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Effect of Preparation Method on Physical Properties of Amorphous Trehalose

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Purpose. To determine the effect of preparation method on the physical properties of amorphous trehalose.

Methods. Amorphous anhydrous trehalose was prepared by four different methods, viz., freeze-drying, spray-drying, dehydration, and melt quenching. The glass transition temperature (T_g), enthalpic relaxation behavior, and crystallization were studied by differential scanning calorimetry, whereas X-ray diffractometry was used for phase identification. The rate and extent of water uptake at different relative humidity values were also obtained.

Results. Though the enthalpic relaxation and crystallization behaviors were influenced by the method of preparation of amorphous trehalose, the T_g and fragility were not. The phase prepared by dehydration showed the highest enthalpic recovery at T_g, indicating that aging may have occurred *during* preparation. Among the four methods used, trehalose prepared by dehydration had the highest tendency to crystallize, whereas there was no crystallization in melt-quenched amorphous trehalose. The method of preparation influenced not only the rate and extent of water sorption but also the phase crystallized. Water vapor sorption removed the effects of structural history in the amorphous phase formed by dehydration.

Conclusions. The method of preparation strongly influenced the pharmaceutically relevant properties of amorphous trehalose. The resistance to crystallization can be rank ordered as trehalose prepared by dehydration < freeze-dried ≈ spray-dried < melt-quenched. The rate of water sorption can be rank ordered as trehalose prepared by dehydration < freeze-dried < spray-dried.

KEY WORDS: amorphous; dehydration; differential scanning calorimetry (DSC); freeze-drying; glass transition temperature (T_g); method of preparation; spray-drying; thermal history; trehalose dihydrate.

INTRODUCTION

Because glasses are in a non-ergodic state, the kinetic properties assume profound importance (1,2). It is a well-established fact that the properties of glasses depend on their previous history. This history is imparted by the preparation method, storage conditions, and thermal, mechanical, or radiation treatment (3,4). Thus, glasses may exist in many possible relaxational states with different kinetic properties (5). This may translate to differences in pharmaceutically relevant properties such as crystallization and vapor sorption (4,6,7). Unfortunately, compared to crystalline forms, it is analytically challenging to distinguish these forms from each other.

There are several routes to the amorphous state, and some of the commonly used methods were summarized by Angell (1). Figure 1 is a schematic representation of some common methods by which pharmaceuticals can be rendered amorphous (8,9). Irrespective of the route of preparation, the underlying effect is the formation of a disordered "solid" phase. However, it has usually been found that the structure of amorphous solids is not random, and there is local or short-range order, despite the absence of long-range order as in crystals (10,11). The method of preparation is one of the most important determinants of this short-range order. As an example, let us consider two completely different routes for the generation of lattice disorder: supercooling of a melt and milling of a crystalline phase. The former method involves rapid removal of the kinetic energy. On the other hand, the direct crystal → glass transformation by milling involves supplying energy to excite an equilibrium crystal and "freeze" it in an energized metastable amorphous state (12). Thus, it is possible that some amorphous materials may be structurally similar to their crystalline counterparts (e.g., silica), whereas others may differ considerably in terms of local order (e.g., sucrose) (13,14).

There are scattered examples in the literature where properties of materials, rendered amorphous by different methods, have been compared. For example, the X-ray diffraction patterns of amorphous cefamandole sodium prepared by freeze-drying and spray-drying showed appreciable differences (4). These results coupled with enthalpy of solution measurements suggested that the spray-dried material was probably more ordered than the freeze-dried material. In another case, amorphous tri-*O*-methyl-β-cyclodextrin prepared by milling and melt-quenching showed differences in the crystallization and relaxation rates (12). However, there were no significant differences, either in the glass transition temperature (T_g) or the heat capacity change associated with glass transition. Crystallization and water sorption behavior of amorphous ursodeoxycholic acid formed by melt quenching and grinding were compared (6). Though the water sorption behavior of the two samples was quite similar, the crystallization rate was considerably higher in the ground sample. From these examples, it is clear that the preparation method can influence the properties of an amorphous phase. However, with the limited data available, it is not possible to clearly relate the observed properties with the preparation method.

This research work is based on the assumption that the local structure in the amorphous phase affects its physicochemical properties, and that the local structure is a function of the preparation method. Andronis and Zografi have suggested that crystallization from the glassy state (i.e., below the T_g) in indomethacin is facilitated by structural features that existed in the amorphous state (7). The higher crystallizing tendency of ground ursodeoxycholic acid compared to the melt-quenched sample was attributed to the differences in their short-range lattice order (6). It is speculated that substances that have similar local structure in the glassy and crystalline states should crystallize relatively easily, whereas, in the absence of this structural similarity, crystallization will be

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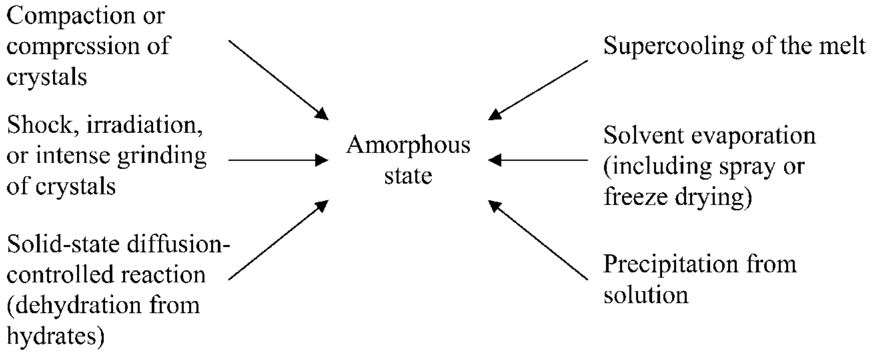


Fig. 1. Some commonly used methods of inducing amorphous character in pharmaceutical systems. Adapted from Angell (1) and Li *et al.* (8).

hindered, particularly below the T_g. If the local structure in the glassy material is similar to that in the crystal, minimal molecular rearrangement would be required for nucleation and crystal growth, whereas if the local structures are different, changes in structure would require significant molecular mobility (11).

Traditionally, the sample history of a glass is "removed" by heating it above the glass transition temperature. It is believed that once the material is heated above its T_g, it is in the equilibrium supercooled liquid region, and all the effects of its history are erased. Though materials like semiconductors, metals, and polymers may be able to endure this high stress, such an approach is not feasible with a majority of pharmaceuticals. In practice, materials that are stored below T_g because of stability concerns are rarely heated above T_g during storage or use. As a result, sample history can play a significant role in the stability of the amorphous formulation, and thus it is practically important to study materials that have been processed differently.

Trehalose is a nonreducing disaccharide used as an excipient in freeze-dried formulations. Its high T_g (~117°C), coupled with a low tendency to crystallize, makes it an attractive model amorphous compound. Moreover, trehalose can be rendered amorphous by different methods including freeze-drying, spray-drying, dehydration of trehalose dihydrate, milling and quenching of the melt (15,16). The aim of this work was to study the effect of preparation method on the physical properties of amorphous trehalose. Our specific objectives were to determine the effects of preparation method on i) T_g and enthalpic relaxation, ii) isothermal and non-isothermal crystallization behavior and activation energy for crystallization, and iii) water vapor sorption and subsequent crystallization in presence of water vapor.

MATERIALS AND METHODS

α,α-Trehalose (α-D-glucopyranosyl α-D-glucopyranoside) dihydrate ($C_{12}H_{22}O_{11} \cdot 2H_2O$; Sigma, St. Louis, MO, USA) was used as obtained. Crystalline trehalose anhydrate (β-polymorph) was prepared by exposing trehalose dihydrate to 20% relative humidity (RH) at 60°C for at least 24 h.

Preparation of the Amorphous Phases

Freeze-Dried Sample

About 25 ml of aqueous trehalose solution (10% w/v) was placed in Petri dishes, cooled to −45°C, and subjected to primary drying (100 mTorr) for 48 h in a tray freeze-drier (Model UNITOP 400L, Virtis, Gardiner, NY, USA). During the next 24 h, the temperature was gradually increased to 50°C. The secondary drying was carried out at 50°C for 24 h, after which the temperature was raised to 60°C and the drying continued for 24 more hours.

Spray-Dried Sample

Aqueous trehalose solution (10% w/v) was spray-dried in a mini spray-drier (Model # B-19, Buchi, Fluwil, Switzerland). A feed rate of 2.6 ml/min was used, the inlet temperature was 125°C, and outlet temperature range was 88 to 91°C.

Dehydrated Samples

The first sample was prepared by dehydrating trehalose dihydrate at ~100°C in a vacuum oven (50 Torr) for 24 h (dehydrated **I**). The second sample was prepared by dehydrating trehalose dihydrate at ~97°C (dehydrated **II**) under partial vacuum (continuous nitrogen flow; pressure ~400 Torr) for 24 h.

Melt-Quenched Sample

The crystalline anhydrate was heated to 220°C under nitrogen flow, held for a few seconds, and cooled rapidly in liquid nitrogen. The quenched liquid thus obtained was milled gently using mortar and pestle in order to obtain a free flowing powder.

The samples were removed from the freeze-drier and stored in a desiccator at room temperature over anhydrous calcium sulfate (RH ~0%) until use. The residual water content was <0.2% w/w (by Karl Fischer titrimetry; KFT). The samples were then handled in a controlled humidity environment (<5% RH, in a glove box) under ambient temperature conditions.

Karl Fischer Titrimetry

The water content was determined coulometrically using a Karl Fischer titrimeter (Model CA-05 Moisture Meter, Mitsubishi Chemical Corp, Kushima, Japan). Accurately weighed samples were directly added to the Karl Fischer cell and the water content was determined.

Thermal Analysis

A differential scanning calorimeter (MDSC, Model 2920, TA Instruments, New Castle, DE, USA) equipped with a refrigerated cooling accessory was used. The instrument was calibrated with pure samples of tin and indium. About 4–5-mg sample was packed in aluminum pans with several pin holes in the lid, crimped, and heated under dry nitrogen purge. Depending on the type of information sought, the samples were pretreated in the differential scanning calorimeter pans. The usual pretreatment was heating to 60°C and holding for 5 h. In conventional differential scanning calorimetry (DSC), the heating and cooling rates were 10°C/min. Modulated DSC experiments were performed with a temperature program having a period of 60 s, an amplitude of ±1°C, and an underlying heating rate of 2°C/min. More specific details, wherever necessary, are provided in the "Results and Discussion" section.

X-ray Powder Diffractometry

Ambient X-ray Powder Diffractometry

About 200 mg of sample was filled in an aluminum holder by the side-drift method (17) and exposed to Cu Kα radiation (45 kV × 40 mA) in a wide-angle X-ray diffractometer (Model D5005, Siemens, Madison, WI, USA). The instrument was operated in the step-scan mode, in increments of 0.05°2θ. The angular range was 5 to 40°2θ, and counts were accumulated for 1 s at each step. The data collection and analyses were performed with commercially available software (JADE, version 5.1 Materials Data Inc., Livermore, CA, USA).

Humidity- and Temperature-Controlled X-ray Powder Diffractometry

An X-ray powder diffractometer (Model XDS 2000, Scintag, Cupertino, CA, USA) equipped with a variable temperature stage (Model 828D, Micristar, R. G. Hansen and Associates, Santa Barbara, CA) was used to control the sample temperature. A humidity-controlling device was built in our laboratory, which permitted us to control the water vapor pressure in the sample chamber of the X-ray powder diffractometer (18). About 50 mg of sample was filled into the holder. The sample chamber was evacuated at 60°C for 30 min to completely dry the sample. For variable temperature scans, the sample was heated at 5°C/min to the temperatures of interest, held isothermally for at least 5 min, and the X-ray powder diffractometry (XRD) patterns were obtained. A continuous flow of nitrogen was maintained over the sample throughout this process. For humidity-controlled experiments, the stage was cooled back to 25°C after the drying process. The RH of the sample chamber was increased to the desired value, and XRD patterns were obtained under isothermal conditions. The samples were exposed to Cu Kα radiation (45 kV × 40 mA) in the continuous mode at chopper increments of 0.05°2θ. The angular range varied between 5 and 40°2θ, and the scanning rate was 3°2θ/min. A small sample size of about 10 mg was used for the *in situ* study of crystallization. This was done to enable a meaningful comparison with vapor sorption results where the sample size is small.

Particle Size Analysis

The sample was sprinkled on a glass slide, suspended in silicon oil, and observed under a compound microscope at a magnification of 400.

Scanning Electron Microscopy

The samples were mounted on scanning electron microscopy (SEM) stubs with double-sided carbon tape, coated with platinum (50 Å), and observed under a scanning electron microscope (Hitachi S-800, Tokyo, Japan).

Surface Area Analysis

Specific surface area was determined by the multipoint (5 points) Brunauer-Emmett-Teller (BET) method using a surface area analyzer (Gemini, Micromeritics, Norcross, GA, USA) with nitrogen as the adsorbate and helium as a carrier gas. Accurately weighed samples were degassed under vacuum at room temperature for at least 12 h followed by repetitively adsorbing and desorbing the adsorbate.

Water Vapor Sorption

About 8 mg of the sample was placed in the sample pan of an automated vapor sorption balance (DVS-1000, Surface Measurements Systems, London, UK). It was first dried at 0% RH, under dry nitrogen flow (flow rate, 200 ml/min) for 24 h, and then exposed to the desired RH. The rate and extent of water uptake was determined over a range of RH conditions. The exposure time at each step varied from 10 to 100 h depending on the time required for equilibration. The microbalance was calibrated using a 100-mg standard weight. The relative humidity sensor was calibrated at 5.0, 11.3, 32.8, 52.8, 75.3, and 84.3% RH (25°C), using saturated salt solutions. Unless stated otherwise, all experiments were performed at 25°C. More specific details, wherever necessary, are provided in the "Results and Discussion" section.

RESULTS AND DISCUSSION

Characterization of Crystalline Trehalose Samples

The XRD pattern of the commercial trehalose dihydrate was in excellent agreement with the published powder pattern (19). The DSC curve of trehalose dihydrate was characterized by a broad endotherm over the temperature range of 60–120°C due to dehydration followed by vaporization of water. This was followed by an endotherm at 218°C, attributed to the melting of the anhydrous β-trehalose. Dehydration under dry nitrogen flow in the vapor sorption balance resulted in a weight loss of ~9.5%, consistent with the stoichiometric water content of trehalose dihydrate. Anhydrous β-trehalose, when subjected to DSC, melted at 214°C (peak temperature). This is in good agreement with the published melting temperatures of 212 and 215°C (20,21).

Baseline Physical Characterization of the Amorphous Samples

The samples prepared by freeze-drying, spray-drying, melt quenching, and dehydration of trehalose dihydrate were all found to be X-ray amorphous with water content <0.2% w/w. Unless differentiated specifically on the basis of the preparation method, these amorphous anhydrous trehalose samples will be identified as amorphous trehalose or amorphous samples. Some of the physical properties including morphology, particle size, and surface area are summarized in

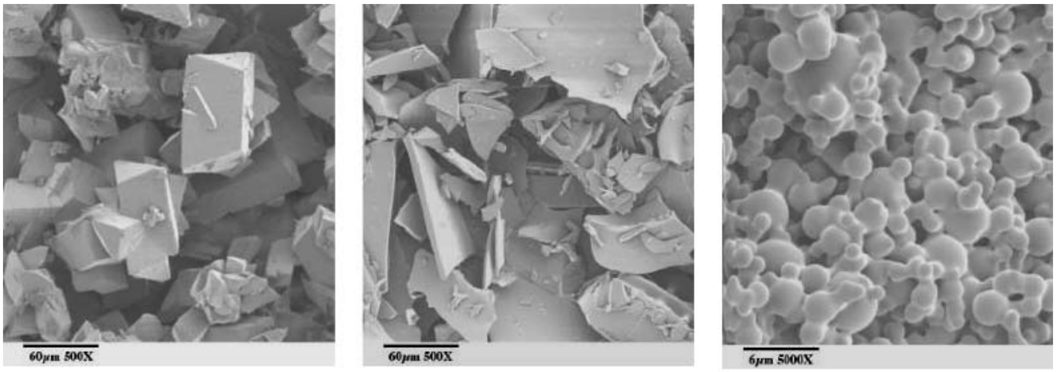
Table I. The SEM photomicrographs of selected samples are presented in Fig. 2.

The "as-is" trehalose dihydrate consisted of prismatic particles. Interestingly, particles in the sample prepared by dehydration at ~97°C (dehydrated **I**) retained the morphology of the crystalline dihydrate, whereas dehydration at a slightly higher temperature (100°C) resulted in irregularly shaped particles (dehydrated **II**). Dehydration of trehalose dihydrate, resulting in particles of the same morphology, is consistent with previous observations (16). However, loss of characteristic morphology on dehydration at a slightly higher temperature of 100°C was not expected. Thus, it was considered important to investigate the effect of dehydration conditions in these two cases. There were two major differences in the preparation method, viz. the temperature and pressure. Until recently, the melting temperature of trehalose dihydrate was considered to be ~100°C (21). However, lately it has been proved that rapid dehydration of the dihydrate occurs at this temperature and that may give the "appearance" of a melting transition (22). This evidence could provide an explanation for the observed differences in the morphology. It is expected that in the sample dehydrated at 100°C, dehydration occurred rapidly, but due to absence of airflow in the vacuum chamber, the liberated water was not removed quickly. The water dissolved in the amorphous anhydrate, decreased the T_g of the amorphous phase (plasticization), and possibly caused a glass → rubber transition. Though exposure to the high temperature dried the sample, the attainment of the rubbery state during preparation resulted in the loss of characteristic morphology. The sample dried at 97°C, on the other hand, dehydrated at a slower rate. Moreover, continuous nitrogen flow during dehydration might have been instrumental in rapid removal of the water. The visual appearance of the two samples supported these conclusions. Though dehydrated **II** was a free-flowing powder, dehydrated **I** was a clumped sample with a higher bulk density (Fig. 2).

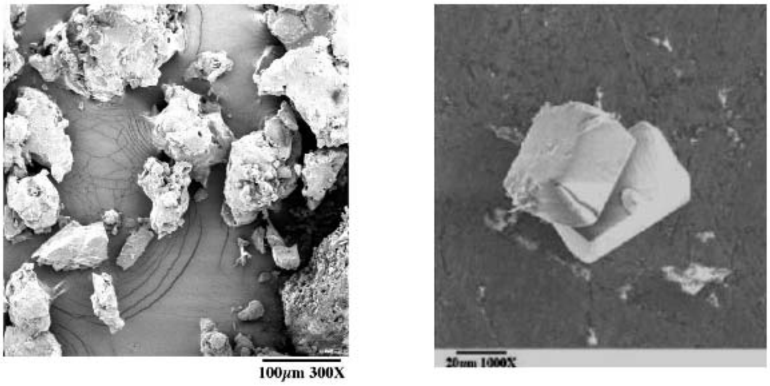
As shown in Table I, thin flakes of freeze-dried particles were about 50–100 μm in size, whereas spherical spray-dried particles were considerably smaller. The particle morphology of melt-quenched samples was considered unimportant as it was prepared by milling of the quenched melt. The surface area values were consistent with the results obtained from microscopy (Table I). The spray-dried sample, characterized by small particles, had the highest specific surface area.

DSC of Amorphous Trehalose

Irrespective of the preparation method, amorphous trehalose was characterized by glass transition at ~117–118°C accompanied by an endotherm due to enthalpic recovery. Figure 3 contains the DSC curves, and the results are summarized in Table II. The glass transition event was followed by crystallization (exotherm) of the anhydrate in all the cases except for the spray-dried trehalose. Finally, the crystalline anhydrate melted at ~214°C.



(a) Trehalose dihydrate (b) Freeze-dried (c) Spray-dried



(d) Dehydrated I (e) Dehydrated II

Fig. 2. The SEM photomicrographs of trehalose samples. The melt-quenched sample could not be analyzed by SEM. These results are also tabulated (Table I).

Table I. Powder Properties of the Amorphous Trehalose Samples Prepared by Different Methods

Sample	Morphology	Particle size	Surface area (m^2/g)*
Freeze-dried	Thin flakes	50–100 μm	0.48 ± 0.12
Spray-dried	Spherical	2–4 μm	1.02 ± 0.22
Dehydrated **I**	Irregular	30–50 μm	0.25 ± 0.10
Dehydrated **II**	Prism shaped	30–50 μm	0.30 ± 0.06
Melt-quenched	Irregular	10–20 μm	Not determined†
Trehalose dihydrate	Prism shaped	50–100 μm	Not determined‡

For the sake of comparison, the properties of trehalose dihydrate are also provided.

* n = 3

† Limited availability precluded surface area determination.

‡ Not determined because of the potential for dehydration during analysis.

The water content of the samples, though small, was a matter of concern. It was important to dry the samples without causing a change in thermal history. As detailed in the MATERIALS AND METHODS section (Thermal Analysis), the samples were therefore heated to 60°C, a temperature significantly below the T_g of ~117°C (Table 1) and held for 5 h. The water content of the samples, when exposed to similar conditions in a vapor sorption balance, was negligible. If the isothermal holding temperature was closer to T_g, it may have caused enthalpic relaxation thus changing the thermal history. However, because the hold temperature was almost 60°C below the T_g, the molecular mobility leading to relaxation was expected to be negligible. As a confirmatory evidence, there was no measurable difference in enthalpic recovery between freshly freeze-dried amorphous trehalose and that held at 60°C for 5 h.

Properties of Trehalose Glass—Effect of Preparation Method

The preparation method had a pronounced influence on the magnitude of enthalpic relaxation (Table II), indicating the differences in the "enthalpy states" of the samples. It is probable that stresses that were imposed during the preparation were responsible for these differences. The phases prepared by dehydration had been exposed to temperatures close to T_g for extended periods of time. Dehydrated **I** and **II** were prepared by exposing trehalose dihydrate to 100 and 97°C, respectively. Because the T_g of amorphous trehalose is ~117°C, it is not surprising that there was considerable relaxation at the dehydration temperatures, with the higher temperature (100°C) causing more relaxation. Amorphous trehalose is known to have very high molecular mobility in this temperature range and undergoes substantial relaxation.

A major step in the preparation of the freeze-dried sample was vacuum drying of the frozen aqueous solution at −45°C for at least 48 h. In frozen solutions, the mobility in a system is assessed by the glass transition temperature of the amorphous freeze concentrate, referred to as T_g'. This is the temperature of glass transition of an amorphous system that has been formed on cooling an aqueous system and from which no more ice will crystallize (23). Though frozen aqueous solutions of trehalose are characterized by two glass transition temperatures ($T_g'_1$ = −40°C and $T_g'_2$ = −31°C), enthalpic recovery has only been observed with $T_g'_2$. The primary drying process effectively resulted in aging of the sample ~15°C below the $T_g'_2$. Similarly, because the secondary drying temperature closely followed the glass transition temperature of the system, more relaxation due to aging was expected. Interestingly, the enthalpic recovery in the freeze-dried material was less than that in the dehydrated samples, though the difference between the T_g and annealing temperature ($T_{annealing}$) was lower in case of freeze-drying (Table II). The annealing occurred ~15°C below the glass transitions temperature in the first case, whereas in the second case it was ~20°C below the T_g. The difference in the annealing temperatures (annealing at 100°C in case of the freeze-dried trehalose and at −45°C in freeze-concentrated trehalose) may have led to a pronounced difference in the mobility, and therefore, extent of relaxation, in the two systems. Moreover, the freeze-concentrated system contains unfrozen water that may cause the relaxation to occur differently (24,25).

The spray-dried and melt-quenched amorphous trehalose underwent the least relaxation during preparation (Table II). This was possibly due to minimal exposure to conditions conducive to relaxation. Spray-drying was performed with the inlet temperature of 125°C and outlet temperature of ~90°C wherein the sample was exposed to higher temperatures for a relatively short period of time. The melt-quenched sample was prepared by cooling the melt using liquid nitrogen wherein the effective cooling rate was several hundred degrees per minute.

Thus, the enthalpic relaxation can be rank ordered according to the preparation method as:

Dehydrated **I** > Dehydrated **II** > Freeze-dried ≈
Spray-dried ≈ Melt-quenched

The heat capacity change (ΔCp) at T_g was independent of the preparation method. The fragility was determined by the method proposed by Moynihan using the dependence of T_g onset on heating rate (26,27). Amorphous trehalose is

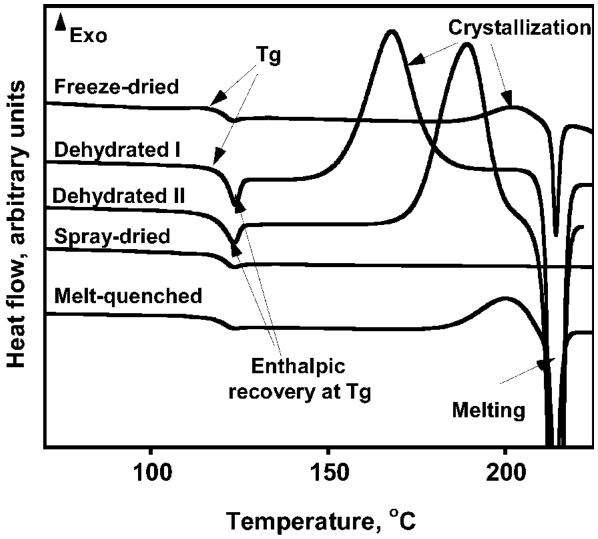


Fig. 3. DSC heating scans of amorphous trehalose prepared by different methods. Following drying at 60°C, the samples were cooled to room temperature and heated to 230°C at 10°C/min. The glass transition, enthalpic recovery, crystallization, and melting events are shown.

Table II. Thermal Analysis of Amorphous Trehalose Prepared by Different Methods

Method of preparation	T_g onset (°C)	Enthalpic relaxation (J/g)	ΔC_p at T_g ($J \cdot g^{-1} \cdot °C^{-1}$)	Crystallization onset (°C)	Fragility
Freeze-dried	117.2 ± 0.2	0.5 ± 0.0	0.55	189.0 ± 0.3	165
Spray-dried	117.7 ± 0.4	0.4 ± 0.1	0.55	No Crystallization	135
Dehydrated **I**	120.1	4.2	0.56	154.1	141
Dehydrated **II**	118.1	3.4	0.55	173.9	153
Melt-quenched	116.1 ± 0.1	0.2 ± 0.1	0.54	178.4 ± 0.2	172

The T_g and crystallization onset and enthalpic relaxation were determined from first DSC scans ($n \geq 2$). The heat capacity change at T_g was determined from modulated DSC ($n \geq 2$), whereas fragility was determined from heating rate dependence of T_g (27).

known to be a fragile glass. No pronounced difference was observed in the fragility parameter in the glasses prepared by the different methods.

Comparison of the Crystallization Behavior

The crystallization behavior of an amorphous material can provide insights into its physical stability. Thus, our next objective was to compare the crystallization behavior of amorphous trehalose prepared by different methods. As a preliminary step, the DSC heating scans (Fig. 3) were used to obtain baseline comparison of the crystallization tendency on the basis of the crystallization onset temperature and enthalpy of crystallization. The crystallization onset temperature in the DSC appeared to be dependent on the preparation method (Fig. 3). As is obvious from the figure, the samples prepared by dehydration had the highest tendency to crystallize followed by the freeze-dried and melt-quenched samples. Crystallization was not observed in the spray-dried material.

Isothermal crystallization studies were performed in the DSC, in the temperature range 140–155°C, wherein crystallization was rapid. Because the spray-dried material did not crystallize readily, it was not possible to study its crystallization behavior. Though crystallization was observed on isothermal annealing at high temperatures and also at very slow heating rates, it was incomplete, and the crystallization exotherms were broad and therefore not quantifiable. Dehydrated **I**, on the other hand, crystallized very readily. Its isothermal crystallization behavior could therefore not be studied. Thus, isothermal crystallization of only dehydrated **II**, freeze-dried and melt-quenched samples was studied. The sample was heated to the desired temperature and held for a predetermined time period, during which anhydrous β-trehalose crystallized. The fraction crystallized, as a function of time, was calculated from the enthalpy of the crystallization exotherm. As a representative example, the isothermal crystallization behavior of freeze-dried sample is shown in Fig. 4. The crystallization data was fitted to common solid-state reaction models. The best fit in all cases was obtained with the three-dimensional nucleation and growth model (Avrami-Erofee'v) (28). The activation energy for crystallization, determined from the Arrhenius plots (Note: It is recognized that crystallization of fragile amorphous substances above T_g may not follow Arrhenius kinetics. This was however done for the sake of simplicity. Moreover, in the narrow temperature range, a linear fit was observed.) was 247, 212, and 238 kJ/mol for dehydrated **II**, freeze-dried and melt-quenched samples, respectively (Fig. 4).

The amorphous samples differed from each other in terms of the morphology and nuclei concentration as well as the extent of relaxation. Because crystallization was studied above the T_g (in the rubbery state), the morphology and the extent of relaxation in the glassy state are irrelevant. Thus, the observed differences may be attributed to varying degrees of nucleation *during* preparation of these samples. However, there appeared to be no effect of preparation method on the activation energy for crystallization. This may be explained in

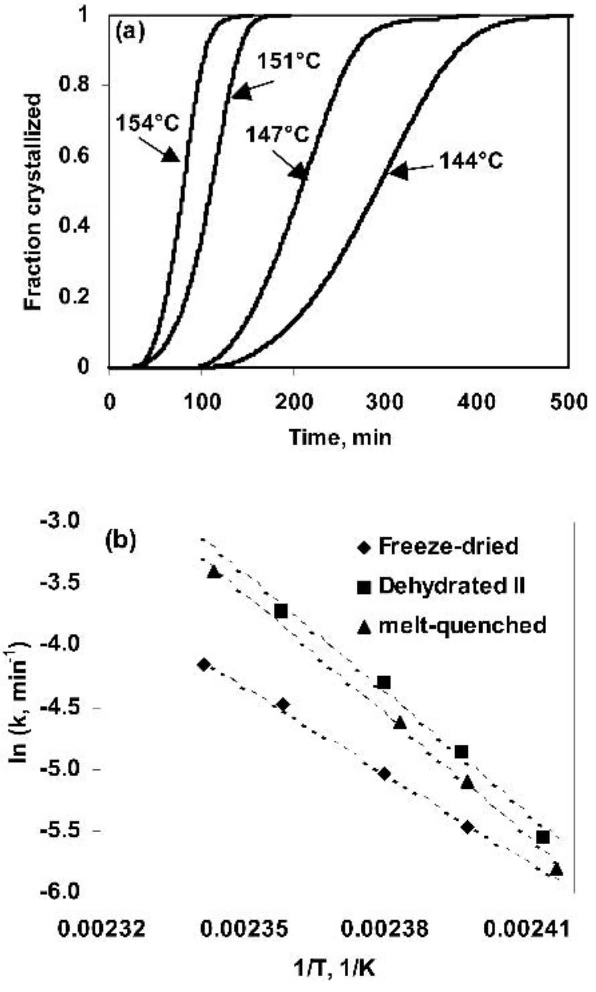


Fig. 4. (a) Isothermal crystallization kinetics of freeze-dried amorphous trehalose in the temperature range of 140 to 155°C. (b) Arrhenius plot of the isothermal crystallization of amorphous trehalose prepared by different methods ($n \geq 3$).

terms of the temperature dependence of the crystallization kinetics. Though difference in nuclei concentration changed the crystallization rate, it did not influence the temperature dependence of the process. The difference in nuclei concentration can also explain the difference in crystallization behavior between dehydrated **I** and spray-dried trehalose. At temperatures where significant molecular mobility existed, rapid crystal growth was observed in dehydrated **I** due to the abundance of nuclei, while spray-dried material could not crystallize readily due to insufficient nucleation. Higher nucleation density in dehydrated **I** was not unexpected in light of our earlier findings, which showed that nucleation occurred in amorphous trehalose on annealing below the Tg (24).

It was mentioned earlier that the spray-dried sample did not crystallize readily. The crystallization was incomplete even after isothermal annealing at temperatures close to melting. However, a rather surprising crystallization behavior was observed after the freshly prepared spray-dried samples had been stored at room temperature at ~0% RH for ~30 days. When these aged samples were subjected to DSC, crystallization was facilitated and occurred to a much greater extent than in the freshly prepared spray-dried samples. One explanation is that nucleation was occurring even at room temperature. This is an important observation, since nucleation was not expected at a storage temperature that was almost 100°C below the T_g. However, this behavior was observed only for the spray-dried material where the extent of nucleation was considerably low in the freshly prepared material. In other samples, storage under similar conditions did not have any effect on the crystallization behavior. An in-depth analysis of this interesting behavior is outside the scope of this manuscript and will be dealt with in future publications.

Water Vapor Sorption

The next objective was to evaluate the effect of preparation method of amorphous trehalose on its interaction with water vapor. Because crystallization was observed at RH $\geq$ 50%, the rate and extent of vapor sorption was studied at RH values ranging from 0% to 40% (25°C). Due to its limited availability, water uptake behavior of trehalose prepared by quenching of the melt could not be studied. Figure 5 compares the water sorption behavior of amorphous trehalose obtained via different routes. In the RH range 0–40%, the freeze-dried and spray-dried materials sorbed almost the same amount of water, with a linear increase in the amount sorbed with RH, whereas the dehydrated phases sorbed less water (Fig. 5a). This difference in the extent of sorption was very pronounced at RH values $\leq$30%. This issue will be discussed in detail later.

Next, the rate of uptake was studied at 10% RH. The method of preparation appeared to have a significant influence on the rate of uptake (Fig. 5b). The initial rate of water uptake at 10% RH, that is, during the first 15 min (marked by a vertical line in Fig. 5b), was maximum in the spray-dried material, whereas it was minimum in the dehydrated **I**. The spray-dried material had sorbed about 2% w/w water, whereas the freeze-dried and dehydrated samples had sorbed <1% and 0.5% w/w water, respectively. Thus, the preparation method can have a strong bearing on the handling and storage of amorphous trehalose. The differences in the initial rates of sorption were also manifested in the extent of water sorption. After exposure for 4 h, the sorbed water content in spray-dried sample was substantially higher than that in the dehydrated trehalose.

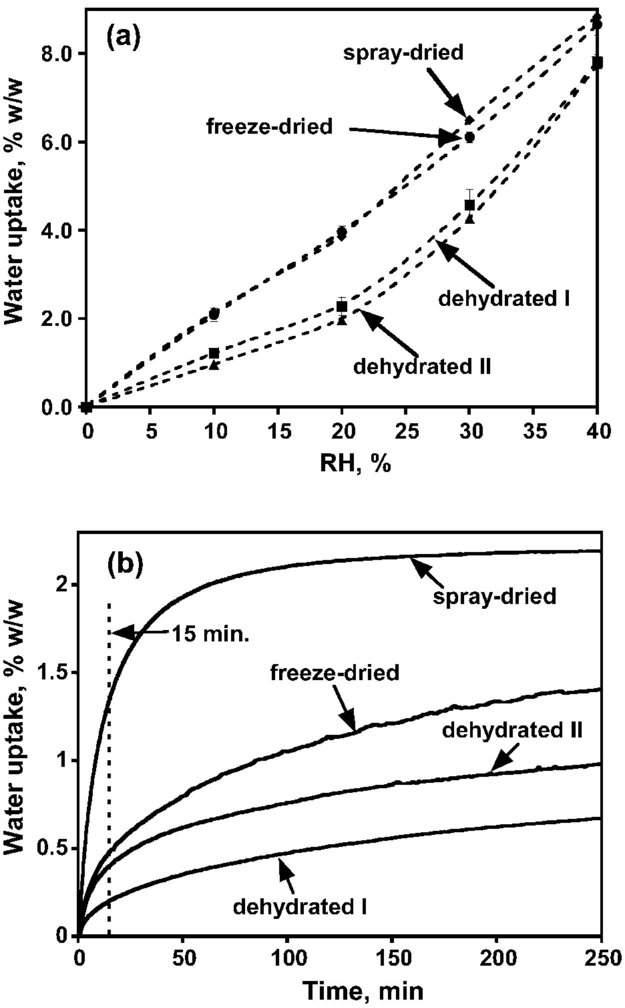


Fig. 5. Water vapor sorption behavior (at 25°C) of amorphous trehalose prepared by different methods. (a) Extent of uptake from 0% to 40% RH. Replicate analyses were performed at selected RH values (mean ± SD; n = 3). (b) Kinetics of uptake at 10% RH.

We have earlier shown that the sample thermal history had a large influence on water sorption in freeze-dried amorphous trehalose wherein relaxation resulted in a decrease in the rate and extent of sorption (24). However, in this case, the comparisons are complicated by the differences in particle size, surface area, density and microstructure of the samples. From Fig. 1, it is evident that the preparation method significantly influenced the particle size and morphology of samples. The higher rate of sorption in spray-dried sample was obvious given its small particle size and high surface area. However, it is expected that the particle size and surface area will influence the rate and not the extent of sorption (24).

As mentioned earlier, although all the samples were X-ray amorphous, there might be differences in the short-range order. The short-range lattice order is possibly one of the factors governing the amount of water sorption. Thus, the material with higher degree of short-range order will sorb less water. It has been postulated that amorphous trehalose prepared by dehydration may be more ordered than that obtained by freeze-drying (22), which would explain the reduced extent of water sorption in dehydrated samples. Moreover, samples prepared by dehydration were characterized by higher degree of relaxation. Based on our earlier studies, this could partly explain the decreased rate and extent of sorption.

Crystallization in Presence of Water Vapor

It is known that amorphous trehalose crystallizes as a dihydrate when stored at RH > 44% at 25°C (29). Figure 6 shows the water uptake of amorphous trehalose samples at 50% RH (25°C). Crystallization following water sorption is usually characterized by a weight loss as was observed for the freeze-dried, spray-dried, and dehydrated **I** samples. This loss in weight during crystallization is attributable to the inability of a crystalline material to retain sorbed water. The water sorption profile of dehydrated **II** was slightly different wherein no weight loss was observed following initial rapid sorption, the details of which will be discussed later. The spray-dried and freeze-dried samples initially sorbed ~12% w/w water and retained ~10.7% water following crystallization, which matches well with the uptake required for conversion to the stoichiometric dihydrate (~10.5% w/w). Humidity-controlled XRD and DSC results (not shown) also revealed that both of them crystallized as the dihydrate.

The water uptake profile of dehydrated **I** was different from that of the freeze-dried and spray-dried samples. It crystallized following approximately 10% w/w water uptake and resulted in a crystalline phase that retained ~8.5% w/w water. This uptake was less than that required for the entire sample to undergo complete conversion to the dihydrate. Humidity controlled XRD revealed that it had crystallized as a mixture of trehalose anhydrate and dihydrate (data not shown). From the DSC studies (Fig. 3), it was deduced that dehydrated **I** contained a higher concentration of the β-polymorph nuclei than any other amorphous trehalose sample. It is therefore not surprising that dehydrated **I** crystallized as a mixture of the two forms. Though the dihydrate is the thermodynamically stable form under these conditions, the mixture of anhydrate and hydrate that crystallized appeared to be kinetically stable in the time scale of our experiments.

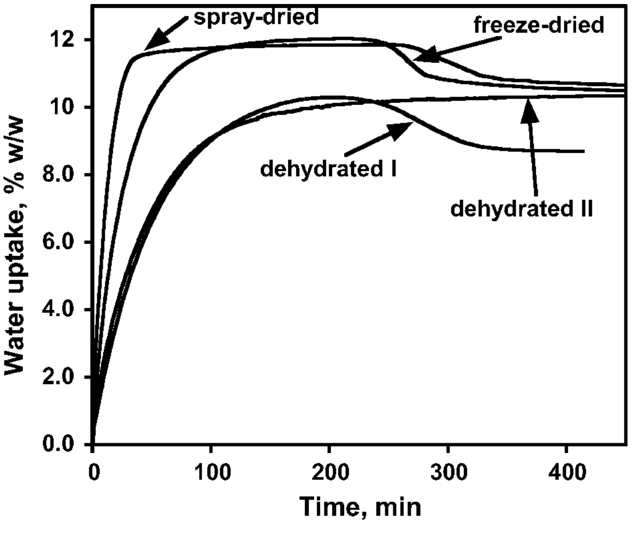


Fig. 6. Water uptake followed by crystallization of amorphous trehalose stored at 50% RH (25°C).

Crystallization of a mixture of two phases from dehydrated **I** may have been a result of the competitive crystallization of the two forms. One possible approach to understand this behavior would be to perform the crystallization experiments at varying temperature and RH conditions. The crystallization behavior at 45, 50, and 55% RH at 25°C was thus compared (results not shown). The weight fraction of the crystalline hydrate increased as a function of the RH, with a simultaneous decrease in the time required for crystallization. Though an increase in the RH is expected to increase the rate of crystallization of both the anhydrate and the hydrate phases, in this case the hydrate form is favored. However, at a fixed RH, an increase in the temperature, led to an increase in the fraction of anhydrate. This observation was also intuitive, as the hydrate formation is usually facilitated by lower temperatures and higher RH values. An increase in temperature was therefore a favorable condition for the crystallization of the anhydrate.

The water uptake behavior of dehydrated **II** was unique in that there was no weight loss after crystallization. It quickly sorbed ~10.4% water and converted to the dihydrate. Such a behavior can be expected in isomorphous dehydrates, wherein water may move in and out of the lattice because of the structural similarity between the anhydrous and hydrate phases. This behavior, however, is not common when conversions are occurring from the amorphous state. It is hypothesized that amorphous trehalose prepared by dehydration may possess some residual structural history of the hydrate from which it was derived and this structural history was probably responsible for a direct conversion to the hydrate phase. Recent studies in our laboratory have shown that, by controlling the dehydration conditions, anhydrous trehalose with varying degrees of lattice order can be obtained. The conversion of this anhydrate, back to the dihydrate, was strongly influenced by the anhydrous "structure" (30).

The differences in the properties of the dehydrated phases could be attributed to their preparation method. As described earlier, dehydrated **I** was prepared under more drastic conditions (higher temperature and lower pressure leading to rapid removal of water) thus resulting not only in complete loss of lattice structure but also in a higher concentration of the β-polymorph nuclei. On the other hand, dehydrated **II** was prepared under milder conditions, thus leading to a residual hydrate structure in the amorphous phase.

Finally, what is the effect of water sorption on any residual structure in the amorphous material? We believe that water sorption (prior to onset of crystallization) should aid in removal of any residual structure. In order to test this possibility, dehydrated **II**, which was expected to have residual structural history of the dihydrate, was equilibrated at 40% RH for ~24 h. This sample was then exposed to 50% RH. Figure 7 compares the crystallization (at 50% RH) of dehydrated **II** pre-exposed to 40% RH, with the "as-is" dehydrated **II**. After exposure to 40% RH, the crystallization behavior of dehydrated **II** changed completely. The sample initially sorbed >11% water and then lost the excess water during crystallization. Thus, after exposure to 40% RH, the residual structure was lost. To further investigate this possibility, dehydrated **II** equilibrated at 40% RH was then dried at 0% RH and subjected to the vapor sorption cycle again. The extent of vapor sorption of this cycled sample was identical to that of the freeze-dried sample, indicating that the

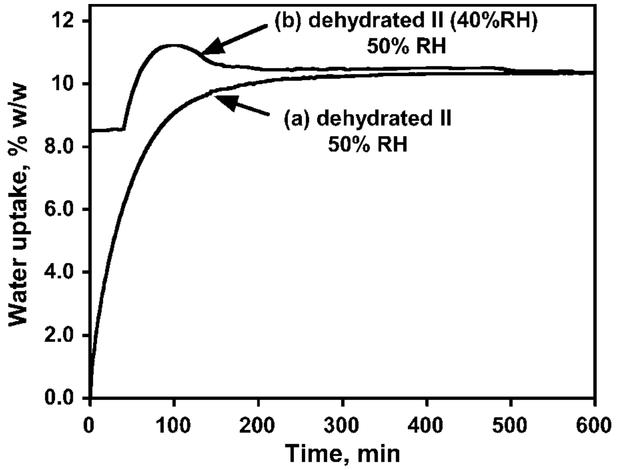


Fig. 7. Crystallization behavior of dehydrated **II** samples at 50% RH, with and without pretreatment. (a) Dehydrated **II** "as-is"; (b) dehydrated **II** equilibrated at 40% RH and exposed to 50% RH.

differences due to preparation method were lost following sorption cycling to 40% RH. This removal of the thermal history may be an effect of the lowering of the glass transition temperature of the plasticized material below the experimental temperature. Once the material goes to the rubbery state, the effects of sample history are lost. The other possible explanation is that the dissolution of water in the amorphous matrix causes the disruption of the residual structure leading to removal of structural history.

Pharmaceutical Significance

Trehalose has been used as a model compound to demonstrate that the method of preparation may considerably influence the pharmaceutically relevant properties. As mentioned earlier, compared to their crystalline counterparts, it is much more challenging to characterize amorphous pharmaceuticals. The glass transition temperature and the heat capacity change at T_g are often used as characteristic properties of amorphous materials. As is evident from Table II, these two properties were incapable of distinguishing between amorphous trehalose samples prepared by different methods.

Crystallization

When an amorphous ingredient is incorporated in a pharmaceutical product, its crystallization, during either processing or storage, is unacceptable. We had earlier demonstrated that aging below T_g caused nucleation, thereby facilitating crystallization (24). From this study, it has become evident that the preparation method can strongly influence the crystallization behavior. Therefore, the thermal history appears to be a critical determinant of the crystallization tendency. For compounds that have a strong tendency to crystallize, a comprehensive knowledge of the thermal history should be an integral part of amorphous phase characterization.

Rate and Extent of Vapor Sorption

In light of the tendency of amorphous materials to sorb water, the rate of sorption has immense practical importance. Rapid water uptake poses major challenges in the handling of the material, from the perspective of manufacture, storage, as well as characterization. If the amount of water sorbed were the only determinant of physical stability, we would expect the spray-dried material to be the least stable at a given RH (Fig. 5b).

Water vapor sorption has been recognized to be an extremely sensitive indicator of the crystallinity in pharmaceuticals (31). Our findings clearly show that the method of preparation affects the rate and, more importantly, the extent of water sorption. Thus, one should be cautious when comparing the vapor sorption of samples and standards if they have been prepared by different methods.

CONCLUSIONS

Thermal history is an important determinant of the physical properties of glassy pharmaceuticals. The method of preparation strongly influences the pharmaceutically relevant properties of amorphous phases. Amorphous trehalose samples prepared by different methods had large differences in particle size, morphology, and surface area. The rate and extent of water sorption, crystallization behavior, and the phase crystallized were all functions of the preparation method. Water vapor sorption was able to remove the effects of structural history in the amorphous phase formed by dehydration.

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