The Physical State of Mannitol after Freeze-Drying: Effects of Mannitol Concentration, Freezing Rate, and a Noncrystallizing Cosolute

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Abstract □ The objectives of this study were to (1) measure the effects of freezing rate and mannitol concentration on the physical state of freeze-dried mannitol when mannitol is present as a single component, (2) determine the relative concentration threshold above which crystalline mannitol can be observed by X-ray powder diffraction in the freeze-dried solid when a variety of noncrystallizing solutes are included in the formulation, and (3) measure the glass transition temperature of amorphous mannitol and to determine the degree to which the glass transition temperature of freeze-dried solids consisting of mannitol and a disaccharide is predicted by the Gordon–Taylor equation. Both freezing rate and mannitol concentration influence the crystal form of mannitol in the freeze-dried solid when mannitol is present as a single component. Slow freezing of 10% (w/v) mannitol produces a mixture of the α and β polymorphs, whereas fast freezing of the same solution produces the δ form. Fast freezing of 5% (w/v) mannitol results primarily in the β form. The threshold concentration above which crystalline mannitol is detected in the freeze-dried solid by X-ray diffraction is consistently about 30% (w/w) when a second, noncrystallizing solute is present, regardless of the nature of the second component. The glass transition temperature of amorphous mannitol measured from the quench-cooled melt is approximately 13 ℃. Accordingly, mannitol is an effective plasticizer of freeze-dried solids when the mannitol remains amorphous. Glass transition temperatures of mixtures of mannitol and the disaccharides sucrose, maltose, trehalose, and lactose are well predicted by the Gordon–Taylor equation with values of *k* in the range of 3 to 4.

Introduction

Mannitol is one of the most commonly used excipients in freeze-dried pharmaceutical products. One of the reasons for the widespread use of mannitol is its tendency to crystallize from frozen aqueous solutions and the high melting temperature of the mannitol/ice eutectic mixture (about −1.5 °C). This property promotes efficient freeze-drying and a physically stable, pharmaceutically elegant freeze-dried solid. However, there have been reports of adverse effects of mannitol on stability of drugs as freeze-dried solids. Herman et al. reported that the rate of hydrolysis of methylprednisolone sodium succinate in the freeze-dried solid state is significantly faster when mannitol is used as the bulking agent versus an amorphous excipient such as lactose.[1] This instability of drug in the presence of mannitol was attributed at least in part to continued crystallization of mannitol from a system which is initially only partially crystalline. This can result in "amplification" of water activity in amorphous regions where the drug is located, with subsequent adverse effects on stability.[2] The physical state of mannitol during and after freeze-drying is particularly important in protein formulations where mannitol is present as a lyoprotectant. Izutsu et al., using three different model proteins, demonstrated that recovery of activity is inversely related to the degree of crystallinity of mannitol.[3,4] In particular, annealing during freeze-drying—which promotes crystallization—was associated with marked loss of activity after freeze-drying of these model systems.

There is a need for a better understanding of the physical chemistry of freeze-drying of mannitol-containing formulations in order to anticipate and avoid adverse effects of mannitol on physical and chemical stability of the freeze-dried solid. The purpose of this report is to identify formulation and processing factors which influence crystallization of mannitol when mannitol is present as both a single solute and in systems containing a second, noncrystallizing solute.

Experimental Section

Materials—The materials used in this study were reagent grade and were used as received. Mannitol, sucrose, and lactose were obtained from J. T. Baker, Inc. (Phillipsburg, NJ). Maltose, trehalose, dextran, and lysozyme were purchased from Sigma Chemical Co. (St. Louis, MO).

Preparation and Characterization of Mannitol Polymorphs—The three known polymorphs of mannitol were prepared using a procedure described by Walter-Levy.[5] Ten milliliter aliquots of mannitol solutions at concentrations of 0.4, 0.8, and 1.2 M were placed in separate watch glasses and evaporated at room temperature. Upon evaporation, three distinct crystal forms were observed. One form, observed primarily at the edge of the watch glass, was opaque, looked like lichens, and grew vertically to about 7 mm in height. The X-ray diffractogram of this material was consistent with the reference diffractogram[6] for the α polymorph (see Figure 1). The second form was observed mostly in the center of the watch glass, and crystals were translucent with a parallelepiped shape about 6−8 mm long. The X-ray powder diffractogram of this form was consistent with the reference diffractogram of the β polymorph (Figure 1). The third form was also translucent, but in the shape of needles in a coarse spherulite morphology. The X-ray powder diffractogram of this material was consistent with the reference diffractogram of the δ form (Figure 1). In general, lower concentrations of mannitol in solution favored formation of the δ form, while higher solution concentrations favored formation of the β form. The α polymorph was observed around the edges of the watch glass.

Thermal Analysis—Thermal analysis was carried out using modulated DSC (Model 2920, TA Instruments, Newcastle, DE). Indium and mercury, with melting points of 156.6 °C and −38.83 °C, respectively, were used for temperature calibration.

Glass transition temperatures of freeze-dried powders were measured by modulated DSC. Samples of freeze-dried powders were equilibrated over phosphorus pentoxide for 3 days and prepared by forming a powder compact in a punch and die with

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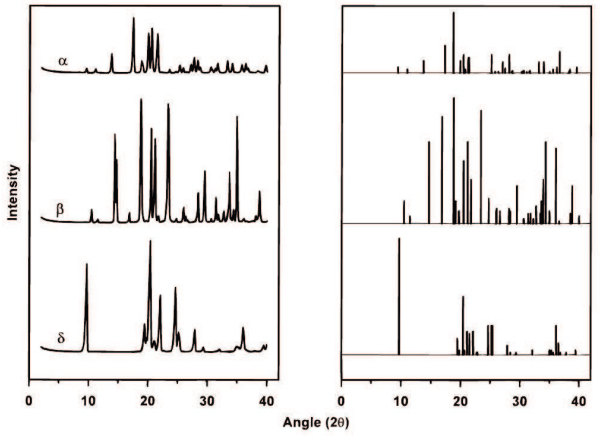

Figure 1—X-ray diffractograms of mannitol polymorphs prepared in this study (left panel) and corresponding reference diffractograms (right panel).

an approximate inside diameter of 4 mm in a dry nitrogen-purged glovebox. A heating rate of 3 °C/min was used with modulation of ±1/°C every 60 s.

Glass transition temperatures of quench-cooled mannitol/sucrose melts were measured by heating mixtures of mannitol/sucrose above the melting point, holding for 5 min, and then quenching in liquid nitrogen. DSC thermograms were recorded at a heating rate of 10 °C/min.

X-ray Powder Diffraction—A Siemens Krystalloflex diffractometer was used with Cu Kα radiation at a voltage of 40 kV and a current of 20 mA. Alignment was verified with a silicon standard using a reflection at 28.466° 2θ before each measurement. Samples were prepared by placing powders on a low background aluminum powder mount and scanning from 2 to 40° 2θ at a rate of 0.1° per second.

Freeze-Drying—Freeze-drying experiments were carried out using an FTS Dura-Stop freeze-dryer (FTS Systems, Inc., Stone Ridge, NY). Two milliliters of solution was filled into 10 mL serum vials, and the vials were placed directly on the shelves of the freeze-dryer. Samples were typically frozen for 6 h at −45 °C. Primary drying was done at a shelf temperature of −25 °C and a chamber pressure of 100 mTorr for 48 h, followed by secondary drying at a shelf temperature of 25 °C and a chamber pressure of 100 mTorr for 12 h. Vials were stoppered under vacuum.

Two freezing rates were used to determine the effect of freezing rate on mannitol crystallization. Slow freezing was carried out by placing vials on the shelf of the freeze-dryer and ramping the shelf temperature at a rate of 0.2 °C/min from room temperature to −45 °C, followed by freeze-drying under the conditions described above. Fast freezing was done by placing vials in liquid nitrogen and transferring them to a precooled shelf at −45 °C.

Measurement of Reconstitution Time—Reconstitution time of fast-frozen versus slow-frozen vials of freeze-dried mannitol was measured by injecting 2.0 mL of sterile water for injection into each vial of freeze-dried powder. The water was added along the side wall of the vial, and the vial was gently swirled. A blank was prepared by adding 2 mL of water to an empty 10 mL vial. Each sample was compared with the blank at 30 s intervals, and the reconstitution time was recorded as the first interval at which the sample and the blank were not distinguishable with respect to visual clarity. Five vials each of slow-frozen and fast-frozen freeze-dried solid were tested.

Preparation of Amorphous Mannitol—Two methods were attempted for preparation of amorphous mannitol. Solutions of 5% and 10% mannitol were added dropwise to liquid nitrogen in a Dewar flask. The frozen pellets were transferred to precooled freeze-dryer shelves at −50 °C and freeze-dried at −50 °C under full vacuum for 5 days. In the second method, a mannitol melt was quench-cooled by placing mannitol powder in an aluminum DSC pan, heating to 200 °C, and holding for 15 min. This sample was then quench-cooled in liquid nitrogen externally to the DSC. The sample compartment of the DSC was then cooled to −70 °C, and the quench-cooled melt was placed back in the instrument. The thermogram was then recorded at a heating rate of 10 °C per minute.

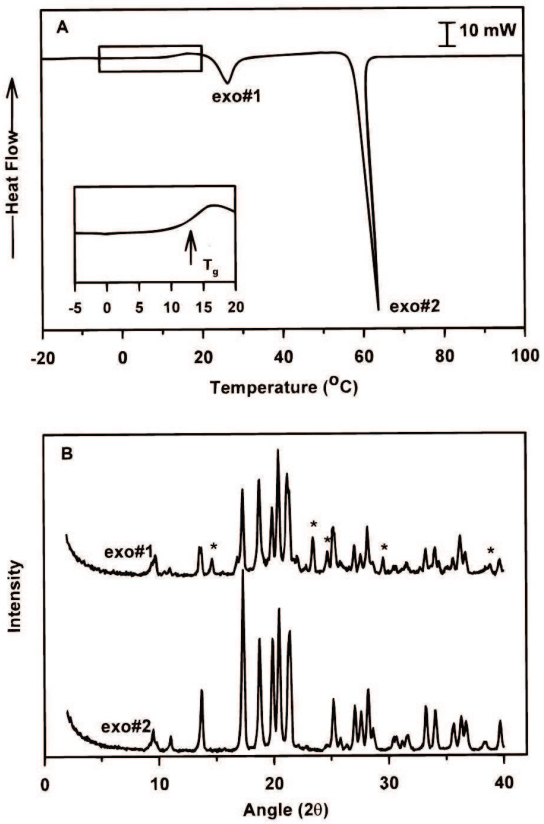

Figure 2—DSC thermogram of quench-cooled mannitol melt, showing the glass transition temperature and two exotherms (A) and X-ray powder diffractograms of quench-cooled mannitol melt after the first and second exotherm (B).

Results and Discussion

Studies on Amorphous Mannitol—Attempts to prepare amorphous mannitol as a single-component freeze-dried solid were unsuccessful. Despite freezing by dropwise addition of mannitol solution to liquid nitrogen and freeze-drying at the lowest attainable temperature, the resulting freeze-dried solid was crystalline by X-ray diffraction.

Preparation of amorphous mannitol by quench-cooling of the melt was successful in producing amorphous mannitol, however. The resulting thermogram is shown in Figure 2 (a). The thermogram consists of a glass transition at about 13 °C, followed by two crystallization exotherms. To better characterize the two exotherms, the DSC experiment was interrupted after each of the exotherms, and samples were held at 4 °C until X-ray powder diffractograms could be measured. As illustrated in Figure 2(b), the quench-cooled mannitol melt formed a mixure of the α and β polymorphs at the first exotherm, which then converted to the α polymorph at the second exotherm, as indicated by the disappearance of the designated peaks (*) at 14.0°, 23.4°, 24.7°, 29.5°, and 38.8° 2θ.

The low glass transition temperature of amorphous mannitol may help to explain our inability to prepare amorphous mannitol as a single-component freeze-dried solid. Even if mannitol were amorphous following freeze-drying, holding the lyophile for even a brief period of time at room temperature would be expected to result in crystallization. Slow crystallization during freeze-drying cannot be ruled out, however. Even though the shelf temperature was controlled at −50 °C, positive control of the sample temperature is uncertain due to lateral heat

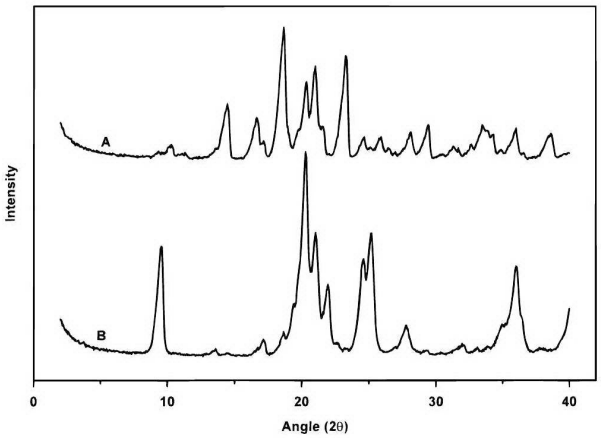

Figure 3—X-ray powder diffractograms of freeze-dried mannitol: (A) 5% and (B) 10%.

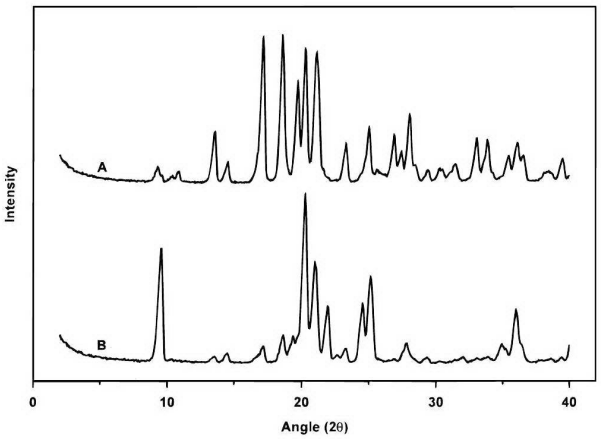

Figure 4—X-ray powder diffractograms of freeze-dried 10% mannitol frozen slowly (A) and fast (B).

transfer from the chamber walls. The glass transition temperature of the freeze-concentrated amorphous phase is approximately −30 °C, and it is well recognized that considerable molecular mobility is present well below the glass transition temperature.

Freeze-Drying of Mannitol as a Single Solute—X-ray powder diffractograms of mannitol freeze-dried from 5% and 10% (w/v) solutions after fast freezing are shown in Figure 3. Freeze-drying was carried out as described above. The β polymorph was formed when 5% mannitol solution was freeze-dried, and the δ polymorph was formed by freeze-drying of 10% solutions. This is in contrast to the behavior observed when mannitol is crystallized from aqueous solutions, where the β polymorph tends to form from more concentrated solutions.

The rate of freezing of mannitol solutions also influences crystallization behavior. Figure 4 shows X-ray powder diffractograms of 10% mannitol solutions frozen slowly and rapidly. The slowly frozen solutions resulted in a mixture of α and β polymorphs, while rapidly frozen solutions produced primarily the δ polymorph.

Reconstitution time was significantly different between fast-frozen and slowly frozen freeze-dried solids. The average of five determinations of reconstitution time for fast-frozen and slowly frozen samples resulted in average reconstitution times of 36 s (SD = 13.4 s) and 78 s (SD = 26.8 s), respectively. However, this cannot be attributed solely to differences in dissolution rates of mannitol polymorphs, since fast freezing would be expected to result in a higher specific surface area of the freeze-dried solid, which would promote more rapid reconstitution.

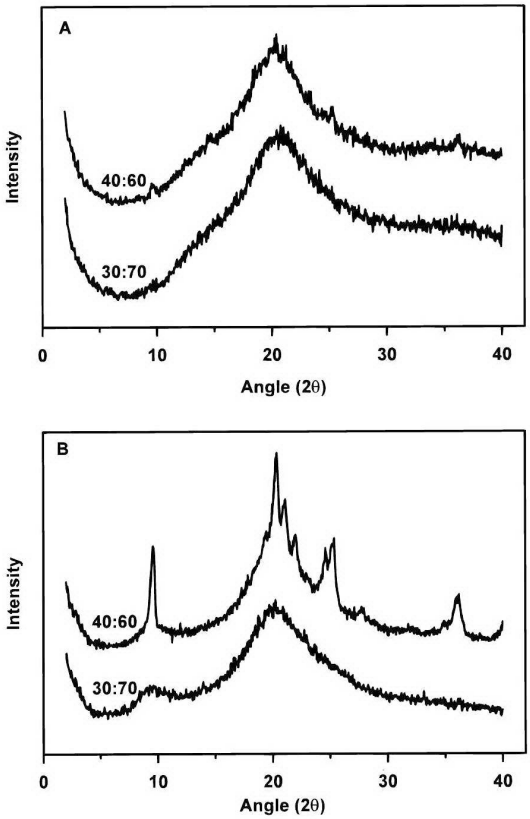

Figure 5—X-ray powder diffractograms of freeze-dried mannitol/sucrose (A) and mannitol/lysozyme (B), showing threshold concentration below which mannitol remains amorphous.

Mannitol Crystallization From a Two-Component System—To determine factors influencing crystallization of mannitol from a two-component system, it is necessary to identify the relative concentration threshold below which mannitol remains amorphous. Mannitol was freeze-dried with several noncrystallizing cosolutes, including sucrose, lactose, maltose, trehalose, dextran, and lysozyme in various ratios at a total solids concentration in the starting solution of 10% (w/w). It was observed that the relative concentration threshold above which crystalline mannitol is detected by X-ray diffraction is about 30% (w/w), and that this ratio is largely independent of the nature of the second solute. Figure 5 illustrates X-ray diffractograms of a freeze-dried mannitol/sucrose mixture (a) and a mannitol/lysozyme mixture (b). These diffractograms illustrate the extremes of the difference in apparent degree of crystallinity between 30:70 and 40:60 ratios. Considering the wide range of molecular weights of these cosolutes, it appears that weight ratios are more important than mole ratios in determining the threshold concentration above which crystalline mannitol is observed by X-ray powder diffraction.

Amorphous Mannitol as a Plasticizer of the Freeze-Dried Solid—The glass transition temperatures of two-component freeze-dried solids were studied by modulated DSC in the range of mannitol concentration below which crystalline mannitol can be detected by X-ray powder diffraction. The effect of mannitol as a plasticizer is clearly illustrated by Figure 6, where the glass transition decreases markedly as the relative concentration of mannitol increases. The observation of only one glass transition is

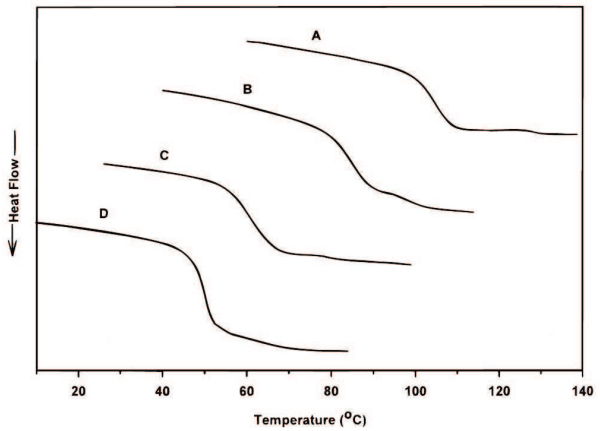

Figure 6—Modulated DSC thermograms (reversing component) of freeze-dried lactose (A), mannitol/lactose (10:90) (B), mannitol/lactose (20:80) (C), and mannitol/lactose (30:70) (D), illustrating plasticizing effect of mannitol.

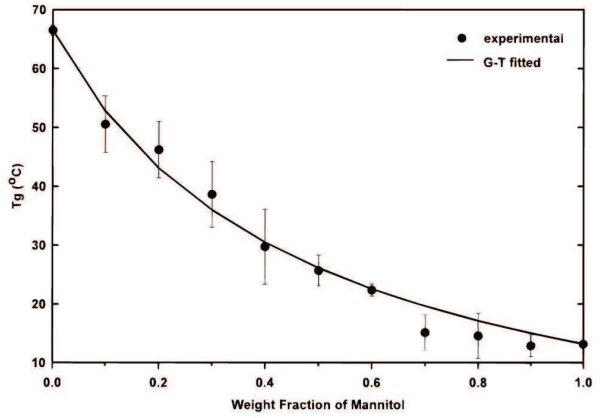

Figure 7—Glass transition temperature of quench-cooled mannitol/sucrose melt vs weight fraction of mannitol (●, experimental; —, Gordon–Taylor equation fit).

consistent with a homogeneous amorphous phase. The composition dependence of the glass transition temperature of a binary mixture can be described by the Gordon–Taylor equation,[7,8]

$$T_g = (w_1 T_{g1} + k w_2 T_{g2})/(w_1 + k w_2) \quad (1)$$

where T_g is the glass transition temperature of the mixture, k is a constant, w_1 and w_2 are weight fractions, and T_{g1} and T_{g2} are glass transition temperatures for component 1 and 2, respectively. The Gordon–Taylor equation assumes ideal volume-mixing in a binary mixture, which means that a mixture is homogeneous and specific volume remains constant.[7] To determine the degree to which the Gordon–Taylor equation can be used to describe the composition dependence of the glass transition temperature of mannitol/disaccharide mixtures over a broad range of composition, T_g data from quench-cooled mannitol/sucrose melts are plotted in Figure 7. Unlike freeze-dried mannitol/disaccharide mixtures, quench-cooling produces an amorphous system over the entire range of compositions. Curve fitting using the Marquardt–Levenberg algorithm results in the illustrated curve, with a best-fit k value of 3.1 ($r^2 = 0.98$). Use of this k value for freeze-dried solids over the composition range for which mannitol remains amorphous is predictive of the glass transition temperature

Table 1—Calculated and Measured Glass Transition Temperatures of Mannitol/Disaccharide Mixtures[a] Using Gordon–Taylor Equation with the Best-Fit Value of *k*

	lit.[9] T_g (°C)	k	calcd T_g (°C)	measured T_g range (°C)[b] (average)
sucrose	67[b]			62.6–66.2 (64.1)
m:S (10:90)		3	51.4	51.6–55.1 (53.2)
(20:80)		3	42.3	44.9–48.9 (46.5)
(30:70)		3	35.5	36.0–40.5 (38.1)
lactose	101[c]			101.4–115.6 (107.1)
m:L (10:90)		4	78.2	78.6–85.2 (82.7)
(20:80)		4	60.2	60.7–63.3 (62.4)
(30:70)		4	47.9	45.8–51.0 (48.9)
maltose	92[b]			88.9–99.2 (94.5)
m:M (10:90)		4	69.5	63.5–68.6 (66.7)
(20:80)		4	53.9	52.5–56.2 (54.4)
(30:70)		4	43.2	41.7–46.7 (43.4)
trehalose	107[b]			106.8–117.0 (112.5)
m:T (10:90)		5	77.1	78.3–83.6 (81.8)
(20:80)		5	57.4	56.4–61.1 (58.8)
(30:70)		5	44.9	43.0–49.3 (47.0)

[a] m: mannitol, S: sucrose, L: lactose, M: maltose, T: trehalose. [b] Midpoint value. [c] Onset value.

of freeze-dried solids, as shown by the data in Table 1. Table 1 also lists the measured glass transition temperatures of disaccharides along with literature values.[9] The best agreement between measured T_g values and calculated values is obtained with values of k in the range of 3 to 5 for all disaccharides listed in Table 1. These are in reasonable agreement with values of k reported by Roos and Karel[8] for frozen solutions of sucrose, lactose, and maltose of 4.7, 7, and 6, respectively. The physical significance of k is uncertain.

Practical Considerations—Given the wide use of mannitol as an excipient in freeze-dried products, pharmaceutical scientists should recognize that the physical state of mannitol in the freeze-dried solid is affected by both formulation and processing parameters. If mannitol is desired as a crystalline component of the formulation, then it is important to ensure that the relative concentration is high enough to result in a crystalline solid. Below the threshold concentration for crystallization, mannitol is an effective plasticizer of the lyophilized solid. This could have adverse effects on both physical and chemical stability of the product as a result of glass transition-associated mobility. In addition, the potential for changes in physical state of the solid due to different processing parameters such as freezing rate should be recognized when carrying out process validation studies intended to identify critical processing variables.

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