Pharmaceutical Research, Vol. 21, No. 5, May 2004 (© 2004)

Effect of Aging on the Physical Properties of Amorphous Trehalose

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Received November 4, 2004; accepted January 22, 2004

Purpose. The purpose of this investigation was i) to study the effect of physical aging on crystallization and water vapor sorption behavior of amorphous anhydrous trehalose prepared by freeze-drying, and ii) to determine the effects of water sorption on the relaxation state of the aged material.

Methods. Freeze-dried trehalose was aged at 100°C for varying time periods to obtain samples with different degrees of relaxation. The glass transition temperature (T_g) and enthalpic relaxation were determined by differential scanning calorimetry, and the rate and extent of water uptake at different relative humidity values were quantified using an automated vapor sorption balance.

Results. Annealing below the T_g caused nucleation in the amorphous trehalose samples, which decreased the crystallization onset temperature on subsequent heating. However, no crystallization was observed below the T_g even after prolonged annealing. Physical aging caused a decrease in the rate and extent of water vapor sorption at low relative humidity values. Moreover, the water sorption removed the effects of physical aging, thus effectively causing enthalpic recovery in the aged samples. This recovery occurred gradually in the glassy phase and was not associated with a glass to rubber transition. We believe this aging reversal to be due to volume expansion during water sorption in the amorphous structure.

Conclusions. Thermal history of amorphous materials is a crucial determinant of their physical properties. Aging of amorphous trehalose led to nucleation below the T_g, and decrease in rate and extent of water sorption. Sorption of water resulted in irreversible changes in the relaxation state of the aged material.

KEY WORDS: aging; amorphous trehalose; crystallization; nucleation below T_g; relaxation; water sorption.

INTRODUCTION

Amorphous substances are characterized by a glass transition temperature (T_g), where there is a transition between a less viscous rubbery phase and a rigid, more viscous, brittle glass (Fig. 1). As the material in the rubbery region (shown as supercooled liquid) is cooled, a temperature is reached where, due to increase in viscosity, it deviates from equilibrium properties to give a nonequilibrium phase that has higher enthalpy, entropy, and specific volume than the corresponding supercooled liquid at that temperature. The material is thus "frozen" in the glassy state and the translational and rotational motions are significantly reduced (1,2).

If there is significant mobility in this nonequilibrium glass, the system tends to lose its "quenched in" excess enthalpy or free volume and densifies with time (3). This process is known as structural relaxation, physical aging, or annealing, and has strong temperature dependence. The relaxation rate is maximal close to the T_g while negligible relaxation occurs at temperatures far below it. The primary effect of aging is the increase in relaxation times. Thus, it is possible to study the relaxation process by following the time evolution or frequency dependence of material properties such as enthalpy, volume, viscosity and dipole relaxation (4). In pharmaceuticals, differential scanning calorimetry (DSC) is routinely used to study relaxation, which is measured as enthalpic recovery associated with T_g.

Relaxation of an amorphous material can occur during pharmaceutical unit operations involving thermal or mechanical stresses. For pharmaceutical systems with T_g near the storage temperature, it is difficult, if not impossible, to prevent physical aging. Time-dependent changes in physical and mechanical properties of glassy polymers are often attributed to relaxation (5,6). This includes increase in density, yield stress, and modulus and decrease in creep and stress relaxation rate (7,8). Aging is also known to increase the brittleness and lower the impact strength of glasses (9). The rate of benzene sorption by PVC decreased with an increase in the aging time (5). However, in pharmaceutical systems, in an effort to correlate the molecular mobility with the shelf-life of a material, aging studies have focused on the measurement of structural relaxation times (10,11).

Aging is reversible, as the aging effects can be completely erased by heating the material above the T_g to the equilibrium rubber state. This reversal can also be achieved by plasticization of the glass, thus decreasing the T_g of the system below the experimental temperature. However, the fundamental requirement for history removal is to reach the equilibrium supercooled liquid state.

The conventional wisdom is that the mobility below T_g is not high enough to cause crystallization. However, it has been observed that several pharmaceuticals including mannitol (12), indomethacin (13), and carbamazepine (14) can crystallize even up to 15–20°C below the glass transition temperature. Moreover, while crystallization may not be observed below T_g, it does not preclude the possibility of nucleation. For example, previous work from our laboratory has shown that nucleation occurs on aging below the glass transition temperature in frozen aqueous solutions of mannitol and trehalose (12). This nucleation during aging may increase the crystallization tendency, which is undesirable if an amorphous end product is desired.

Given the influence of physical aging on the amorphous material characteristics, it is necessary to understand its impact on the properties of pharmaceutical relevance, specifically water sorption and crystallization. As mentioned earlier, it is possible to remove the effect of thermal history by heating the material above its T_g. However, this is not a viable option both for compounds that readily crystallize at temperatures close to T_g and for thermolabile materials. It is therefore important to study the effects of the time dependent changes on the properties of glassy pharmaceuticals.

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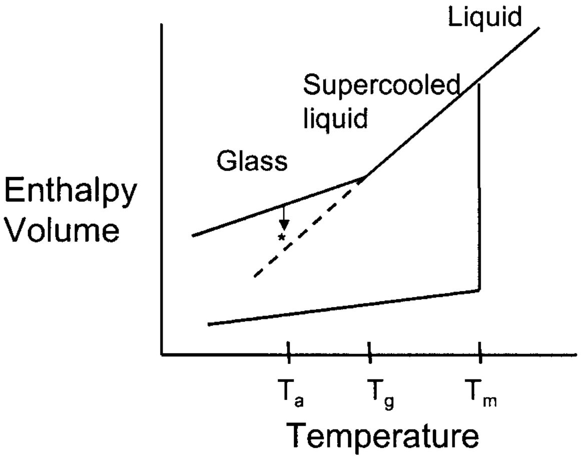


Fig. 1. Schematic representation of enthalpy and free volume curves of a material in the amorphous (upper curve) and crystalline (lower curve) states. T_a = annealing temperature; T_g = glass transition temperature; T_m = melting temperature. The arrow pointing downwards indicates the direction of change in thermodynamic properties toward equilibrium supercooled liquid (discontinuous line) during isothermal aging below the T_g. The material properties following aging are shown by the asterisk (*).

Amorphous materials have a much higher affinity for water than their crystalline counterparts. Sorption of water is known to influence both their physical and chemical properties. Water causes plasticization, leading to a concentration dependent lowering of T_g, thus considerably increasing the possibility of crystallization. This in turn may result in a pronounced decrease in the dissolution rate. Moreover, the increased molecular mobility due to water sorption also makes an amorphous phase more prone to chemical changes (15). Interestingly, crystallization of a stabilizing excipient can lead to chemical instability of the amorphous active ingredient (16,17).

It is well accepted that sorption of water or other small molecules in the glassy matrix results in an increase in the free volume (18), an effect opposite to physical aging. Thus, water sorption may change the relaxation state of a material. This is an important issue since aged glassy pharmaceuticals are routinely exposed to water, both during processing and storage. By studying the water sorption in aged materials, we will not only develop an understanding of the effect of aging on the sorption behavior, but will also obtain insight into the effect of water incorporation on the state of relaxation of an aged material.

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 (19–21).

The objectives of this work were i) to study the effect of physical aging on crystallization and water vapor sorption behavior of amorphous trehalose prepared by freeze-drying, and ii) to determine the effects of water sorption on the relaxation state of the aged material.

MATERIALS AND METHODS

Preparation of Amorphous Trehalose

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. Freeze-dried trehalose was obtained by lyophilization in a bench-top freeze drier (Unitop 400L, Virtis, Gardiner, NY, USA). About 25 ml of aqueous trehalose solution (10% w/v) was placed in Petri dishes, cooled to -45°C, and subjected to vacuum (100 mTorr) for 48 h. Over 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. 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). The samples were then handled in a controlled humidity environment (<5% RH, in a glove box).

In order to obtain the physically aged samples, the freshly freeze-dried amorphous trehalose (also referred to as "unaged" sample), was annealed at 100°C in a vacuum oven (at 25 Torr). The annealing times were 8, 20, and 120 h.

Karl Fischer Titrimetry (KFT)

The water content was determined using a Karl Fischer titrimeter (Model CA-05 Moisture Meter, Mitsubishi). 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) with a refrigerated cooling accessory was used. The instrument was calibrated with pure samples of tin and indium. A 4–5 mg sample was packed in aluminum pans, with several pinholes in the lid (for easy removal of any residual water), crimped, and heated under dry nitrogen purge. Depending on the type of information sought, the samples were pretreated in the DSC pans. The usual pretreatment was heating to 60°C followed by isothermal hold for 5 h. Unless otherwise specified, in conventional 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.

X-Ray Powder Diffractometry (XRD)

About 200 mg of sample was filled in an aluminum holder by the side-drift method and exposed to Cu Kα radiation (45 kV × 40 mA) in a wide-angle X-ray powder 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).

Scanning Electron Microscopy (SEM)

The samples were mounted on 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

Specific surface area was determined by the multipoint (5 points) BET method using a surface area analyzer (Gemini, Micromeritics, Norcross, GA, USA). Accurately weighed samples were de-gassed under vacuum at room temperature for at least 12 h, and measurements were made using nitrogen as an adsorbate and helium as carrier.

Water Vapor Sorption

About 8–10 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% relative humidity (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. More specific details, wherever necessary, are provided in the "Results and Discussion" section.

RESULTS AND DISCUSSION

Characterization of the Amorphous Samples

The unaged trehalose was confirmed to be amorphous by XRD. SEM revealed that the particles were thin flakes, about 50–100 μm in length. Aging did not lead to perceptible changes in morphology, particle size, or in the XRD pattern. The surface area of the unaged sample was approximately 0.7 m^2/gm, which was lower than that reported for many other amorphous pharmaceuticals (14,22). Because of their limited availability, surface area of the aged samples was not determined. Because the particle size and morphology were unchanged by aging, large changes in surface area were not expected. The water content of all the samples was <0.2% w/w.

Differential Scanning Calorimetry

Figure 2 contains the DSC scans and the results are summarized in Table I. The freeze-dried trehalose exhibited onset of glass transition at 117°C. This was followed by onset of crystallization of anhydrous trehalose (β-polymorph) at 174°C (23). The endotherm attributable to melting of the anhydrate was observed at ~214°C (peak temperature). In the aged samples, the glass transition was accompanied by enthalpic recovery. Both the T_g and enthalpic recovery increased as a function of aging time (Table I).

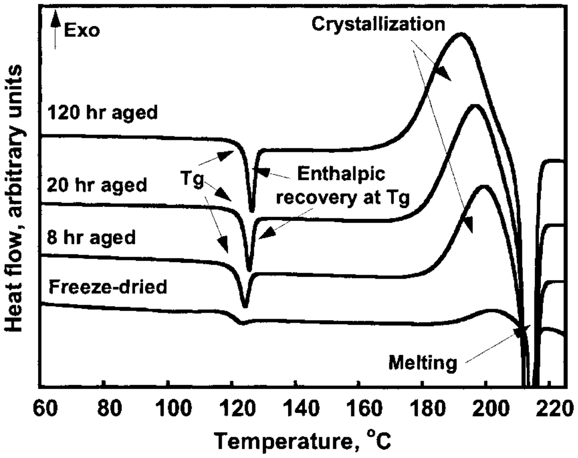


Fig. 2. DSC heating curves of amorphous trehalose samples aged at 100°C. The aging time is given above each of the curves. The unaged (freshly freeze-dried) sample served as control.

The water content of the samples, though small, was a matter of concern. It was very important to dry the samples without causing a change in thermal history. As detailed in the experimental section, the samples were therefore heated to 60°C, a temperature significantly below the T_g of ~117°C (Table I) 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 unaged amorphous trehalose and that held at 60°C for 5 h.

Relaxation leads to an increase in the structural relaxation time and thus increases the observed glass transition temperature. Similar effects were observed as a function of annealing time. With an increase in the annealing time, the enthalpic recovery associated with T_g increased (Table I). As expected, this increase was not linear, and the maximum effect was observed in the first 12 h. With increase in aging time, there appeared to be a small increase in the heat capacity change at T_g (ΔC_p). This trend is expected, as the heat capacity of the glass decreases with aging, with a consequent increase in the heat capacity difference between the glassy and the rubbery states.

With an increase in the aging time, very interestingly, the crystallization onset temperature decreased (Table I). Because crystallization occurred above T_g, the effects of physical aging are expected to be lost. Thus relaxation, per se, would not be responsible for this observation. Decrease in the crystallization temperature following aging indicates that nucleation occurred *during* the aging process and longer the aging, higher the nucleation density. Because all the samples came from the same batch of the unaged material, the observed differences must be a consequence of the aging process.

The highly viscous glass is characterized by significantly reduced mobility compared to the rubbery state. However, crystallization has been observed below the glass transition

Table I. Effect of Aging at 100°C on the Thermal Properties of Amorphous Trehalose

Aging time	$T_g$onset* (°C)	ΔC_p at T_g† (J/g/°C)	Enthalpic recovery* (J/g)	Crystallization onset temperature* (°C)
Unaged	117.2 ± 0.2	0.55	0.5 ± 0.0	189.0 ± 0.3
8 h	120.9 ± 0.3	0.57	4.4 ± 0.4	184.4 ± 0.2
12 h	121.8 ± 0.1	0.57	6.4 ± 0.1	183.4 ± 0.5
20 h	122.8	0.59	7.0	180.4
120 h	123.7 ± 0.1	0.59	8.3 ± 0.1	173.7 ± 0.3

The results are an average of n ≥ 2 determinations. Standard deviations are provided whenever n ≥ 3.
* Determined by conventional DSC.
† Determined by modulated DSC.

temperature (12–14), indicating that sufficient molecular mobility may exist in the glassy state. Mobility in the glassy state is responsible for the enthalpic relaxation observed in a variety of glasses and it is possible that some materials have enough mobility for molecular rearrangements leading to nucleation and crystallization. Even after prolonged annealing, there was no indication of crystallization in glassy trehalose. Seemingly, there was sufficient mobility for nucleation but not for crystallization. Nucleation, as a consequence of physical aging below the T_g, has interesting connotations. The conventional wisdom, that all the effects of physical aging can be "erased" by heating above T_g, may be true only for properties related to structural relaxation. Heating above T_g is unlikely to "erase" nucleation that may have occurred along with structural relaxation as a consequence of aging. Unfortunately, since there are not many studies in the organic material science literature, relating crystallization to aging below the T_g, it is difficult to draw definitive conclusions.

Crystallization Behavior

The crystallization behavior of an amorphous material can provide insights into its physical stability. Thus, our next objective was to compare the isothermal crystallization behavior of the samples aged for different time periods. Crystallization studies were performed in the DSC, in the temperature range of 140–155°C, wherein crystallization was rapid. 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. A representative example of isothermal crystallization behavior at 151°C is shown in Fig. 3a. As the aging time increased, there was a pronounced decrease in the time required for complete crystallization. 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 (Avrami-Erofee'v) model (24). The activation energy for crystallization, determined from the Arrhenius plots, was 212 kJ/mol for the unaged sample (Fig. 3b). For the samples aged for 8, 20, and 120 h it was 206, 269, and 253 kJ/mol, respectively.

The rate of crystallization was lowest in the unaged sample and increased with aging time (Fig. 3a). Aging is expected to increase the nuclei concentration as well as the extent of relaxation of the amorphous phase. Because crystallization was studied above the T_g, the extent of relaxation in the glassy state is irrelevant. Thus, the observed effect is attributed to the increase in nuclei concentration as a function of the aging time. However, there was no systematic effect of aging time on the activation energy for crystallization. This may be explained in terms of the temperature dependence of the crystallization kinetics. Though an increase in nuclei concentration accelerated crystallization, it did not influence the temperature dependence of the crystal growth rate.

Water Vapor Sorption

The next objective was to evaluate the effect of thermal history of amorphous trehalose on its interaction with water vapor. Because crystallization was observed at RH ≥ 50%,

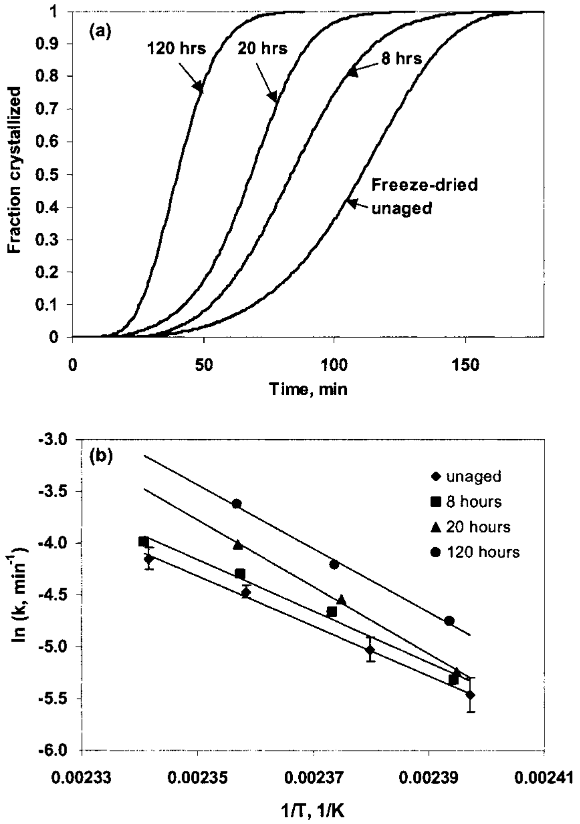


Fig. 3. (a) Isothermal (151°C) crystallization kinetics of amorphous trehalose samples aged at 100°C for different time periods. (b) Arrhenius plots of the isothermal crystallization kinetics between 140 and 155°C. Data plotted are the mean of n ≥ 2 determinations. Standard deviations are shown where n ≥ 3. In some instances, the error bars are smaller than the size of the symbol.

the rate and extent of vapor sorption was studied at RH values ranging from 0% to 40% (25°C). Figure 4 compares the water sorption behavior of the unaged sample with that of the samples aged for 20 and 120 h. Aging affected both the rate and the extent of water sorption. In unaged samples, there was a linear increase in the amount sorbed with RH (Fig. 4a). At 10% and 20% RH, the aged samples sorbed less water than the unaged trehalose. The difference became more pronounced with increase in the aging time. However, at 30% and 40% RH, the amount sorbed was identical in all the cases. This issue will be discussed in detail later.

The rate of sorption at 10% RH was also affected by aging (Fig. 4b). The longer the aging time, slower was the rate of sorption. It is recognized that the rate of sorption may be influenced by the surface area of the sample. However aging did not have any discernible effect on particle size and morphology. Thus, the observed differences are attributed to relaxation. A decrease in rate of sorption following aging has been observed in polymer systems. The rate of benzene sorption on PVC decreased with physical aging, and was attributed to a decrease in free volume (5).

It is evident from Fig. 4a that at RH ≥ 30%, the water sorption behavior of amorphous trehalose was independent of its thermal history. In other words, exposure to these RH values seems to remove the effects of aging. The most common method for the removal of thermal history of a glass is to transform it to a rubber. It can be done either by heating the sample to a temperature above T_g or by plasticizing the system and lowering the T_g below the experimental temperature ("plasticization theory"). Because the T_g of dry trehalose is 117°C, which is ~90°C above the room temperature, plasticization is the likely mechanism. By extrapolating the published results on the water sorption behavior of amorphous trehalose, the amount of water required for lowering the T_g to 25°C was calculated to be ~9% w/w (25). From Fig. 4a, it is evident that such a high amount of water was sorbed only following storage at ~40% RH. However, water sorption of 6.4% w/w at 30% RH (calculated T_g = 64°C) was sufficient to completely remove the effects of aging (Table II). In light of this discrepancy, it may not be appropriate to invoke the plasticization theory to explain our observations. This was confirmed by investigating the system in greater detail.

The shape of the water sorption profile revealed interesting information. As pointed out earlier, the water sorption profile of the unaged sample was quite linear, while a considerable curvature was observed in case of the aged samples (Fig. 4a). With an increase in RH, the sorption profiles of the aged samples gradually shifted toward that of the unaged sample. Let us assume that water sorption at a certain RH lowered the T_g of the aged sample to the experimental temperature. If this plasticization theory were true, the system being in the aged glassy state below this RH would sorb only a small amount of water. Above this RH, because the material is in the rubbery state, a significantly higher water sorption will be expected. In other words, the system will exhibit an abrupt change in its water sorption behavior at this RH value analogous to the enthalpic recovery of an aged material at T_g. At and above this RH value, the sorption profiles of aged and the unaged materials would become identical. However, experimentally, the shift in the sorption profile of the aged material toward that of the unaged material was not abrupt and occurred gradually with increase in RH. This observation leads to the interesting possibility that the reversal of physical aging by water sorption might be gradual and continuous and not an abrupt change associated with glass to rubber transition.

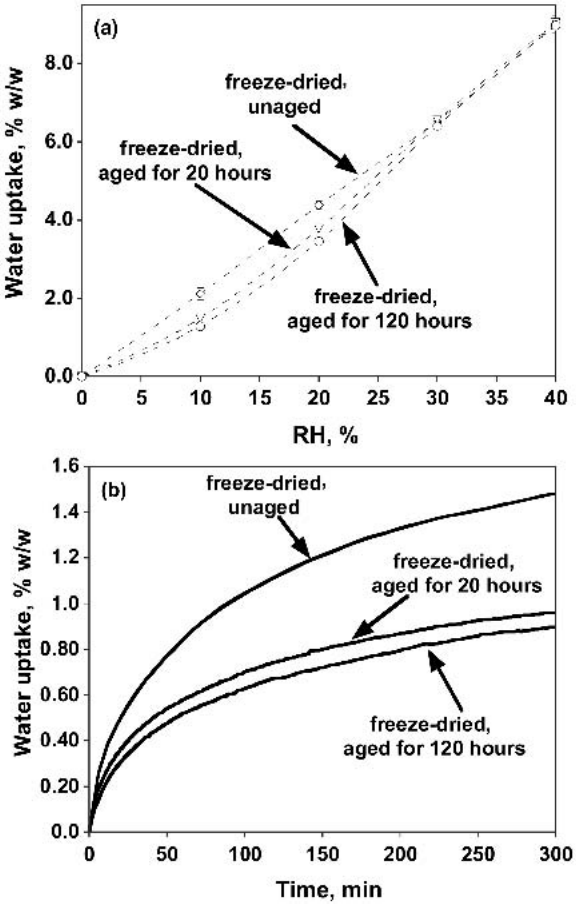
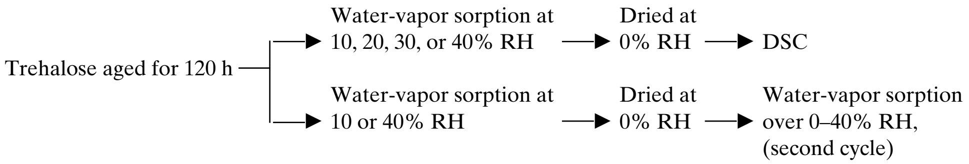


Fig. 4. Water vapor sorption behavior of unaged and aged amorphous trehalose at 25°C. (a) Extent of uptake in the RH range of 0–40%. For selected data points, mean ± SD (n = 3) are provided. In some instances, the error bars are smaller than the size of the symbol. (b) Kinetics of uptake at 10% RH at initial time points. Time to equilibrium has not been shown.

Table II. The Amount of Water Sorbed and the T_g of Aged Trehalose (at 100°C for 120 h) After Exposure to Different RH Conditions

	Sorbed sample		Dried at 0% RH	
RH (%)	Water sorbed (% w/w)	Plasticized T_g* (°C)	Enthalpic recovery† (J/g)	T_g† (°C)
0	0	124	8.3	123.7
10	1.3	110	6.5	121.7
20	3.4	89	2.2	118.5
30	6.4	64	0.4	117.3
40	9.0	45	0.4	117.0

The last two columns show the enthalpic recovery and T_g, determined by DSC, after drying at 0% RH.
* Calculated using the Gordon-Taylor equation.
† Determined by DSC, at 10°C/min, following drying.



Scheme 1. Experimental design to evaluate the effect of sorption-desorption pretreatment cycle on the enthalpic recovery and water-sorption behavior of trehalose aged for 120 h.

In order to investigate this proposition, the effect of sorption-desorption pretreatment on the relaxation state of the sample aged for 120 h was studied. Scheme 1 illustrates the experimental design. In the first set of experiments, the samples were equilibrated at 10, 20, 30, or 40% RH in the vapor sorption balance, dried, and the enthalpic recovery was determined (Table II). There was a gradual decrease in the T_g as well as enthalpic recovery as a function of the RH of sorption cycling (Table II). This effect became more pronounced with an increase in the cycling RH, and following exposure to 30% or 40% RH, the behavior was nearly identical to that of the unaged material (Tables I and II and Fig. 5). These results clearly indicated that the reversal of physical aging effects occurred gradually with an increase in the RH of cycling.

The next question is: Is this reversal of physical aging manifested in the water sorption behavior of the cycled sample? If relaxation (due to aging) leads to decreased interaction with water, a gradual reversal of aging effects should progressively restore the glass-water interaction. In the second set of experiments (Scheme 1), the sorption behavior was investigated following sorption cycling at two extreme RH values of 10% and 40%. Cycling through 40% RH caused a pronounced increase in the amount of water sorbed when compared with the uncycled trehalose (freeze-dried, aged for 120 h). The sorption profile following this cycling was nearly identical to that of the unaged sample (Fig. 5a). This indicated that all the effects of physical aging were lost following this cycling treatment. The effect of cycling through 10% RH, though readily discernible, was much less pronounced (Fig. 5a). The water sorption and DSC results are in excellent agreement. While cycling through 40% RH resulted in a very low enthalpic recovery reflecting complete reversal of the aging effect, there was only partial reversal following exposure to 10% RH (Fig. 5a; Table II). Our results so far have been based on the extent of water sorption (Fig. 5a). The same conclusions can be drawn based on the rate of water sorption (Fig. 5b).

As mentioned earlier, plasticization is an unlikely explanation for this behavior, as reversal of physical aging occurred even when the T_g was not lowered below the experimental temperature. Our belief is that the reversal of aging is due to volume expansion during water sorption in the amorphous structure. One of the major consequences of aging is a decrease in free volume and increase in density. If water sorption increases the free volume (as shown schematically in Fig. 6), it can potentially reverse the effect of physical aging. The relaxed matrix, with decreased free volume and increased density, expands due to vapor sorption (Fig. 6c). When the water leaves the system at a low temperature, the matrix does not have enough molecular mobility to relax again and it stays expanded. The state of this resulting matrix (Fig. 6d) is comparable to that of the unaged matrix (Fig. 6a), reflecting removal of the aging effects. The effect of sorption and desorption, investigated in annealed polymers, forms the basis of our postulate (26). Sorption of carbon dioxide by annealed polycarbonate resulted in dilation of the polymer (27). This increased the enthalpy of the polymer with respect to the annealed state. Though desorption led to contraction, the kinetics of desorption and contraction exhibited pronounced differences. Gravimetric studies have revealed that while the sorbed vapor may be readily removed from a polymer pow-

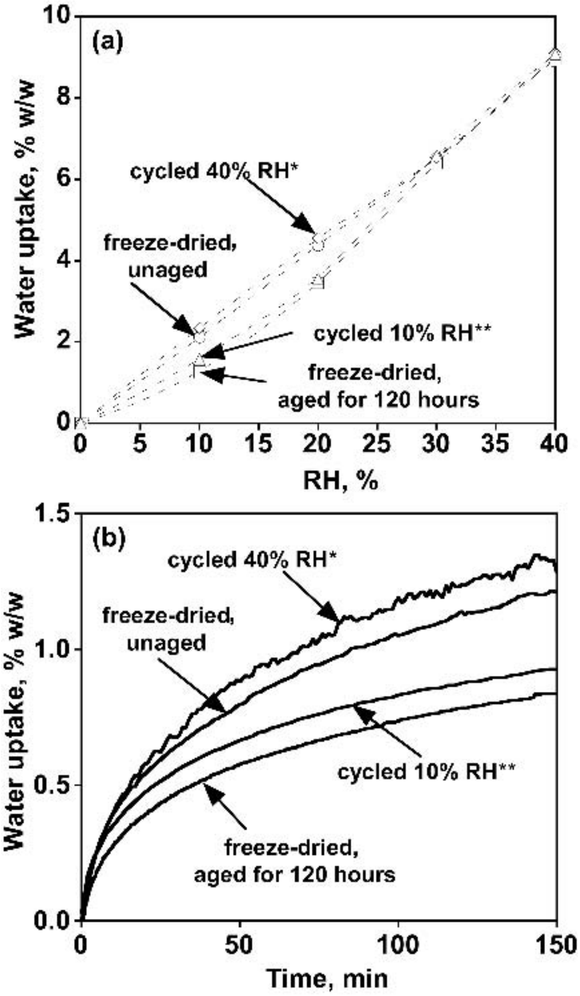


Fig. 5. Water uptake in the aged (for 120 h) samples, before and after sorption cycling. (a) Amount sorbed as a function of RH at 25°C. (b) Rate of water uptake at 10% RH. *Sample pretreated by equilibrating at 40% RH followed by drying at 0% RH. **Sample pretreated by equilibrating at 10% RH followed by drying at 0% RH. The water sorption profiles of the freeze-dried (unaged) samples have also been provided.

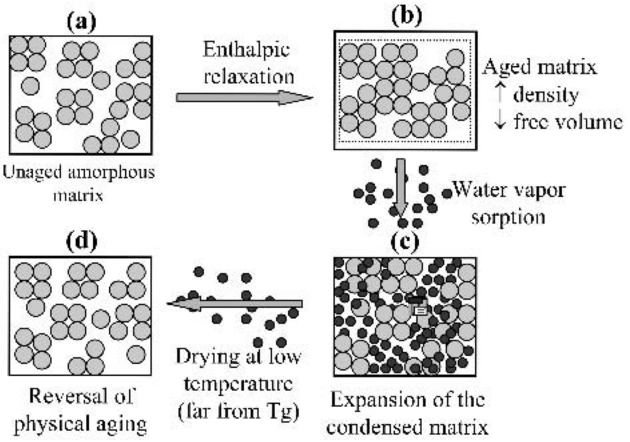


Fig. 6. Schematic representation of the effect of enthalpic relaxation and water vapor sorption on an amorphous matrix. The large and small circles represent the molecules of the amorphous phase and water respectively. The amorphous matrix (a), when aged, undergoes relaxation resulting in a matrix with lower free volume and higher density (b). Water sorption results in matrix expansion (c). When dried at a low temperature, the matrix does not have enough molecular mobility to relax again (d). (*In case of trehalose, for complete reversal, RH ≥ 30% at room temperature; details in the text.)

der, reversal of volume expansion tends to occur much more slowly (28,29).

In order to get a better understanding of the mechanism of this aging reversal, additional sorption-desorption cycling experiments were performed. Three samples of the aged material (120 h) were used. Whereas the first one was subjected to sorption at 10% RH followed by desorption at 0% RH once, the second and third samples were subjected to this cycle twice and thrice, respectively. The cycled samples were subjected to DSC. The enthalpic recovery of the samples cycled once, twice, and three times were 6.5, 5.8, and 5.1 J/g, respectively. The recovery in the sample aged for 120 h and not subjected to sorption cycling was 8.3 J/g (Table I). As shown in Table II, sorption of 1.3% w/w water (10% RH) led to a decrease of about 1.8 J/g in enthalpic recovery, whereas 3.4% w/w water sorption (20% RH) resulted in a decrease of ~6 J/g. If each sorption cycle (through 10% RH) resulted in a 1.8 J/g decrease in enthalpic recovery, the enthalpic recovery of the sample cycled three times (through 10% RH) should have been approximately 2.9 J/g [$8.3 - (1.8 \times 3) = 2.9$]. However, the observed enthalpic recovery of 5.1 J/g was much higher indicating that the second and third cycling had much less pronounced effect than the first cycle. According to the volume expansion theory, if the water-solid interaction is unchanged by repeated sorption-desorption cycling, then the second and subsequent cycles should have no effect on the enthalpic recovery. While first cycle had a pronounced effect, there was a further decrease in recovery (albeit small) following the second and third cycles. In light of the complex nature of the water-solid interaction, this issue can only be understood with more detailed investigation.

From Fig. 1, it is evident that the relaxation has similar effects on the volume and enthalpy of the amorphous phase. The aging reversal by water sorption, which has been explained as being due to volume expansion (Fig. 6), can also be understood in terms of changes in enthalpy. Though the enthalpy of the dry relaxed trehalose is less than that of the unrelaxed material, following water sorption at and above 30% RH, their enthalpy values will be the same. This can be explained by a lower enthalpy of sorption for the aged trehalose.

Implications on Processing of Organic Amorphous Materials

It is the general consensus that the effects of physical aging of a glass can be removed by transforming it to the rubbery state. This is conventionally accomplished by heating above the glass transition temperature. Our work demonstrates that it is possible to remove the effects of physical aging while retaining the material in the glassy state by controlled water sorption followed by desorption. A complicating factor is that the amount of water sorbed by the glass is a function of its relaxation state. Thus, there is a complex interplay between the relaxation state of a glass and its interaction with water. While aging causes a decrease in the amount of water sorbed, the sorbed water reverses the effect of aging. These observations have numerous implications, particularly in the pharmaceutical arena.

1) The handling of amorphous materials becomes difficult in light of their strong tendency to sorb water. In addition to the possible consequences of water sorption discussed earlier, any interaction with water during handling can also lead to the reversal of aging effects.

2) An aged material when compared with its unrelaxed counterpart is characterized by decreased physical and chemical reactivity. This decreased reactivity is brought about by the increase in structural relaxation time due to aging. Water vapor sorption, by removing the effects of physical aging may increase the molecular mobility thus increasing the possibility of physical and chemical changes.

3) Water vapor sorption has been recognized to be an extremely sensitive indicator of the crystallinity in pharmaceuticals (30). Because aging affects the rate and extent of water sorption, quantitative crystallinity evaluation by this technique will be meaningful only when there is precise control of the sample thermal history.

CONCLUSIONS

The thermal history of amorphous trehalose was varied by aging it below T_g for different time periods. The water sorption and crystallization behavior were studied as a function of aging time. Aging below T_g caused nucleation leading to a decrease in the crystallization temperature. However, crystallization did not occur below the T_g even after prolonged aging. Aging caused a decrease in the rate and extent of water sorption but only at low RH. More importantly, the effects of aging were reversed, partially or completely, by controlled water sorption. The reversal is believed to be due to volume expansion during water sorption in the amorphous structure. Though heating above T_g is the conventional approach for erasing thermal history, the water sorption-desorption method enables the same outcome without going through the glass transition process.

ACKNOWLEDGMENTS

We thank Dr. Evgenyi Y. Shalaev for his insightful comments. Rahul Surana was partially funded by a USP Fellowship, Novartis Fellowship, and ISWOP, University of Minnesota. Abira Pyne was partially funded by a grant from PDA and ISWOP.

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