Existence of a Mannitol Hydrate during Freeze-Drying and Practical Implications

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Abstract □ We report thermal and crystallographic evidence for a previously unknown mannitol hydrate that is formed in the process of freeze-drying. The mannitol hydrate was produced by freeze-drying pure mannitol solutions (1–4% w/v) using the following cycle: (1) equilibration at −5 °C for 1 h; (2) freezing at −40 °C; (3) primary drying at −10 °C for 15 h; and (4) secondary drying at 10 °C for 2 h and then 25 °C for 5 h. This crystal form was also observed upon freeze-drying in the presence of sorbitol (1% w/v). The mannitol hydrate showed a distinct X-ray powder diffraction pattern, low melting point, and steplike desolvation behavior that is characteristic of crystalline hydrates. The mannitol hydrate was found to be metastable, converting to anhydrous polymorphs of mannitol upon heating and exposure to moisture. The amount of the mannitol hydrate varied significantly from vial to vial, even within the same batch. The formation of mannitol hydrate has several potential consequences: (1) reduced drying rate; (2) redistribution of the residual hydrate water during accelerated storage to the amorphous drug; and (3) vial-to-vial variation of the moisture level.

Introduction

The crystalline[1−11] and amorphous[12] forms of D-mannitol, a commonly used pharmaceutical excipient, have been extensively studied. The crystallization and polymorphic behaviors of mannitol have also been investigated in frozen aqueous solutions,[13−17] with an aim to understand and control the freeze-drying process. Unlike many excipients (e.g., sorbitol and disaccharides), mannitol has a strong tendency to crystallize from a frozen aqueous solution, both during cooling and reheating. The vial breakage phenomenon[13,14] is a striking illustration of this tendency. Mannitol has been observed to continue to crystallize after freeze-drying, especially as a result of heat and moisture,[18,19] which indicates that the freeze-drying process can produce a partially amorphous and partially crystalline material. The crystallization of mannitol during freeze-drying can lead to different anhydrous polymorphs (α, β, and δ) and their mixtures as a result of different formulation and processing conditions,[15] which leaves room for polymorphic transformations during storage. To the best of our knowledge, hydrated crystal forms of mannitol have not been reported.[20]

The formation of a crystalline hydrate by an excipient during freeze-drying may have several practical consequences. The difficulty of removing bound water from a crystal lattice can significantly limit the drying rate. Certain hydrates lose water without significantly altering the initial lattice structures ("isomorphic dehydration")[21] and lead to materials that are highly hygroscopic and undergo structural relaxation during storage. The residual water that is not removed by freeze-drying may be a potential threat to product stability if it is released during storage, especially under "accelerated" conditions.

We present here thermal and crystallographic evidence for a mannitol hydrate that is formed during freeze-drying and discuss the practical implications in terms of process design and product stability.

Experimental Section

Freeze-Drying—A laboratory freeze-drier (FTS Systems Inc.) was used. The following conditions produced the "mannitol hydrate" as characterized below: (1) equilibration at −5 °C for 1 h; (2) freezing at −40 °C; (3) primary drying at −10 °C for 15 h; and (4) secondary drying at 10 °C for 2 h and then 25 °C for 5 h. The cooling rate not controlled during freezing, and it took approximately 3 h to reach −40 °C. The chamber pressure was set to 100 μm of Hg throughout the drying process. Mannitol solutions were prepared at several concentrations (1, 2, and 4 w/v %) by dissolving mannitol (99+%, ACS reagent, Sigma; USP quality) in deionized water. The freeze-drying vials (tubing type manufactured by Wheaton) were 5 mL with 18.4 mm i.d. The fill volume was 2.0 mL/vial. The samples were stored at −20 °C before analysis.

XRD—A Siemens D5000 X-ray diffractometer was used, which was equipped with a Cu Kα source(λ = 1.54056 Å) operating at a tube load of 50 kV and 40 mA. The divergence slit size was 0.6 mm, the receiving slit 1 mm, and the detector slit 0.1 mm. Data were collected by a Kevex solid-state (SiLi) detector. The freeze-dried cake was broken, spread over the sample holder, and gently pressed before analysis. Each sample was scanned between 4 and 35° (2θ) with a step size of 0.03° and a maximum scan rate of 2 s/step.

DSC—Differential scanning calorimetry (DSC) was conducted in sealed Al pans at 10 °C/min using a Seiko DSC 210 under 50 mL/min nitrogen purge. Samples (5−10 mg) were either loosely packed into sample pans or first pressed into pellets using a stainless steel pellet-maker of local design. Sample preparation was carried out in a dry glovebag maintained at <5% RH.

TG/DTA—Simultaneous thermal gravimetric analysis (TGA) and differential temperature analysis (DTA) were conducted at 10 °C/min in open Al pans using a Seiko TG/DTA 220 under 150 mL/min nitrogen purge. Three to five milligrams were used for each analysis.

Results and Discussions

Figure 1 (curve 1) shows the XRD pattern of a freeze-dried mannitol sample from a 4% w/v solution. In addition to peaks that belong to the δ (major) and β (minor) polymorphs of mannitol,[22] additional peaks (marked by asterisks and listed in Table 1) were observed that could not be attributed to any known mannitol polymorphs. Heating this sample to 70 °C for 30 min eliminated the additional peaks (Figure 1, curve 2), with the remaining peaks attributable to the δ and β mannitol. These observations indicate the existence of a metastable crystal form of mannitol that was produced during freeze-drying and

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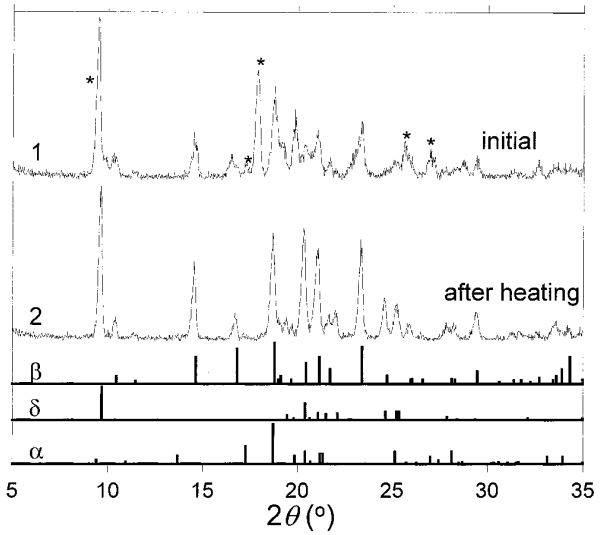

Figure 1—X-ray powder diffraction patterns of a freeze-dried mannitol sample (curve 1) and the same sample after heating at 70 °C for 30 min (curve 2). Curve 1 contains peaks that do not belong to any known mannitol polymorphs, whereas all peaks in curve 2 are attributable to the δ and β polymorphs of mannitol. The α-polymorph pattern is also shown for comparison.

Table 1—X-ray Powder Pattern for the Mannitol Hydrate

2θ, deg	9.6	16.5	17.9	25.7	27.0
I/I_0	0.8[a]	0.2	1[b]	0.3	0.2

[a] Overlapping the (020) reflection of δ-mannitol (2θ = 9.7°). [b] Best i.d. peak.

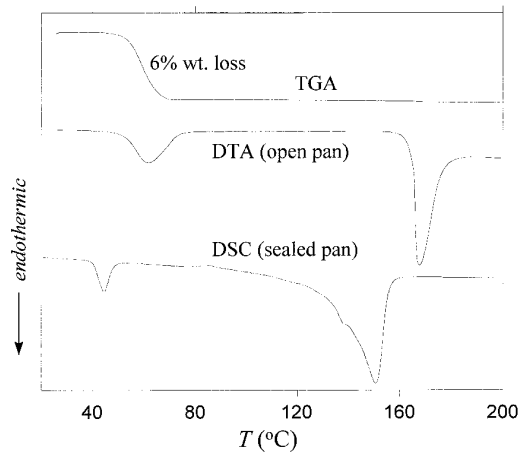

Figure 2—TGA (top), DTA (middle), and DSC (bottom) data of a freeze-dried mannitol sample. The TGA and DTA data were recorded simultaneously in an open sample pan, which show the melt/desolvation behavior of a crystalline hydrate. The DSC data was recorded in a hermetically sealed sample pan, which shows the homogeneous melting of the hydrate.

capable of converting to anhydrous mannitol polymorphs upon heating.

Figure 2 shows the thermal characteristics of the same sample described above. TGA (top curve) showed a steplike weight loss near 50 °C. The simultaneously conducted DTA (middle curve) showed a well-defined endotherm coinciding with the weight loss, which was followed by the melting of the anhydrous mannitol (mp 169 °C). DSC conducted in hermetically sealed pan (bottom curve) showed a sharp endotherm slightly below the desolvation onset.

The thermal data indicate that the crystal form transformation during heating (Figure 1) was accompanied by the loss of solvent (water). The steplike TGA loss and the well-defined DTA endotherm suggest the removal of structural water from a crystalline hydrate, rather than loosely bound ("free") water. This interpretation is supported by the sealed-pan DSC data. The use of sealed sample pans prevented the simultaneous evaporation of water during melting, making it possible to observe a sharp, homogeneous melting of the crystalline hydrate. The broad endothermic event following the sharp hydrate melting in the DSC trace can be attributed to the temperature-depressed melting of anhydrous mannitol crystals in the presence of water. We conclude therefore that a mannitol hydrate was formed in the process of freeze-drying and survived what appeared a "typical" drying cycle. This crystal form was metastable, converting to anhydrous polymorph(s) of mannitol upon heating.

Conditions of Formation—Using the same drying cycle, we have observed the mannitol hydrate under different formulation conditions: mannitol concentrations ranging from 1 to 4% w/v, with or without sorbitol (1% w/v), and in a drug formulation.[23] The same X-ray pattern assigned to the mannitol hydrate (Table 1) has also been observed by Cavatur and Suryanarayanan using in situ powder X-ray diffractometry.[24]

Anhydrous mannitol crystals are nonhygroscopic at room temperature, gaining less than 1% moisture at 90% RH.[25] This indicates that hydrate formation from anhydrous crystals is unlikely upon moisture exposure at the room temperature. The DSC data of Martini et al.[16] indicate that upon cooling a 10% w/v mannitol solution, ice forms (with substantial supercooling) before mannitol crystallizes. These observations, along with the previous failure to crystallize the mannitol hydrate at the room temperature or above,[20] suggest that the formation of the mannitol hydrate is likely a low-temperature phenomenon, relevant in particular to freeze-drying.

We have observed significant vial-to-vial variations in the amount and stability of the mannitol hydrate, even within the same batch. The relative intensity of the hydrate pattern ranged from comparable to that shown in curve 1 (Figure 1) to barely detectable or rapidly diminishing during measurement; the TGA losses ranged from 1 to 6%, and the onset of TGA loss ranged from 40 to 60 °C. The mannitol hydrate produced in certain vials could withstand mild heat (40 °C), humidity (60% RH) and compression (in DSC sample preparation), while in others it was easily destroyed, even by gentle compression. Although the drying cycle used in this study seemed a reasonable one, more aggressive secondary drying (same temperature but longer drying time) did eliminate traces of the mannitol hydrate and resulted in anhydrous mannitol crystals.

Several factors may contribute to the vial-to-vial variation in the amount and stability of the mannitol hydrate. First, the hydrate is formed in a low-temperature, concentrated solution, conditions that are not favorable for producing well-developed crystals in a reasonable processing time. Second, the ice crystallization temperature can vary as a result of the cooling rate[16] and thus the vial location in a drier. Other more subtle factors can also affect the onset of ice crystallization (e.g., defects on the vial surface). Such variability can influence the degree of freeze concentration and the ice structure and, in turn, the subsequent crystallization of mannitol. Finally, the hydrate formation is in competition with the crystallization of the anhydrous polymorphs (α, β, or δ), directly or through subsequent solid–solid conversion. Depending on the rate of anhydrous crystallization, the hydrate amount will vary. We suspect that these effects acting in concert contributed to the vial-to-vial variation and the previous failure to detect the mannitol hydrate.

Practical Implications—The formation of the mannitol hydrate during freeze-drying has several practical implications. First, the rate of water removal may be reduced due to strongly bound water that resides in the crystal lattice. In certain hydrates, such as the mannitol hydrate, the structural water cannot be fully removed without destroying the crystal lattice, a process that may require more aggressive or atypical drying conditions (e.g., longer drying time and higher temperature). Depending on the detailed mechanism of formation, it may be necessary, for example, to anneal the frozen solution to promote crystallization of the anhydrous form, thereby reducing or eliminating the mannitol hydrate.

Second, the residual hydrate water that is not fully removed by freeze-drying may pose a long-term threat to product stability, as the hydrate water can be released and redistributed, especially in "accelerated" storage, to the often amorphous drug and thus increases the potential for chemical and physical changes. Herman et al. have shown that methylprednisolone sodium succinate (MPSS) stored at accelerated conditions and formulated with mannitol is chemically less stable than materials formulated with lactose.[26] The difference in chemical stability was attributed to mannitol crystallization and the redistribution of water within the freeze-dried cake which promoted the hydrolysis of MPSS. Roos and Karel have shown that as amorphous lactose crystallizes and releases water, the glass transition temperature of the remaining amorphous material is depressed and the crystallization process accelerated.[27] It is conceivable, therefore, that the release of hydrate water during accelerated storage may also complicate the interpretation of stability data and cause a premature termination of an otherwise promising formulation.

Finally, our observations indicate that certain vial-to-vial variation in the water level may be associated with the formation of the mannitol hydrate.

Conclusions

We have reported thermal and crystallographic evidence for the formation of a previously unknown mannitol hydrate in the freeze-drying process. The mannitol hydrate can survive a "typical" drying cycle, but can be converted to anhydrous polymorph(s) of mannitol on gentle heating and more aggressive secondary drying. Potential consequences of the mannitol hydration formation include reduced drying rate, moisture release during accelerated storage, and vial-to-vial variation in the water level.

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