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Phase Behavior of Amorphous Molecular Dispersions I: Determination of the Degree and Mechanism of Solid Solubility

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Purpose. To understand the phase behavior and the degree and mechanism of the solid solubility in amorphous molecular dispersions by the use of thermal analysis.

Methods. Amorphous molecular dispersions of trehalose-dextran and trehalose-PVP were prepared by co-lyophilization. The mixtures were exposed to 23°C, 40°C, and 50°C [75% relative humidity (RH)] and 23°C (69% RH) storage conditions, respectively. Thermal analysis was conducted by modulated differential scanning calorimeter (MDSC).

Results. Upon exposure to moisture, two glass transition temperatures (T_{gS}), one for phase-separated amorphous trehalose ($\mathbf{T_{g1}}$) and the other for polymer-trehalose mixture ($\mathbf{T_{g2}}$), were observed. With time, $\mathbf{T_{g2}}$ increased and reached to a plateau ($\mathbf{T_g^{eq}}$), whereas $\mathbf{T_{g1}}$ disappeared. The disappearance of $\mathbf{T_{g1}}$ was attributed to crystallization of the phase-separated amorphous trehalose. It was observed that $\mathbf{T_g^{eq}}$ was always less than T_g of pure polymer. The lower $\mathbf{T_g^{eq}}$ when compared to T_g of pure polymer may be the result of solubility of a fraction of trehalose in the polymers chosen. The miscible fraction of trehalose was estimated to be 12% and 18% wt/wt in dextran at 50°C/75% RH and 23°C/75% RH, respectively, and 10% wt/wt in PVP at 23°C/69% RH.

Conclusions. Mixing behavior of trehalose-dextran and trehalose-PVP dispersions were examined both experimentally and theoretically. A method determining the "extent of molecular miscibility," referred to as "solid solubility," was developed and mechanistically and thermodynamically analyzed. Solid dispersions prepared at trehalose concentrations below the "solid solubility limit" were physically stable even under accelerated stability conditions.

KEY WORDS: glass transition; miscibility; phase separation; solid dispersions; thermal analysis.

INTRODUCTION

In the pharmaceutical literature, publications on the use of solid dispersions to enhance the dissolution rate and bioavailability of poorly water-soluble compounds are in abundance (1). However, despite the extensive R&D efforts only a few solid dispersion products are commercially available. One of the reasons for this rarity is the thermodynamic instability of amorphous active component in dispersed systems (2,3). Although recrystallization of amorphous drugs from solid dispersions is reported in several publications, the exact mechanism of such recrystallization is not clear. The investigators, who extensively evaluated the physical stability of amorphous materials, attributed the recrystallization phenomenon mainly to the molecular motions of amorphous components even below the glass transition temperature (T_g) (4–8).

It is generally understood that polymers improve the physical stability of amorphous drugs in solid dispersions by either increasing the T_g of the miscible mixture, thereby reducing the molecular mobility at regular storage temperatures, or specifically interacting (e.g., hydrogen bonding) with functional groups of amorphous drug substance (9–11). For a polymer to be effective in preventing crystallization by these mechanisms, it has to be molecularly miscible with the amorphous substance. For complete miscibility, interactions between the two components are necessary (12). A miscible system can phase separate and become unstable if specific interactions between the components are adversely affected by a third component like water. The extent of moisture uptake depends upon factors like hygroscopicity of the drug and/or carrier, the concentration of the active substance in the drug product and the storage temperature. Due to this complexity, the prediction of physical stability of a drug substance in a solid dispersion becomes challenging. In order that the solid dispersion systems can be effectively used by the pharmaceutical industry, it is critical to understand the role of time, temperature, and humidity in determination of the physical stability of solid dispersion during its shelf-life.

The objective of this study was two-fold: To study the phase behavior of the selected solid dispersions via thermal analysis, in order to determine the extent of solid state miscibility under accelerated stability conditions, and to understand the mechanism of physical stability of these amorphous molecular dispersions.

Molecular dispersions of trehalose-dextran and trehalose-poly (vinylpyrrolidone) (PVP) were chosen as models to understand the phase behavior of a crystallizable amorphous substance that was molecularly mixed with a polymer. Trehalose is a well characterized compound (13). Amorphous trehalose, which is highly susceptible to moisture, crystallizes to a stable dihydrate that can be characterized thermally (13). Although hydrogen-bond interactions between trehalose and PVP have been cited (14), the influence of water on these interactions, the phase behavior of the mixtures, and the solid-state miscibility has not yet been demonstrated in the literature.

MATERIALS AND METHODS

Materials

Dextran (polymer containing sucrose having α-1, 6-glucosidic linkage), with M_n of 70 kDa, and trehalose (α-D-glucopyranosyl α-D-glucopyranoside) dihydrate were purchased from Sigma Chemical Co., (St. Louis, MO). Poly (vinylpyrrolidone) (PVP-K29/32), (M_w of 58 kDa) was obtained from ISP Technologies (Wayne, NJ). Sodium chloride (Fisher Chemicals Co., Fair Lawn, NJ), was used to prepare saturated salt solution providing 75% RH conditions.

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Methods

Preparation of Amorphous Mixtures by Lyophilization

Dextran and PVP-K29/32 were dried in a vacuum oven overnight at 105°C until a constant weight was obtained. Trehalose dihydrate was used without any further treatment. Physical mixtures of each polymer and trehalose dihydrate were made in dry glove bags (RH<15%) to obtain weight proportions of trehalose dihydrate in the polymer from 0–100% at 10% wt/wt intervals. These mixtures were dissolved in purified water to obtain 10% wt/vol concentration and stirred overnight at 35°C. Aqueous solutions (2 ml) were freeze-dried using a commercial freeze dryer (Dura-Stop µP, FTS System, Stone Ridge, NY). Vials containing solutions were transferred onto pre-cooled shelves (–45°C). Primary drying was carried out at shelf temperature of –27°C for 22–24 h, followed by drying at –12°C for 2 h, 0°C for 16 h, and secondary drying at 30°C for 20 h. Vacuum pressure of 15 mTorr was applied throughout the drying. All samples were stored over anhydrous calcium sulfate at room temperature, after capping the vials. X-ray analysis of these samples showed that all the mixtures were amorphous.

Isothermal Stability Studies

Lyophilized amorphous mixtures of dextran containing 10%, 20%, 30%, 40%, and 70% by weight of trehalose were accurately weighed (8–12 mg) in standard DSC pans in a glove bag (RH <15%). These pans were placed in desiccators containing saturated sodium chloride solution (75% RH) to expose the miscible mixtures to excess humidity. The desiccators were kept at room temperature (23 ± 1°C), and incubators (Precision Scientific Inc., Chicago, IL) at 40 ± 1°C and 50 ± 1°C. Saturated NaCl solution provides relative humidity of 75 ± 0.5% over the temperature range of 23° to 50°C (15). By storing the samples in discrete DSC pans, the reproducibility of all measurements was ascertained without any interference of sample handling. Samples were taken out for analysis at pre-determined time intervals for a period of up to six months. They were crimped with aluminum lids having 10 pinholes to facilitate removal of absorbed and bound water liberated upon melting of any recrystallized trehalose.

Thermal Analysis

A modulated differential scanning calorimeter (TA Instruments 2920, New Castle, DE), with a liquid nitrogen cooling accessory was used for thermal analysis. The analysis was performed under a purge of dry nitrogen gas (60cc/min). High purity indium and sapphire were used frequently to calibrate for the heat flow and heat capacity of the instrument. Samples were initially cooled to –30°C for 10 min and were heated to 245°C at 1°C/min with modulations of 0.266° every 50 s.

Modulated differential scanning calorimetry (MDSC) offers an advantage over conventional DSC because it can detect reversible glass transition phenomenon in the presence of several other non-reversible relaxation processes. Heat capacity changes can be resolved from moisture removal endotherm, thereby improving the signal to noise ratio and improving the elucidation of the T_g.

RESULTS

Modulated DSC Analysis of Trehalose and Trehalose-Polymers Mixtures

Characterization of Trehalose

DSC scans of trehalose before and after lyophilization are shown in Fig. 1a. A heating rate of 1°C/min was used to minimize polymorphic conversion of trehalose dihydrate during heating (16). Upon heating trehalose dihydrate, an endotherm at 96° ± 1°C (T_h) was observed. This was followed by a T_g at 122°C and recrystallization at 171°C. The recrystallized anhydrous trehalose melted at 212°C. Thermogravimetric analysis (TGA) performed on trehalose dihydrate showed a 9.5% weight loss at 96°C confirming that the endotherm at 96° ± 1°C was due to the removal of water of hydration. Hot-stage microscopy was conducted on trehalose dihydrate by heating at 1°C/min and it was observed that water removal from trehalose resulted in a structural collapse and loss of birefringence (indicating amorphous state). At approximately 135°C, trehalose liquefied and recrystallized to the anhydrate form. These results agreed with DSC characterization and with observations of Taylor and York (13).

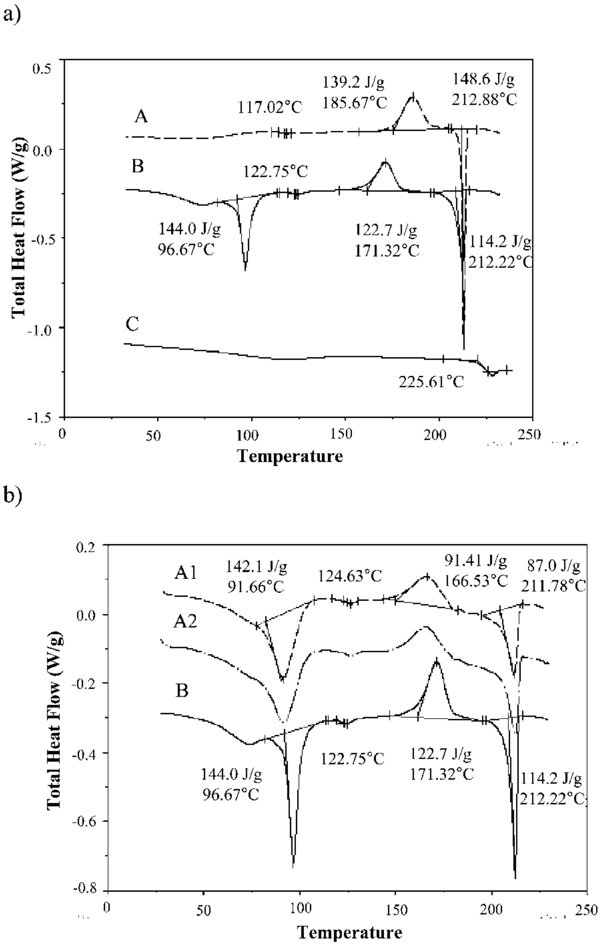

Fig. 1. DSC total heat flow scans of (a) **A**, lyophilized trehalose; **B**, untreated trehalose dihydrate, and **C**, lyophilized dextran; (b) heat flow due to recrystallization of amorphous trehalose by storing it at **A1**, 50°C/75% RH and **A2**, 40°C/75% RH.

Lyophilized trehalose was amorphous as determined by X-ray diffraction. DSC scan of lyophilized trehalose (Fig. 1a) showed no endotherm at 96°C, indicating the absence of crystalline hydrate structure. For verification, amorphous trehalose that was exposed to 50°C and 40°C with 75%RH were heated in DSC and examined. One would expect the amorphous trehalose to crystallize upon storage and indeed as seen in Fig. 1b, the thermograms are similar. In conclusion, the water-loss endotherm observed at 96°C is the characteristic of crystalline trehalose.

Molecular Dispersions of Trehalose and Polymer

All the freshly prepared co-lyophilized mixtures of trehalose-dextran or trehalose-PVP were amorphous exhibiting a composition-dependent single T_g indicating their molecular miscibility. The T_g of the mixtures decreased with increasing concentration of trehalose as shown in Figs. 2a and 2b, indicating the plasticizing effect of trehalose on the polymers. The T_g values were plotted as a function of weight fraction of trehalose in polymer and are shown in Figs. 2c and 2d for the determination of solid solubility.

Physical Stability Studies

MDSC reversing heat flow scans of mixtures containing 40% wt/wt trehalose-in-dextran, which were stored at 50°C/75% RH for a period of 2–50 days are shown in Fig. 3a. A freshly prepared sample showed a single T_g at 169°C, indicating miscibility. Amorphous miscible mixtures when exposed

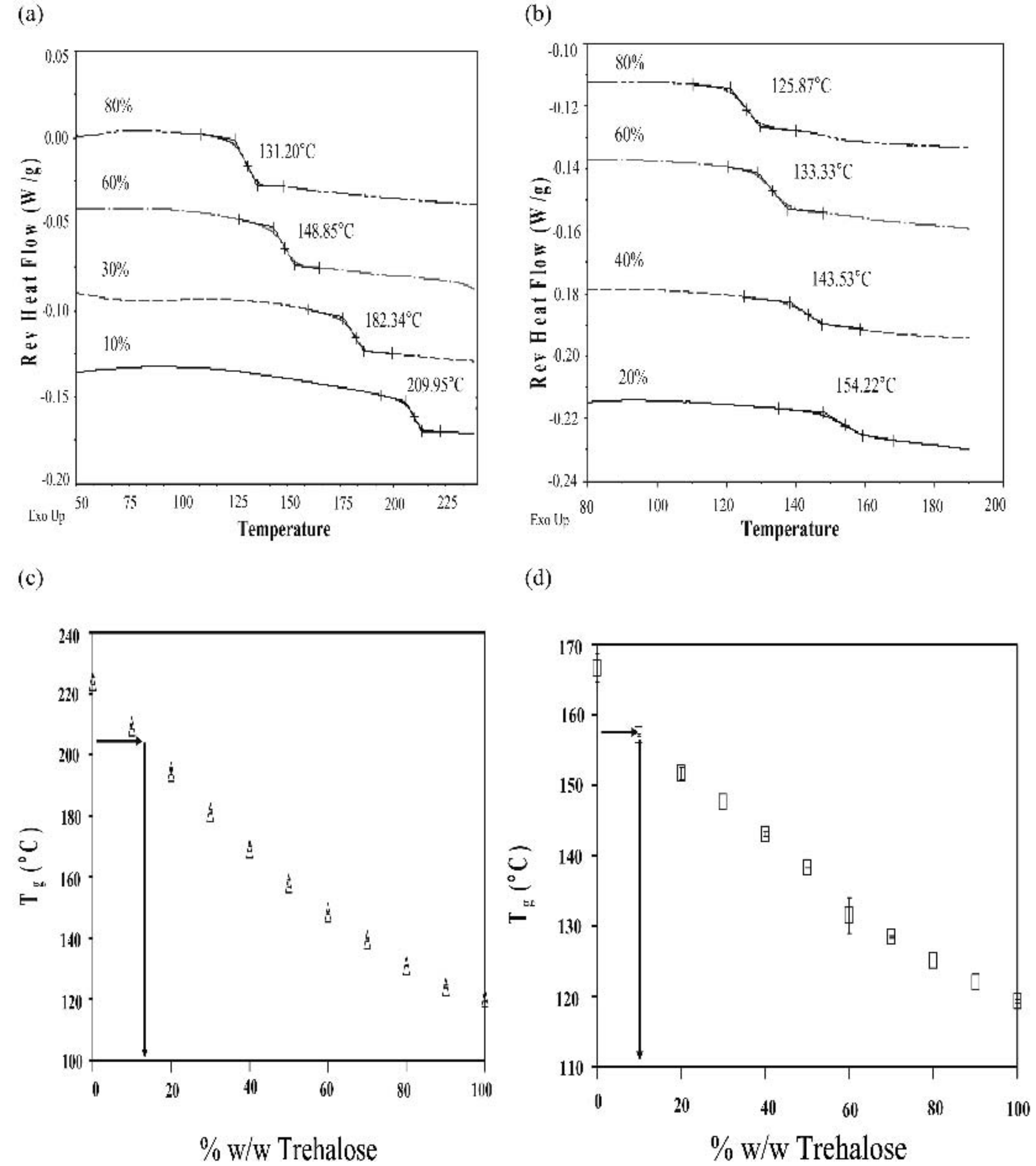

Fig. 2. MDSC reversing heat flow scans of mixtures of trehalose with (a) dextran and (b) PVP, showing a composition dependent single T_g. Plots of T_g of mixture of trehalose with (c) dextran and (d) PVP as function of trehalose composition. Arrows represent the solid-solubility limits of trehalose in the polymers as explained in the text.

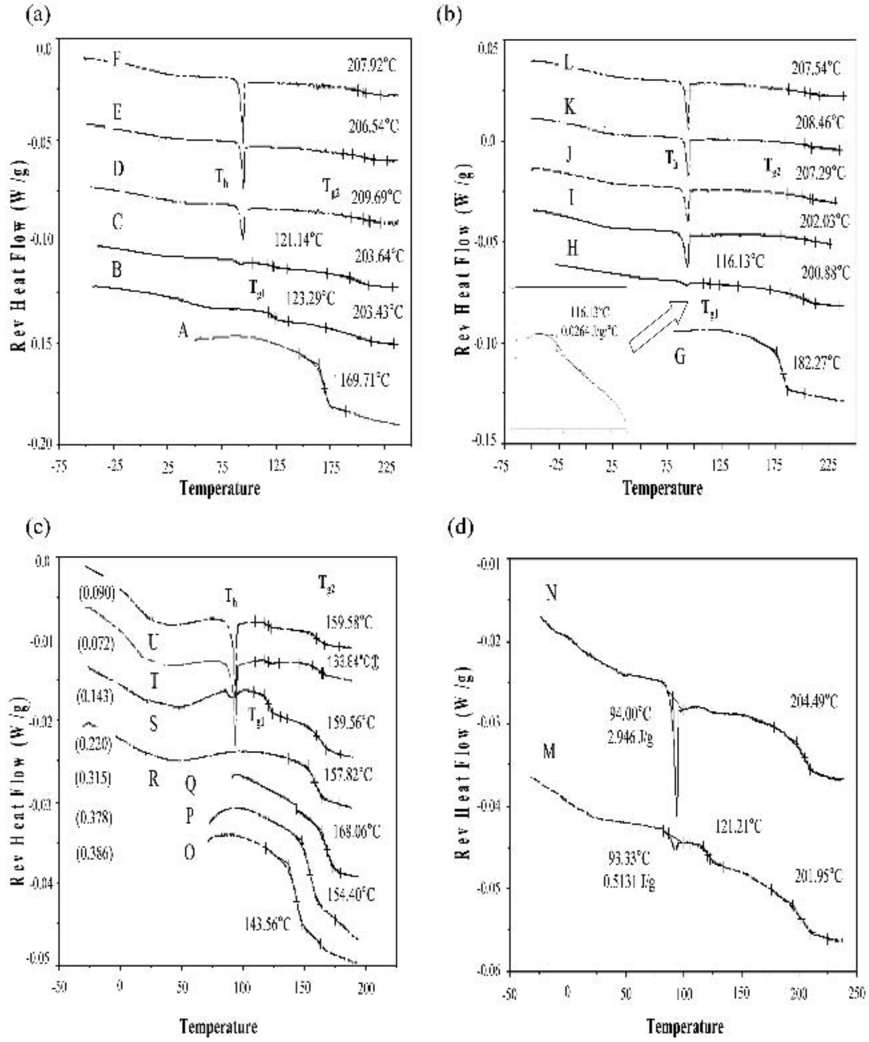

Fig. 3. MDSC reversing heat flow scans of trehalose-dextran mixtures exposed to 50°C/75% RH conditions for different time intervals: (a) 40% wt/wt trehalose (**A**: 0 days, **B**: 2 days, **C**: 4 days, **D**:13 days, **E**: 23 days, **F**: 34 days), (b) 30% wt/wt trehalose (**G**: 0 days, **H**: 5 days, **I**: 8 days, **J**: 19 days, **K**: 31 days, **L**: 50 days), (c) trehalose-in-PVP stored at 23°C/69% RH (**O**: 40% wt/wt, 0 days; **P**: 20% wt/wt, 0 days; **Q**: 0% wt/wt, 0 days; **R**: 20% wt/wt, 200 days; **S**: 40% wt/wt, 190 days; **T**: 40% wt/wt, 195 days; **U**: 40% wt/wt, 200 days), and (d) 40% wt/wt, trehalose at **M**: 4 days, **N**: 34 days [$\mathbf{T_{g1}}$: T_g of phase separated amorphous trehalose; $\mathbf{T_{g2}}$: T_g of polymer-trehalose mixture; $\mathbf{T_h}$: water-loss endotherm of crystalline trehalose. The numbers within the brackets in Fig. 3c represents the change in heat capacity (ΔC_p) at $\mathbf{T_{g2}}$].

to 50°C/ 75% RH, absorbed moisture and the plasticized T_g of such a system, from the total heat flow scan (data not shown), was detected at 22° ± 3°C. The absorbed moisture was removed from the mixtures as the samples were heated in MDSC. As opposed to a single T_g of 169°C in the original mixture, two distinct T_gs were observed in the 2- and 4-day-old samples; T_g values at 123° and 121°C (referred to as $\mathbf{T_{g1}}$), and T_g at 203°C ($\mathbf{T_{g2}}$). In the 16–34 day samples, $\mathbf{T_{g1}}$ was not observed. However a water-loss endotherm at 96° ±1°C ($\mathbf{T_h}$), characteristic of crystalline trehalose; had appeared. In addition, $\mathbf{T_{g2}}$ values had increased and seemed to plateau, which is termed as equilibrium T_g ($\mathbf{T_g^{eq}}$). There was also a distinct reduction in the heat capacity step change (ΔC_p) at $\mathbf{T_{g2}}$. These values decreased initially and seemed to reach a plateau as seen from data in Table I. The thermal events in dextran mixtures containing 30% wt/wt trehalose were also similar to those that were observed for 40% wt/wt mixtures when stored at 50°C/75% RH (Fig. 3b).

MDSC reversing heat flow scans of mixtures containing 20% and 40% wt/wt trehalose-in-PVP which were stored at 23°C/ 69% RH are shown in Fig. 3c. With this polymer, the phase separation of amorphous trehalose was observed ($\mathbf{T_{g1}}$ at 120°C) in the 190-, 195-, and 200-day-old samples. A water-loss endotherm at 96° ± 1°C ($\mathbf{T_h}$) was also observed. For 20% wt/wt samples, the $\mathbf{T_{g1}}$ and $\mathbf{T_h}$ were not noticeable even after 200 days of storage. A reduction in ΔC_p values at $\mathbf{T_{g2}}$ (given within brackets in Fig. 3c) followed by a plateau was noticed in these systems as well. The heat flow scans that are depicted in Fig. 3d are to demonstrate the presence of $\mathbf{T_{g1}}$ and $\mathbf{T_{g2}}$ values for some selected mixtures.

A plot of $\mathbf{T_{g2}}$ values of dextran mixtures containing different weight fraction of trehalose, stored at 50°C, 40°C, and

Table I. Effect of Storage Conditions on Thermal Behavior of Selected Trehalose-Dextran Mixtures

Storage condition	Percent trehalose (wt/wt)	Storage period (days)	T_h* (J/g)	T_{g2}† (°C)	ΔC_p‡ at T_{g2} (J/g/°C)
50°C/75% RH	40	0		169	0.3727
		2		203	0.1710
		4		203	0.1564
		13	17.7	209	0.1095
		23	28.6	206	0.1189
		34	34.9	207	0.0981
	30	0		182	0.3329
		5		200	0.2428
		8	12.9	202	0.1314
		19	13.8	207	0.1214
		31	14.7	208	0.1176
		50	15.1	207	0.1055
40°C/75% RH	40	0		169	0.3727
		6	7.6	196	0.1807
		13	23.9	213	0.1076
		23	27.0	207	0.1136
		34	30.6	207	0.0741
	30	0		182	0.3329
		8	6.2	192	0.1579
		19	9.5	202	0.0971
		31	12.2	206	0.1913
		52	12.5	203	0.1192
		90	17.9	205	0.1620

* Heat of fusion of water-loss-endotherm for recrystallized trehalose dihydrate.

† Glass transition temperature of polymer-trehalose mixtures.

‡ Heat capacity change.

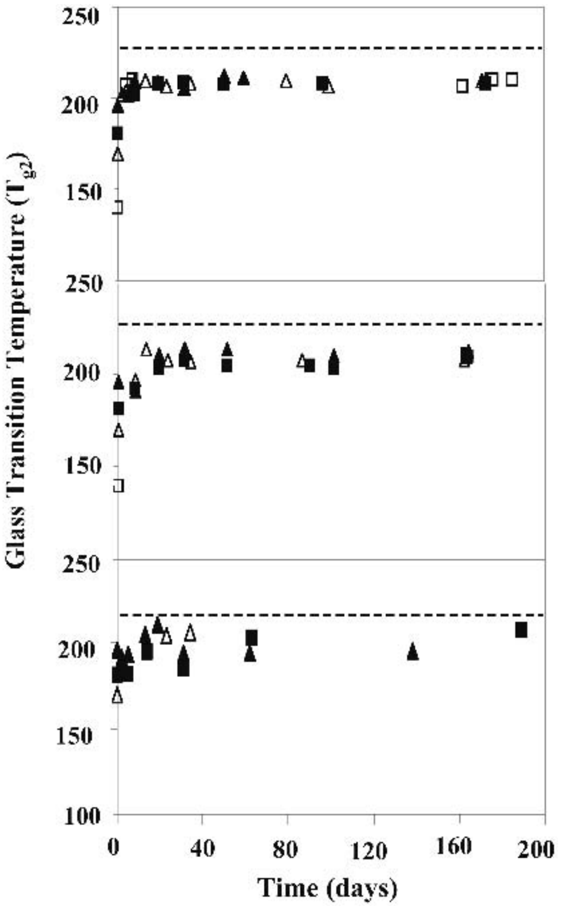

Fig. 4. Plot of T_{g2} of trehalose-dextran mixtures: 70% wt/wt (□), 40% wt/wt (△), 30% wt/wt (■), and 20% wt/wt trehalose (▲) at (a) 50°C/75% RH; (b) 40°C/75% RH; (c) 23°C/75% RH. Dotted line indicates the T_g of pure dextran.

23°C with 75% RH is shown in Fig. 4. It should be noted from the figure that $\mathbf{T_g^{eq}}$ is always less than 225°C, which is the T_g of pure amorphous dextran (dotted line). This reduction in T_g indicated that a fraction of trehalose remained miscible with the polymer even after being exposed to excessive temperature and humidity conditions for up to six months. This fraction was identified as the "solid solubility" of trehalose in dextran. The $\mathbf{T_g^{eq}}$ of all amorphous mixtures ranged from 207°C to 210°C, when they were stored at 40°C and 50°C with 75% RH, and within 196° C to 203°C for mixtures stored at 23°C/75% RH conditions. These findings suggested that, at equilibrium, irrespective of the initial trehalose-concentrations, the solid solubility limit of trehalose in dextran was the same for a specified storage condition. For instance, when stored at 50°C/75% RH, 70% wt/wt trehalose mixture which was initially miscible with dextran, formed a dihydrate and demonstrated $\mathbf{T_{g2}}$ values at 207°C to 210°C upon "equilibrium." This concentration was accepted as its solid solubility limit.

To exclude the possible interference of moisture on reduction of $\mathbf{T_{g2}}$, amorphous dextran that was equilibrated at 50°C/75% RH for a month and contained 11% wt/wt water was heated in MDSC. The T_g of the sample was 225°C, which was identical to the T_g of the original sample with 3% wt/wt water content. These findings proved that moisture was not the cause of $\mathbf{T_{g2}}$ reduction.

On the other hand, trehalose that was liquefied above its T_g of 118°C could have plasticized dextran and lowered the T_g below 225°C. Such a possibility was suggested by Six *et al.* (17) for HPMC- itraconazole dispersions. The MDSC scan of 1:1 physical mixture of amorphous trehalose and dextran, demonstrated T_gs at 119°C ± 0.5°C and 223°C ± 0.9°C (n = 3) upon heating at 1°C/min rate. The two T_g values coincided with the T_gs of pure trehalose and dextran, respectively indicating that heating in MDSC did not cause any mixing. The reduction in T_{g2} values from 225°C to T_g^{eq}, is indicating the presence of the miscible fraction of trehalose in the polymer.

Crystallization of Trehalose

A plot of percent crystallinity of trehalose from the miscible mixtures containing 40% and 30% wt/wt trehalose as a function of storage time is shown in Fig. 5. The amount of trehalose crystallized as the dihydrate was calculated from the heat of fusion under the water-loss-endotherm ($\mathbf{T_h}$) of the total heat flow scan. Amorphous trehalose by itself when exposed to 75% RH had enthalpy of fusion equal to untreated crystalline trehalose dihydrate, indicating 100% crystallization (Fig. 1b). On the other hand, solid dispersion samples showed a maximum of 84% crystallization for samples stored at 50°C/75% RH for 6 months. It was also seen that the rate of crystallization, after an initial rapid increase, was much less in 30%wt/wt dispersions when compared to 40% wt/wt dispersions. These findings are consistent with results which sug-

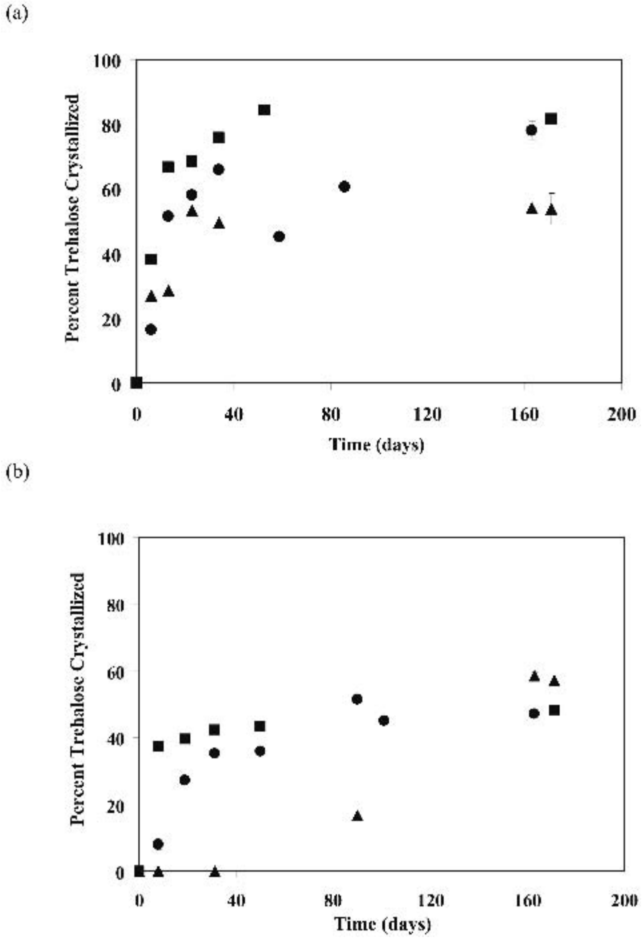

Fig. 5. Percent crystalline trehalose dihydrate present in trehalose-dextran mixtures containing (a) 40% wt/wt trehalose and (b) 30% wt/wt trehalose under storage conditions: 50°C/75% RH (■), 40°C/75% RH (●), and 23°C/75% RH (▲). Error bars represent the range for (n = 3) measurements. The percent crystallinity was calculated from the heat of fusion values of water-loss endotherm of the total heat flow and has been adjusted for the differences in initial trehalose content.

gest that a fraction of trehalose remains miscible with the polymer.

Quantifying the Limit of Solid Solubility

To determine the molecularly dispersed trehalose concentration in the polymer, the plot of T_g of the freshly prepared mixtures against the % wt/wt trehalose concentration was used, (Figs. 2c and 2d). In this graph, the $\mathbf{T_g^{eq}}$ values were used to determine the fraction of trehalose that remained miscible with dextran (Fig. 2c) and PVP (Fig. 2d). In these graphs, the solubility limits are shown by arrows. The corresponding value on the x-axis represented the solid solubility of trehalose in the polymer. Table II demonstrates the solid solubility limits of trehalose in dextran at different storage conditions**.** In this table the difference between the storage temperature and the plasticized T_g due to presence of moisture (T_g^w) is shown as ΔT_g. It is important to show that, the samples were stored at temperatures above their plasticized T_g. At this region, the system has a high degree of molecular mobility and consequently, a higher rate of destabilization. The solid solubility of trehalose-in-dextran decreased from 18% wt/wt at 23°C to 12% wt/wt at 50°C, whereas the total water content decreased from 16.5% wt/wt to 11% wt/wt. However, no appreciable difference in solid solubility was observed between 40°C and 50°C storage conditions, although total water content decreased from 13% to 11%.

DISCUSSIONS

Mechanism of Phase Separation and Solid Solubility

The hypothesized mechanism for phase separation and solid solubility is depicted schematically in Fig. 6 for a dextran-trehalose model. The repeat units of dextran and trehalose are labeled as 1D, 2D, and 1T, 2T, and so forth, respectively.

In the absence of moisture, freshly prepared mixtures have high T_g values and hence the molecular mobility is very low at the storage temperatures. Trehalose, therefore, remains in a kinetically frozen state of miscibility. Upon exposure to moisture, the mixtures are plasticized, and the molecular mobility increases. The exact role of water is not clear yet. Water may either weaken the H- bond interaction (between –OH of trehalose and –OH or –O- groups in dextran, or C=O groups in PVP) by bridging with polymer and trehalose structural units, or may merely increase the molecular mobility by plasticizing the mixtures. In either case, diffusion of trehalose through the polymeric matrix could result in separation of trehalose into an amorphous phase, and subsequent crystallization. As more and more trehalose phase separates, increasing amounts of "free" polymer units are left to interact with the remaining trehalose. Such units possibly orient around and arrest the non-diffused trehalose molecules

Table II. Solid Solubility of Trehalose in the Polymers Used as a Function of Storage Condition

Polymer	Storage condition (°C/% RH)	Water content at equilibrium* (%wt/wt)	$(T_{storage} - T_g^w) = \Delta T_g$§ (°C)	T_g^{eq}† (°C)	Solid solubility‡ (% wt/wt trehalose)
Dextran	23/75	16.5% ± 1.0	1	197.8 ± 4.4	18 ± 2.9
	40/75	13% ± 1.0	18	208.7 ± 3.8	12 ± 2.5
	50/75	11% ± 0.8	28	208.3 ± 1.0	12 ± 0.7
PVP	23/69	19.9 ± 1.1	5–15	158 ± 2.1	10 ± 2.5

* Determined by thermogravimetric analysis by heating the sample at 5°C/min.

§ ΔT_g is the difference between storage temperature and the plasticized T_g due to presence of moisture (T_g^w), when the samples were exposed to stability conditions.

† Mean value of $\mathbf{T_{g2}}$ (i.e., T_g of polymer-trehalose mixture) after $\mathbf{T_{g2}}$ reaches a steady state.

‡ Calculation of solid solubility limit is described in Figs. 2c and 2d and the text.

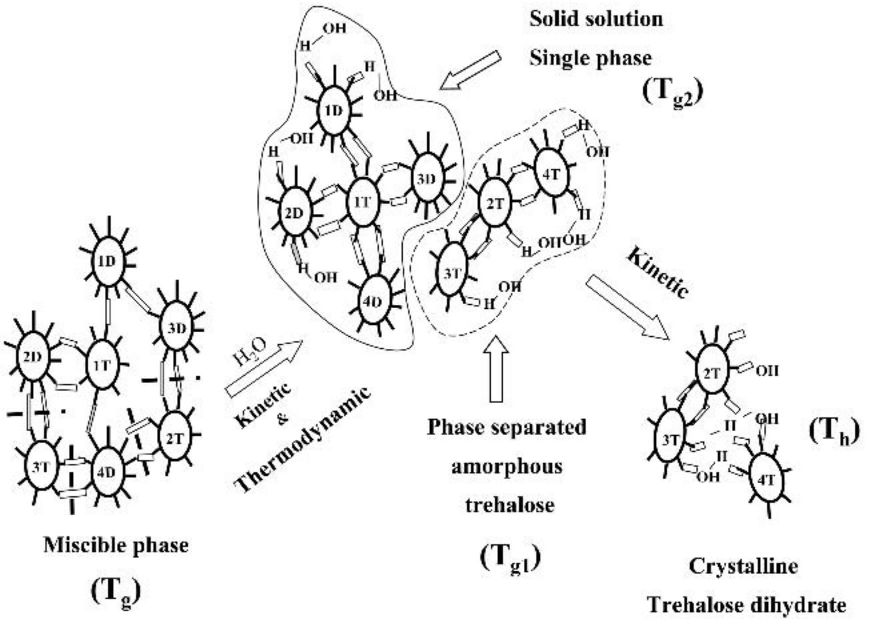

Fig. 6. Hypothetical depiction of solid solubility between trehalose (1T, 2T, 3T, 4T) and dextran (repeat units: 1D, 2D, 3D, 4D). Bridging cylinders represent the hydrogen bonds between the acceptor and donor groups of the components.

by satisfying their H-bond requirements (1T, in Fig. 6). At equilibrium, localized pockets of trehalose molecules are almost entirely bonded to the polymer, reaching solid solubility as characterized by $\mathbf{T_{g2.}}$

Thermodynamic Interpretation of Solid Solubility

To analyze the solid-solubility of trehalose in the polymers thermodynamically, the energetics of interactions between trehalose and polymers was calculated using the Couchman & Karasz theoretical equations (18). Similar approach was used by Shamblin *et al.* to determine the excess thermodynamic properties of mixing in the binary mixtures (19). Using the equations described in detail in the appendix, the enthalpy (ΔH_{mix}) and free energy (ΔG_{mix}) of mixing as a function of trehalose weight fraction were calculated and plotted in Fig. 7.

Since the influence of water on the free energy of mixing is not understood, water was not taken into consideration in the thermodynamic analysis. The enthalpy of mixing (ΔH_{mix}) shown in Fig. 7 were positive for the entire composition range and were similar in magnitude to those reported for mixtures of sucrose-PVP or sucrose-dextran (19). The positive ΔH_{mix} at all compositions indicates that greater numbers of H-bonds are broken between trehalose-trehalose and polymer-polymer than those that are formed between trehalose-polymer during mixing. Since lesser number of bonds was formed between trehalose and polymer when compared to the bonds that they had in their individual states, the positive ΔH_{mix} would favor trehalose and polymer to self-associate or phase separate. The excess entropy of mixtures was positive (not shown) throughout the trehalose composition favoring mixing. The negative free energy values for low trehalose-containing mixtures are thus driven by the excess entropy. Although the dextran-trehalose mixtures studied contained moisture, and a three-phase system could be significantly different from the two-phase system, the analysis conducted supports the hypothesis that a component dispersed in a solid dispersion with certain solubility can be thermodynamically favored.

Mechanism of Crystal Growth

Upon phase-separation, amorphous trehalose, as characterized by $\mathbf{T_{g1}}$ crystallizes in the presence of water, and is

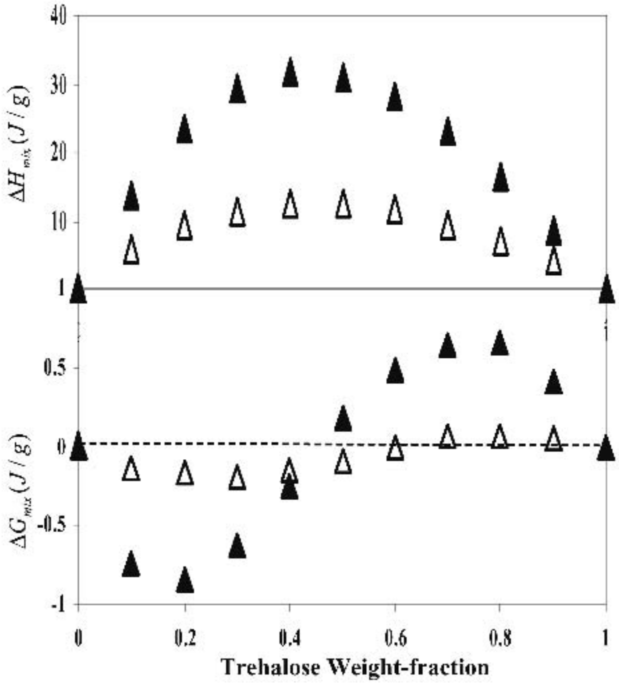

Fig. 7. Enthalpy of mixing (ΔH_{mix}) and free energy of mixing (ΔG_{mix}) of trehalose with dextran (▲) and PVP (△). The broken line indicates zero ΔG_{mix}.

characterized by **T$_h$** (Fig. 3). The crystallization is dependent upon the initial concentration of trehalose in the mixture and the storage conditions used (Fig. 5).

The portion of polymer located at the amorphous-crystalline interface has low molecular mobility and is termed as "rigid amorphous fraction" as suggested by Lee and Kim (20). This fraction does not contribute significantly to the heat capacity step change values at **T$_{g2}$** (ΔC_p) (20–22). The fraction of amorphous bulk distant from crystalline region is the "mobile amorphous phase" and could be responsible for the ΔC_p at **T$_{g2}$** as indicated by Craig *et al.* (21). As the phase separated trehalose crystallizes, the fraction of polymer in close contact with the crystalline surface increases. This explains for the reduction in the ΔC_p values at **T$_{g2}$** (Table I), until no more "rigid amorphous fraction" is formed.

Formation of rigid amorphous fraction may also explain the decrease in the rate and extent of crystallization in 30% and 40% wt/wt mixtures with time (Fig. 5). As suggested by Crowley and Zografi (23), the rigid amorphous fraction impedes the kinetics of crystallization of trehalose that has already phase separated. This kinetic phenomenon is different from the solid solubility, which is characterized by **T$_{g2}$**, as described in the previous section.

Effect of Temperature and Moisture on Solid Solubility

Table II demonstrates the effect of storage temperature and moisture content on the solid solubility of trehalose. Reduction of solid solubility limit with an increase in the temperature is probably due to the higher molecular mobility of the system at elevated temperatures, leading to an enhanced phase separation and crystallization. In addition, at 50°C, thermal expansion of the mixtures may reduce the degree of interaction between the sugar and polymer thereby decreasing the miscibility limit as suggested by Tang *et al.* (24). Finally, since the miscible mixtures have a higher enthalpy and free energy, it is not surprising to see that at higher temperatures, the system proceeds for de-mixing causing trehalose to crystallize. Although with an increase in temperature from 23°C to 40°C solubility reduction was seen, no difference in solubility was noticed between 40°C and 50°C. This is possibly due to an associated reduction in water content, where the reduced water level compensates the temperature effects. Therefore, water seems to have an indirect effect on **T$_{g2}$** reduction and hence solid solubility. Overall, the data obtained suggest that both temperature and moisture affect the phase behavior and extent of solid solubility.

CONCLUSIONS

In this paper, we have shown application of MDSC in studying the phase separation and crystallization behavior of trehalose from its amorphous miscible mixtures, at ambient temperature and accelerated stability conditions. Upon exposure to moisture, trehalose separated as an intermediate amorphous phase which later crystallized to the dihydrate form. Based on the experimental data and thermodynamic as well as mechanistic assessment, it is postulated that a fraction of trehalose remained miscible with the polymers due to extensive hydrogen bonding between the two components. This fraction was analyzed by the changes in the glass transition temperatures and was referred to as the "solid solubility" of trehalose in the polymer. Molecular mixtures containing 10% wt/wt trehalose (i.e., trehalose concentration below solid solubility) did not phase separate or crystallize during the 6 months storage at accelerated stability conditions, which substantiated our findings. We also propose that the MDSC technique described can be applied to determine the phase behavior and solid solubility limits of hydrophobic drugs that are dispersed in water-soluble carriers. This method can be useful in identifying the solid dispersions that will remain physically stable during the shelf-life.

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APPENDIX

Calculation of Enthalpy, Entropy, and Free Energy of Mixing

For a miscible blend containing polymer P$_1$ and sugar S$_2$ with weight fractions w$_1$ and w$_2$, respectively, and undergoing a single glass transition temperature T$_{g12}$, the enthalpy of mixture, H_{12} (J/g) can be described by the relation:

$$H_{12} = w_1H_1 + w_2H_2 + \Delta H_{mix} \tag{A1}$$

where H_1 and H_2 are the enthalpy in either liquid or glassy states of polymer and sugar respectively, and ΔH_{mix} is the enthalpy of mixing.

The enthalpy of mixing can be described as a function of temperature (T) and weight fraction (w), in the liquid or glassy states. By following Ehrenfest's theory of second order transition, the function $H(T,w)$ is continuous at T$_{g12}$, (i.e., H^1_{12} (T_{g12}) = H^g_{12} (T_{g12})), and hence the following equations can be given:

$$H^1_{12}(T_{g12}w) = w_1 \int_{T_{g1}}^{T_{g12}} \overline{C}^l_{p1}dT + w_2 \int_{T_{g2}}^{T_{g12}} \overline{C}^1_{p2}dT + \Delta H^1_{mix} \tag{A2}$$

$$H^g_{12}(T_{g12}w) = w_1 \int_{T_{g1}}^{T_{g12}} \overline{C}^g_{p1}dT + w_2 \int_{T_{g2}}^{T_{g12}} \overline{C}^g_{p2}dT + \Delta H^g_{mix} \tag{A3}$$

Also, since $\Delta H_{mix} = \Delta H^E_{mix} + \Delta H_{idealmix}$ and $\Delta H_{idealmix} = 0$, by rearranging Eqs. A2 and A3 at T$_{g12}$, Eq. A4 is obtained, (30).

$$\begin{aligned} -\Delta H_{mix}(J/g) &= -\Delta H^E_{mix} \\ &= \Delta H^g_{mix} - \Delta H^l_{mix} \\ &= w_1\int_{T_{g12}}^{T_{g1}} \Delta C_{p1}dT + w_2 \int_{T_{g12}}^{T_{g2}} \Delta C_{P2}dT \end{aligned} \tag{A4}$$

where the excess enthalpy of mixing ΔH^E_{mix} (J/g) = $\Delta H^l_{mix} - \Delta H^g_{mix}$; $\Delta C_p = C^l_p - C^g_p$; ΔH_{mix} is the enthalpy of mixing, and T$_{g1}$ and T$_{g2}$ are the glass transition temperatures of polymer and sugar respectively.

The entropy of mixing can also be calculated in a manner similar to the above performed calculations, and is described as:

$$\begin{aligned}-\Delta S_{mix}(J/g/^{\circ}K) &= \Delta S^{g}_{mix} - \Delta S^{l}_{mix}\\ &= w_1\int_{T_{g12}}^{T_{g1}} \Delta C_{p1} d\ln T + w_2\\ &\quad \int_{T_{g12}}^{T_{g2}} \Delta C_{p2} d\ln T - R\sum X_i \ln X_i \qquad \text{(A5)}\end{aligned}$$

where $\Delta S_{mix} = \Delta S^{E}_{mix} + \Delta S_{idealmix}$; $\Delta S_{idealmix} = -R\sum X_i \ln X_i$; R is the gas constant and X_i is the mole fraction of component i of the mixture. X_i has been converted to weight fractions to adjust the units for $\Delta S_{idealmix}$ to J/g/°K.

In order to numerically calculate the thermodynamic functions, it is necessary to obtain the heat capacity of individual components of the mixture as a function of temperature in their glassy and liquid states. The heat capacity function of a polymer is typically in the form of, $C_p = a + bT$, where a and b are constants.

By substituting the expression for C_p in Eqs. A4 or A5, the enthalpy (or the entropy) of mixing can be calculated:

$$\begin{aligned}-\Delta H_{mix} &= w_1\int_{T_{g12}}^{T_{g1}} [(a_1 + b_1T)^1 - (a_1 + b_1T)^g]dT\\ &\quad + w_2\int_{T_{g12}}^{T_{g2}}[(a_2 + b_2T)^1 - (a_2 + b_2T)^g]dT \qquad \text{(A6)}\end{aligned}$$

Upon calculating the enthalpy and entropy, the free energy of mixing can be calculated using the following equation:

$$\Delta G_{mix} = \Delta H_{mix} - T_{g12}\Delta S_{mix} \qquad \text{(A7)}$$

REFERENCES

1. A. T. M. Serajuddin. Solid dispersion of poorly water-soluble drugs: early promises, subsequent problems, and recent breakthroughs. *J. Pharm. Sci.* **88**:1058–1066 (1999).
2. C. Doherty and P. York. Accelerated stability of an X-ray amorphous furesemide-poly (vinyl pyrrolidone) solid dispersion. *Drug Dev. Ind. Pharm.* **15**:1969–1987 (1989).
3. H. Suzuki and H. Sunada. Some factors influencing the dissolution of solid dispersions with nicotinamide and hydroxypropylmethylcellulose as combined carriers. *Chem. Pharm. Bull.* **46**: 1015–1020 (1998).
4. E. Y. Shalaev and G. Zografi. How does residual water affect the solid-state degradation of drugs in the amorphous state? *J. Pharm. Sci.* **85**:1137–1141 (1996).
5. V. Andronis, M. Yoshioka, and G. Zografi. Effects of sorbed water on the crystallization of indomethacin from the amorphous state. *J. Pharm. Sci.* **86**:346–351 (1997).
6. K. Khougaz and S. D. Clas. Crystallization inhibition in solid dispersions of MK-0591 and poly (vinylpyrrolidone) polymers. *J. Pharm. Sci.* **89**:1325–1334 (2000).
7. Y. Aso, S. Yoshioka, and S. Kojima. Relationship between the crystallization rates of amorphous nifedipine, Phenobarbital, and flopropione, and their molecular mobility as measured by their enthalpy relaxation and ^{1}H NMR relaxation times. *J. Pharm. Sci.* **89**:408–416 (2000).
8. M. Yoshioka, B. C. Hancock, and G. Zografi. Crystallization of indomethacin from the amorphous state below and above its glass transition temperature. *J. Pharm. Sci.* **83**:1700–1705 (1994).
9. M. Yoshioka, B. C. Hancock, and G. Zografi. Inhibition of indomethacin crystallization in poly(vinylpyrrolidone) coprecipitates. *J. Pharm. Sci.* **84**:983–986 (1995).
10. T. Matsumoto and G. Zografi. Physical properties of solid molecular dispersions of indomethacin with poly(vinylpyrrolidone) and poly(vinylpyrrolidone-co-vinylacetate) in relation to indomethacin crystallization. *Pharm. Res.* **16**:1722–1728 (1999).
11. L. S. Taylor and G. Zografi. Spectroscopic characterization of interactions between PVP and indomethacin in amorphous molecular dispersions. *Pharm. Res.* **14**:1691–1698 (1997).
12. M. M. Coleman, J. F. Graf, and P. C. Painter. *Specific interactions and the miscibility of polymer blends*. Technomic Publishing, Lancaster, Basel, 1991.
13. L. S. Taylor and P. York. Characterization of phase transitions of trehalose dihydrate on heating and subsequent dehydration. *J. Pharm. Sci.* **87**:347–355 (1998).
14. L. S. Taylor and G. Zografi. Sugar-polymer hydrogen bond interactions in lyophilized amorphous mixtures. *J. Pharm. Sci.* **87**: 1615–1621 (1998).
15. H. Nyqvist. Saturated salt solutions for maintaining specified relative humidities. *Int. J. Pharm. Tech. Prod. Mfr.* **4**:47–48 (1983).
16. F. Sussuch, R. Urbani, F. Princivalle, and A. Cesaro. Polymorphic amorphous and crystalline forms of trehalose. *J. Am. Chem. Soc.* **120**:7893–7899 (1998).
17. K. Six, H. Berghmans, C. Leuner, J. Dressman, K. V. Werde, J. Mullens, L. Benoist, M. Thimon, L. Meublat, G. Verreck, J. Peeters, M. Brewster, and G. V. Mooter. Characterization of solid dispersions of itraconazole and hydroxypropylmethylcellulose prepared by melt extrusion, part II. *Pharm. Res.* **20**:1047–1054 (2003).
18. P. R. Couchman and F. E. Karasz. A classical thermodynamic discussion on the effect of composition on glass-transition temperatures. *Macromolecules* **11**:117–119 (1978).
19. S. L. Shamblin, L. S. Taylor, and G. Zografi. Mixing behavior of colyophilized binary systems. *J. Pharm. Sci.* **87**:694–701 (1998).
20. H. S. Lee and W. N. Kim. Glass transition temperatures and rigid amorphous fraction of poly(ether ether ketone) and poly (ether imide) blends. *Polym.* **38**:2657–2663 (1997).
21. D. Q. M. Craig, V. L. Kett, J. R. Murphy, and D. M. Price. The measurement of small quantities of amorphous material—should we be considering the rigid amorphous fraction? *Pharm. Res.* **18**:1081–1082 (2001).
22. Y. S. Chun, Y. S. Han, J. C. Hyun, and W. N. Kim. Glass transition temperatures and rigid amorphous fraction of poly (ether ether ketone) and polyarylate blends. *Polym.* **41**:8717–8720 (2000).
23. K. J. Crowley and G. Zografi. The effect of low concentrations of molecularly dispersed poly(vinylpyrrolidone) on indomethacin crystallization from the amorphous state. *Pharm. Res.* **20**:1417–1422 (2003).
24. X. C. Tang, M. J. Pikal, and L. S. Taylor. The effect of temperature on hydrogen bonding in crystalline and amorphous phases in dihydropyrine calcium channel blockers. *Pharm. Res.* **19**:484–490 (2002).
25. K. K. Chee. Thermodynamic study of glass transitions in miscible polymer blends. *Polym.* **36**:809–813 (1995).