properties, such as enhanced dissolution rate (Chiou and Kyle, 1979; Burt and Mitchell, 1981), as well as undesirable properties such as increased chemical instability (Pikal et al., 1978; Oberholzer and Brenner, 1979; Waltersson and Lundgren, 1985) and a potential for solid-state transformation to lower energy crystalline forms upon storage (Makower and Dye, 1956; Pikal et al., 1978; Kontny et al., 1987). Ahlneck and Zografi (1990) have demonstrated how water vapor taken up at relatively low overall levels, can concentrate in regions of disorder, thus producing significant effects on molecular mobility, which in turn, can result in enhanced physical and chemical instabilities.

In view of the significant effects that the state of disorder in crystalline solids can have on the properties of pharmaceutical solids, it would be important to be able to assess the extent of disorder in a solid quantitatively, down to very low levels. By far, the most widely used technique is X-ray powder diffraction (Klug and Alexander, 1974), where the characteristic peak intensities or integrated peak intensities are measured as the crystallline sample is mixed with various proportions of an amorphous sample (Hermans and Weidinger, 1948; Black and Lovering, 1977) to provide a calibration plot. Other techniques used in this way to assess the percent disorder in crystalline samples include: density (Suryanarayanan and Mitchell, 1985; Duncan-Hewitt and Grant, 1986), heat of solution (Pikal et al., 1978; Hendriksen, 1990), infrared spectroscopy (Black and Lovering, 1977), dissolution rate (Hendriksen, 1990) and water vapor absorption (Pikal et al., 1978). Once a calibration curve with the pure crystalline and amorphous samples and their known mixtures is established, similar measurements are then made on the sample of interest to assign the corresponding percent disorder.

In evaluating the various approaches used to assess disorder in crystalline solids, a number of questions or uncertainties arise. First, there is the question of what constitutes 100% crystallinity and 100% amorphous nature in a given sample. Second, most techniques reported so far rarely were able to discern percent disorder of a physically mixed system of less than about 10%. Third, in the few cases where more than one technique has been used with the same processed sample, very different estimates of percent disorder often have been determined (Pikal et al., 1978; Duncan-Hewitt and Grant, 1986). It has been suggested that part of this latter uncertainty may arise because of differences in what properties these techniques actually measure. X-ray powder diffraction and density, for example, reflect the 'average' degree of order directly detected by the measurement (throughout the bulk), while heats of solution or water vapor absorption are measurements of the higher state of energy associated with the disordered state. Thus, different techniques may be more sensitive to different characteristics of the solid. It also is possible that the exact nature of an amorphous region may be different depending on the method used to cause activation, and, therefore, various techniques could respond differently to such samples. It has been pointed out that the use of a physical mixture of amorphous and crystalline particles represents a 'two-state' model, where each particle is almost entirely crystalline or amorphous, whereas in processed samples, it is very likely that all or most of the solid particles are only partially crystalline or amorphous, in what is termed a 'one-state' model (Hüttenrauch et al., 1985).

In this study, we wish to examine these issues more closely, using a model substance, sucrose, capable of being prepared in highly anhydrous crystalline and amorphous forms, and processed by milling to various extents in a controlled manner. Four different techniques have been compared: X-ray powder diffraction, density from helium pycnometry, heats of crystallization from the amorphous state by DSC measurements and water vapor absorption. Particular attention has been given to establishing the extent of agreement or disagreement for the same samples, milled to different extents, using the four tech-

niques, and to probing the lower limits of detection and precision for each technique.

Experimental

Materials

Sucrose, analytical grade, 99 + % (Aldrich Chemical Co., Milwaukee, WI) and lithium fluoride, reagent grade (Aldrich Chemical Co.) were used as received. The salts chosen for preparation of saturated salt solutions used to control relative humidity were described previously (Oksanen and Zografi, 1990). Water used in these solutions and in any freeze-drying procedure was doubly distilled after treatment with a Millipore, Milli Q filtering system. Krypton, used in the surface area measurements, was obtained from Matheson Co. (Division of Searle Products U.S.A., Inc.) at greater than 99.999% purity.

Procedures

Sample preparation Amorphous samples of sucrose were obtained by first freezing a 10% sucrose solution in water at −45°C (T'_g of sucrose is −32°C). The solid formed was then evacuated for 48 h at this temperature in a freeze dryer (Dura-Stop DC, FTS Systems, Stone Ridge, NY), after which the lyophilization was continued by gradually increasing the temperature of this system. The excess water was removed to below 0.1% by further drying the cake at 60°C for at least 48 h. Samples taken to be 100% crystalline were milled in a high energy impact mill (Shatter Box, Spex Industries, Edison, NJ) for 5 s to reduce the particle size for X-ray powder diffraction measurements. The milled samples were then sieved and transferred to desiccators containing magnesium nitrate, at a relative humidity of 56% at 30°C (Nyqvist, 1983), where they were stored for 1 week. This relative humidity and storage time have been shown to result in complete recrystallization of any amorphous sucrose present (Makower and Dye, 1956). The crystalline samples were further dried at 90°C in a vacuum oven (Precision®, Precision Scientific, Chicago, IL) for 3 h and stored in desiccators containing phosphorus pentoxide (relative humidity ≈ 0%). Amorphous samples of sucrose were found to exhibit a glass transition temperature of 74°C by DSC measurements, in good agreement with a previously reported value (Korey, 1991).

Milled samples were prepared by placing approx. 10 g of dried sucrose in a high energy impact mill, described above, for various time periods up to 900 s. The milled samples were sieved and the portion passing through a 230 mesh screen ($d < 63\ \mu m$) was collected. Samples were stored over phosphorus pentoxide and later manipulations were carried out either in a glove box at a relative humidity close to 0% or in environments where the ambient relative humidities were less than 20% to prevent recrystallization of any amorphous material produced by milling.

X-ray powder diffraction X-ray powder diffraction procedures were carried out using an X-ray diffractometer (45 kV × 40 mA, Scintag, Scintag, Santa Clara, CA) in an atmosphere of below 20% relative humidity and at room temperature. Approx. 2 g of each sucrose sample was loaded into a sample holder with 2 mm thickness and scanned at a rate of 5° 2θ per min. No particle size effects on peak intensities and integrated peak intensities were observed with samples that had been sieved below the equivalent of 63 μm. For those systems where an internal standard was included, LiF, previously sieved to equivalent particle diameters of less than 63 μm, was used at a level of 11% w/w; its characteristic peak at 45° 2θ was shown not to interfere with the peaks of interest for sucrose.

Density measurement A helium pycnometer (Model MVP-1, Quantachrome Corp., Syosset, NY) was used to measure the true density of all samples. Each value reported is the mean of at least eight independent measurements. Measurements were carried out in an atmosphere of less than 10% relative humidity.

Differential scanning calorimetry Measurements of heat of crystallization were carried out on a differential scanning calorimeter (TCII/DSC 30, Mettler, Hightstown, NJ), using hermetically sealed aluminum pans, at a scanning rate of 10° 2θ per min. Manipulation of materials before

sealing the sample pans was carried out at less than 10% relative humidity.

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 (Brunauer et al., 1938).

Water vapor sorption Water vapor sorption experiments were carried out at 30°C by gravimetric measurements of water uptake at relative humidities of 7.6, 11.3, 21.5 and 32.4%. No crystallization from any amorphous sample of sucrose could be detected, using X-ray powder diffraction, after exposure to relative humidities at or below 32.4% over the time period of any experiment. The samples with high levels of amorphous form were stored in desiccators containing different saturated salt solutions to give the desired relative humidities (Nyqvist, 1983). For samples with high degrees of crystallinity, hence low levels of disorder, measurements were made using a vacuum assembly ($< 10^{-4}$ Torr), containing a Cahn, C2000 electrobalance (Cahn Instruments, Cerritos, CA), as previously described (Kontny et al., 1987). The balance was connected to a computer (Model 386/SX, Gateways, ND) that used a Cahn instrument software program to collect and analyze the data.

Results and Discussion

Mixtures of crystalline and amorphous samples

X-ray powder diffraction Fig. 1 shows the X-ray powder diffraction pattern for samples of sucrose used in this study taken to be completely amorphous and crystalline. The X-ray pattern of crystalline sucrose was compared to and confirmed using data from the Joint Commission for Powder Diffraction Standards, Monograph no. 74 (1977). Comparison of the peak intensities and

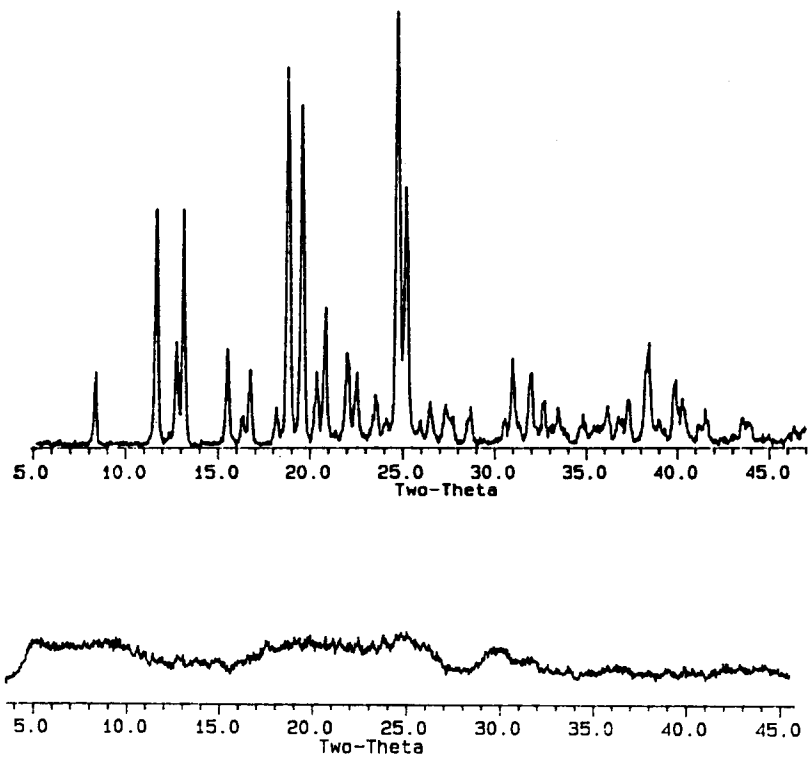

Fig. 1. X-ray powder diffraction patterns for sucrose: (top) crystalline form; (bottom) amorphous form.

the area under the largest crystalline peaks for mixtures of amorphous and crystalline samples, calculated directly from the data generated using the computer software program available (Pad V, Scintag, Scintag, CA), showed a linear relationship as a function of percent disorder. In all cases, with mixtures, the diffraction pattern was corrected point-by-point for scattering of the amorphous portion using the Pad V, Scintag computer program indicated above. The integrated peak intensity values showed better reproducibility and lower standard deviations than the peak intensities, thus only these values are reported in Fig. 2. Various peaks at different angles showed good agreement, so only the results for the crystalline peak of sucrose at 18.8° 2θ (one of the most intense peaks of sucrose) are reported. Alternatively, an internal standard (lithium fluoride was chosen since its characteristic peak does not interfere with the sucrose crystalline peaks) was added to each sample. The ratio of integrated peak intensity for the crystalline peak of sucrose at 18.8° 2θ to that of lithium fluoride at 45° 2θ was measured and plotted as a function of percent disorder as shown in Fig. 2. For sucrose, integration was carried out over a range of 18.3° 2θ to 19.0° 2θ, while for LiF the range was 44.7° 2θ to 45.2° 2θ. The angular range of integration

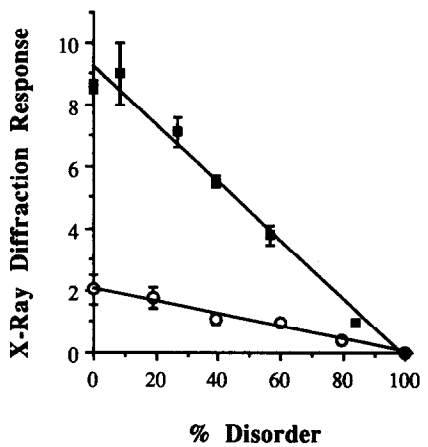

Fig. 2. X-ray powder diffraction responses vs percent disorder for mixtures of amorphous and crystalline sucrose: (■) integrated peak intensity $\times 10^4$ at 18.8°; (○) integrated peak ratio, sucrose at 18.8° and LiF at 45° as an internal standard.

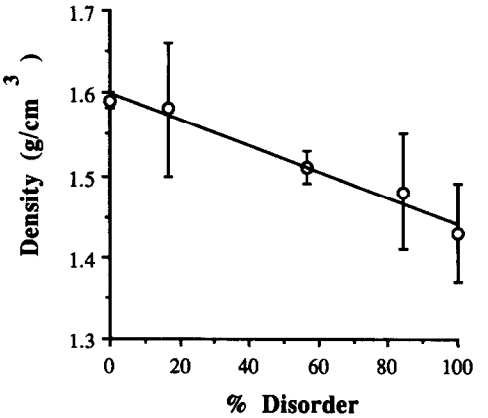

Fig. 3. Density vs percent disorder for mixtures of amorphous and crystalline sucrose.

did not change significantly as a function of percent disorder. Background subtraction for all samples was performed using the Pad V, Scintag software program. The standard curves obtained from both measurements with three independent experiments resulted in linear plots with correlation coefficients of better than 0.99. From these plots, it is apparent that X-ray powder diffraction measurements show lower standard deviations for samples with higher percentages of amorphous content; as the percentage of amorphous material decreases the sensitivity of this technique is reduced, so that one cannot distinguish between the sample that is 90% crystalline and that which is 100% crystalline for this system.

Density Fig. 3 shows the standard curve as prepared from density measurements with various mixtures. As the concentration of percent amorphous solid increases there is a decrease in density that is linear with concentration. However, due to the small change that occurs in density and the large standard deviations associated with such measurements, this technique clearly is less capable of detecting low levels of disorder than X-ray powder diffraction.

Heat of crystallization Fig. 4 shows a DSC scan of amorphous sucrose, where a distinct exotherm due to crystallization occurs above its glass transition temperature and below its melting point endotherm. The exotherm, with a peak temperature of 130°C, gave a heat of crystalliza-

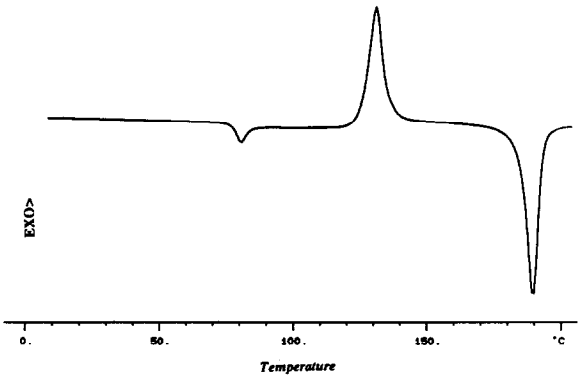

Fig. 4. Typical differential scanning calorimetry profile for sucrose showing glass transition endotherm, crystallization exotherm and fusion endotherm.

tion that was very close, but opposite in sign, to the heat of fusion, indicating that essentially complete crystallization had occurred. The heat of crystallization values for mixtures of amorphous and crystalline sucrose were calculated by estimating the area under the exothermic peak, observed to be reduced to 115°C for all mixtures. Fig. 5 shows the standard curve prepared from these values as a function of percent amorphous solid present. This technique, carried out on three independent samples, appears to be able to estimate the degree of disorder with an overall sensitivity of ±5%. However, for highly crystalline samples, as the concentration of amorphous solid decreases below 10%, this method is not able to differentiate the low levels of disorder.

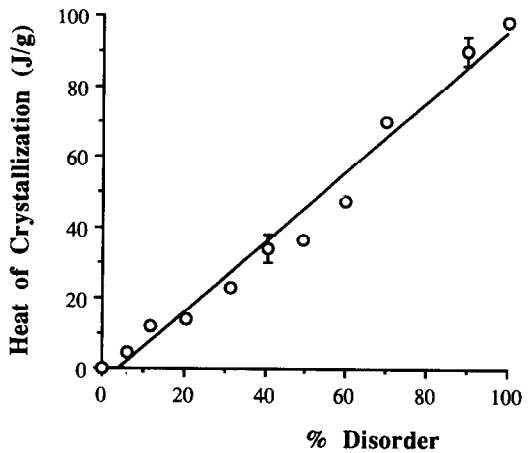

Fig. 5. Heat of crystallization vs percent disorder for mixtures of amorphous and crystalline sucrose. (Error bars represent one standard deviation for all data.)

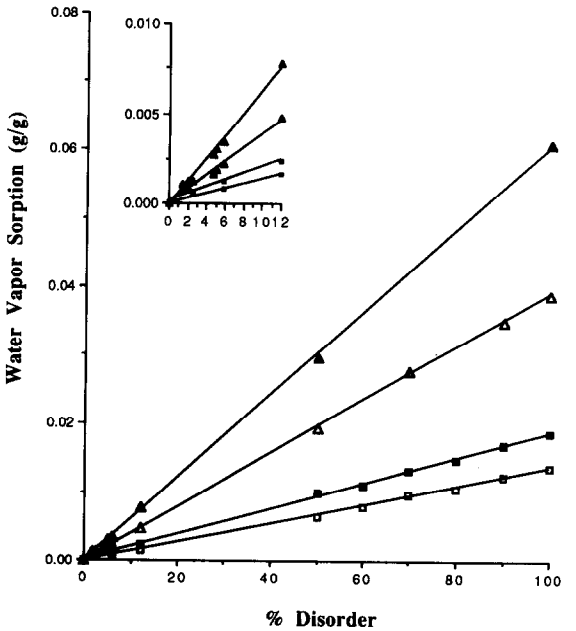

Fig. 6. Water vapor absorption at 30°C and various relative humidities vs percent disorder for mixtures of amorphous and crystalline sucrose. Inset focuses on 0–12% disorder. Relative humidities: (□) 7.6%; (■) 11.3%; (△) 21.5%; (▲) 32.4%.

Water vapor sorption The equilibrium water vapor sorption of various samples was measured at relative humidities of 7.6, 11.3, 21.5 and 32.4%, as described above. Fig. 6 depicts the water vapor absorption vs percent amorphous sample for all four relative humidities at 30°C, showing excellent linearity over the entire range of percentages for the average of three independent measurements. The inset included shows that this is still true over the region from 0 to 12% amorphous material. Values of water vapor absorption for the completely amorphous sucrose sample at each relative humidity were in excellent agreement with those in the literature (Makower and Dyer, 1956). Measurements of water adsorption with the 100% crystalline sample indicated that such adsorption was negligible when compared to the amounts of water taken up for the amorphous

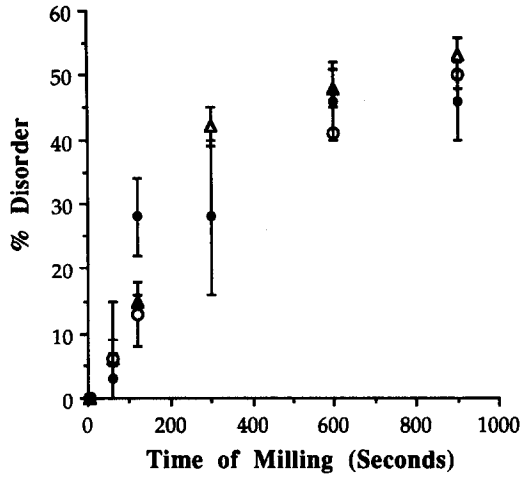

Fig. 7. Percent disorder of milled samples vs milling time estimated by: integrated X-ray peak intensity (○); integrated X-ray peak intensity ratio with LiF (△) and density (●).

samples, and thus for purposes of calculation, it was assumed that the water vapor sorption was exclusively due to the amount of amorphous solid present. From the specific surface area of the amorphous sample, estimated to be 0.71 ± 0.01 m^2/g, and the overall amounts of water vapor taken up at the various relative humidities, we also can conclude that the fraction of water adsorbed on the surface is negligible compared to that taken up into the amorphous structure. As seen in Fig. 6, excellent linearity was observed over the entire range of percent amorphous mass. Furthermore, this technique is much more sensitive than the other techniques in detecting the concentration of amorphous solid at very low values, i.e., $\approx 1.0\%$, with a higher degree of accuracy ($\pm 0.5\%$).

Percent disorder in milled samples of sucrose

X-ray powder diffraction and density Fig. 7 presents the percent disorder for samples of crystalline sucrose milled for various time periods, estimated from X-ray powder diffraction, with and without an internal standard present, and from density measurements, using the standard curves in Figs 2 and 3. As might be expected, essentially the same results were obtained by using corresponding values for the '100%' crystalline and amorphous samples in the following equation;

$$\%\ disorder = 100 - [(X - X_a)/(X_c - X_a)] \times 100 \qquad (1)$$

where X is the area under the curve or density value measured for the sample, and X_a and X_c denote the same values for completely amorphous and crystalline samples, respectively. From Fig. 7, it can be seen that the estimates of disorder for milled samples show good agreement for these different methods, particularly at longer milling times. However, as expected, very large standard deviations are associated with the estimates made from density measurements due to the small difference in the density values of the crystalline and amorphous sucrose samples. The results of three independent milling experiments for X-ray powder diffraction and milling measurements showed good reproducibility of percent disorder estimates at the longer milling times, but significant error at shorter milling times. It is believed that the larger error associated with shorter milling times is due to the variation in the actual milling operation over short time periods, some variation in particle size distribution produced with such short milling time, and lower levels of accuracy in the X-ray measurements in detecting the lower amounts of disorder.

Heat of crystallization

In order to translate the results of measuring the heats of crystallization of crystalline and amorphous sucrose mixtures to those of milled samples, it is necessary to take into account the fact that the temperature at which crystallization occurred varied with these two samples; the temperature of crystallization for mixtures of amorphous and crystalline samples occurred at 115 ± 5°C, whereas with the milled samples the heat of crystallization was measured at 74 ± 3°C which is the T_g for amorphous sucrose. As shown schematically in Fig. 8, the heat of crystallization from the amorphous state would be expected to increase as a function of temperature. Thus, proper corrections for the effect of temperature should be made before the heat of crystallization

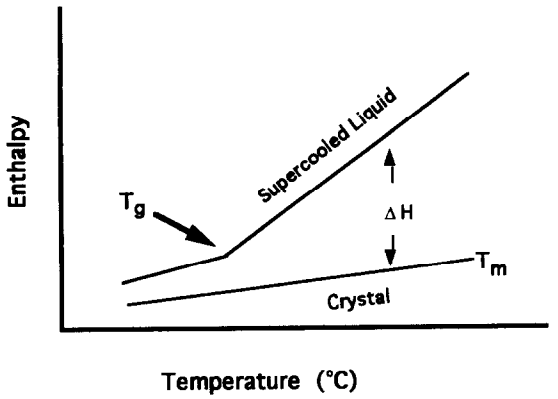

Fig. 8. Schematic representation of enthalpy vs temperature for crystalline and amorphous forms of a solid.

values measured at two different temperatures are compared on an equivalent basis for estimating the percent disorder. In 1958, Hoffman published an equation, describing the free energy change, ΔG, for crystallization at temperatures other than the melting temperature. This equation is expressed as:

$$\Delta G = (\Delta H_f \Delta T / T_m)(T/T_m) \quad (2)$$

where ΔH_f is the heat of fusion at the melting point, ΔT represents the difference between the crystallization temperature and the melting temperature, T_m is the melting temperature and T denotes the crystallization temperature. In the derivation of this equation, it was assumed that crystallization ideally occurs from the liquid state at the melting point. However, if one assumes that an amorphous solid in a supercooled liquid state behaves similarly to the liquid state, by analogy the change in the driving force of crystallization for a partially crystalline sucrose sample at a lower temperature may be expressed as follows:

$$\Delta G = (\Delta H_c \Delta T / T_c)(T/T_c) \quad (3)$$

where T_c is the crystallization temperature for mixtures of amorphous and crystalline sucrose prepared from a standard curve and ΔT denotes the temperature difference between T_c and T, the crystallization temperature for milled sucrose samples. Consequently, ΔH_f and T_m are replaced by ΔH_c and T_c, respectively. From the Gibbs-Helmholtz equation, one may express the Gibbs free energy ΔG as:

$$\Delta G = \Delta H - T\Delta S \quad (4)$$

where ΔH is the enthalpy of crystallization at a temperature T and ΔS represents the entropy contribution due to the crystallization process. Assuming the entropy change over the small temperature range of study to be very small (Hoffman, 1958), the free energy change for the crystallization process may be assumed in such cases to be dependent primarily on the contribution from the temperature dependence of enthalpy values. Eqn 3 then, may be expressed as:

$$\Delta H = (\Delta H_c \Delta T / T_c)(T/T_c) \quad (5)$$

In the present study, this equation was utilized to correct the measured heats of crystallization for milled sucrose samples at 74°C, ΔH, to that at the higher temperature of 115°C, observed with mixed samples, ΔH_c, in the manner described with the following example. It was assumed that the entropy change, proportional to $\ln(T_2/T_1)$, was negligible over the temperature range of 388–347 K used in this analysis. For a sample milled at 900 s, the ΔH was measured to be 4.65 J/g, where ΔT is 41, T_c is 388 and T is 347. From Eqn 5, therefore, ΔH_c is estimated to be 49.2 J/g, and from the standard curve the sample is estimated to be about 52% amorphous. As shown in Fig. 9, the estimates of disorder based on the heat of crystallization values for three independent samples of milled sucrose are in very good agreement with the results obtained from X-ray powder diffraction measurements.

Water vapor sorption Fig. 9 also presents the estimated degree of disorder for sucrose samples for various time periods using data obtained from water vapor sorption at 7.6 and 32.4% relative humidity and 30°C, and from the calibration curves for predetermined mixtures obtained at these relative humidities. The relative humidities of 7.6 and 32.4% were chosen to compare the accuracy possible using the highest and lowest

possible relative humidities, and hence the highest and lowest possible amount of water sorbed. The percent disorder values estimated at these two relative humidities for three independent samples were in excellent agreement and, hence, the average obtained is given in Fig. 9. Two important observations may be made first for shorter milling times and then for the longer milling times. At shorter milling times, i.e., up to about 1 min, no statistical difference could be observed with other techniques. However, not shown on this plot was a comparison made between the sample of crystalline sucrose milled for only 5 s and one where this sample was equilibrated at 56% relative humidity, where any amorphous material formed would completely recrystallize (the '100%' crystalline sample in this study). Here, it was possible to observe that at 7.6% relative humidity, the sample milled for 5 s picked up $0.01 \pm 0.001\%$ water, equivalent to about 1% disorder, while no measurable water uptake was detected for the sample first equilibrated to 56% relative humidity to promote complete crystallization. The same percent disorder was estimated when measurements at 32.4% relative humidity were carried out with the 5-s milled sample. Thus, the water sorption technique clearly is superior as a probe of low levels of disorder in such samples. As seen in Fig. 9, however, the use of water vapor sorption for sucrose samples milled for longer than about 200 s resulted in significant differences with the other techniques, wherein estimates of disorder based on water uptake values in this region were significantly lower. The most likely explanation for such low levels of water uptake by samples milled at longer time periods would be the inaccessibility of the water vapor to a large proportion of the sample. Such inaccessibility might arise because of surface crystallization that could occur during milling (Hüttenrauch, 1978) due to the resulting increased temperature and mechanical force. Indeed, as shown in Fig. 10, sucrose samples milled up to approx. 2 min had their specific surface areas increased to a maximum value, while samples milled for longer time intervals showed a significant reduction in their specific surface area values. Agglomeration, which could cause such a decrease in specific surface area, was physically observed for these samples. Thus, it would appear most likely that enough surface crystallization of sucrose occurred during such periods of milling to significantly prevent water accessibility to the remaining amorphous portions. Although this might be seen as a disadvantage in using water vapor sorption for detecting disorder under such conditions, these experiments also illustrate the sensitivity of water vapor sorption as a diagnostic tool in detecting the formation of crystallinity on the sur-

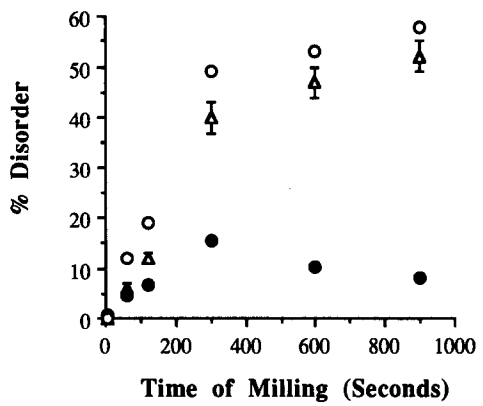

Fig. 9. Percent disorder of milled samples vs milling time estimated by: X-ray powder diffraction (△); heat of crystallization (○) and water vapor absorption (●).

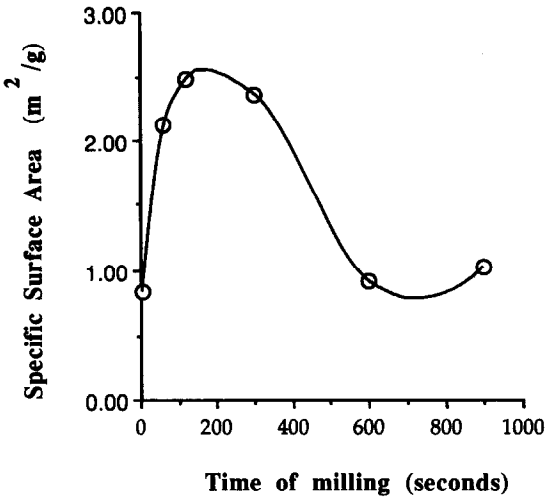

Fig. 10. Specific surface area of sucrose samples milled for various time periods. (The line is drawn simply to follow the data points.)

face of such particles during processing, where disorder might be desirable. X-ray powder diffraction detects an overall crystallinity, whereas water vapor sorption might indicate the extent to which the crystallization occurs on the available surfaces.

It also should be mentioned that the results given in Figs 9 and 10 at milling times up to 150 s allow us to assess the relative influences of increasing surface area and increasing disorder on water vapor adsorption vs absorption. For example, whereas the specific surface area increases by a factor of about 2, the amount of water taken up increases by a factor of about 10. Hence, we see an uptake of water 5-times greater than might be expected if only surface adsorption was involved because of an increase in surface area, and presumably because of the disorder introduced by the milling.

Summary and Conclusion

Comparison of X-ray powder diffraction, density, heat of crystallization and water vapor absorption measurements for mixtures of amorphous and crystalline sucrose particles reveals that a linear relationship with percent amorphous composition exists over the entire range of concentration. However, whereas the X-ray powder diffraction, density, and heat of crystallization methods could not accurately detect disorder below about a 10% level, as expected from previous reports, it was possible to detect the amorphous state down to a level of about 1%, with a high level of precision, using a Cahn microbalance vacuum assembly and measuring the specific surface area of all components. Such measurements also provided a basis for ensuring that the standard 100% crystalline samples used in this study were indeed very crystalline, relative to mildly processed samples normally thought to be highly crystalline.

Estimates of percent disorder above 10% for variously milled samples of sucrose obtained from calibration curves with predetermined mixtures of amorphous and crystalline sucrose were in very good agreement with each other when determined by the X-ray, density and heat of crystallization methods. This confirms the general validity, for this model system at least, of using predetermined mixtures of amorphous and crystalline particles, a two-state system, to assess disorder in milled samples, which most likely represent more of a one-state system.

Water vapor absorption measurements of samples milled for short times and, hence, levels of disorder below about 10% gave results that were generally consistent with the other techniques, but they were very much more precise. Results obtained with water vapor absorption at longer time periods, where extensive particle agglomeration had taken place, however, were significantly different than with the other techniques, i.e., the percent disorder detected was significantly lower. It is concluded that the agglomeration process most likely led to surface crystallization, too small to be detected by the other techniques, but great enough to block access of water to some of the amorphous structure. However, the unlikely possibility cannot presently be excluded that the milling process produced a disordered sample for which water had a different affinity, relative to an amorphous freeze-dried sample. This possibility currently is being explored with a wider range of systems and conditions.

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