Effects of Formulation and Process Factors on the Crystal Structure of Freeze-Dried *Myo*-Inositol

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ABSTRACT: The objective of this study was to elucidate effects of formulation and process variables on the physical forms of freeze-dried *myo*-inositol. Physical properties of *myo*-inositol in frozen solutions, freeze-dried solids, and cooled heat-melt solids were characterized by powder X-ray diffraction (PXRD), thermal analysis (differential scanning calorimetry [DSC] and thermogravimetric), and simultaneous PXRD–DSC analysis. Cooling of heat-melt *myo*-inositol produced two forms of metastable anhydrate crystals that change to stable form (melting point 225°C–228°C) with transition exotherms at around 123°C and 181°C, respectively. Freeze-drying of single-solute aqueous *myo*-inositol solutions after rapid cooling induced crystallization of *myo*-inositol as metastable anhydrate (transition at 80°C–125°C) during secondary drying segment. Contrarily, postfreeze heat treatment (i.e., annealing) induced crystallization of *myo*-inositol dihydrate. Removal of the crystallization water during the secondary drying produced the stable-form *myo*-inositol anhydrate crystal. Shelf-ramp slow cooling of *myo*-inositol solutions resulted in the stable and metastable anhydrous crystal solids depending on the solute concentrations and the solution volumes. Colyophilization with phosphate buffer retained *myo*-inositol in the amorphous state. Crystallization in different process segments varies crystal form of freeze-dried *myo*-inositol solids. J Pharm Sci
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INTRODUCTION

The increasing therapeutic significance of biopharmaceuticals and molecular assembly drug delivery systems (e.g., liposomes) has brought attention to freeze-dried formulations that improve the storage stability required for their wide clinical use.[1] Various active pharmaceutical ingredients (APIs) and excipients in the freeze-dried formulations are in either the crystalline or the amorphous state, depending on their anticipated functions in the particular formulations.[1–5] Crystalline solids are preferable to confer chemical stability of small API molecules (e.g., antibiotics) and structural integrity of dried cakes. Contrarily, certain noncrystalline excipients (e.g., sucrose, trehalose) are necessary to protect structural integrity of the proteins and the liposomes from stresses during the freeze-drying process and subsequent storage through water-substituting heteromolecular interactions and reduction of molecular mobility.[6,7] Some polymers form amorphous microporous matrix that improve dissolution behavior of colyophilized lower-solubility small-molecule APIs.[8] Multiple ingredients in pharmaceutical freeze-dried formulations affect their crystallization profiles in complex manner.[9] Appropriate control of the physical properties through formulation and process optimization should be a prerequisite for the production of pharmaceuticals based on the quality by design (QbD) concept.[10]

Some APIs (e.g., pentamidine isothianate[11–13] and hydroxytetracaine hydrochloride[14] and excipients (e.g., D-mannitol[15–21] and glycine)[22–25] exist in multiple polymorphic forms, including polymorphs and pseudepolymorphs upon freeze-drying. Freeze-drying of some other APIs (e.g., paracetamol)[26] results in particular structure crystals that are different from those obtained by other production methods (e.g., recrystallization from organic solvents). Controlling the physical properties of D-mannitol and glycine are particularly important to ensure stability of many lyophilized formulations containing the bulking agents.[9,16–19,22,27–29] Extensive studies indicated the significance of the freezing segment to affect physical forms of D-mannitol in the dried solids. Cosolute composition (e.g., addition of pH buffer) and freezing methods (e.g., cooling speeds, postfreeze heat treatment) affect distribution of mannitol polymorphs in freeze-dried solids.

Many other formulation ingredients being maintained in the high viscosity supercooled phase should have varied opportunities to crystallize during the following primary drying (ice sublimation), secondary drying (removal of water from solid phase), and solid storage because of the intrinsic thermodynamic instability of the amorphous state. Information on the crystallization propensities of components in each segment of the lyophilization would contribute to rational design of the formulations and processes.

The main objective of the present study was to identify and characterize the formulation and process conditions that

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affect physical forms of freeze-dried *myo*-inositol. *Myo*-inositol is a small sugar alcohol GRAS excipient that protects the native conformation of proteins in aqueous solutions by the same mechanism as many sugars and sugar alcohols.[30,31] However, varied crystallinity of *myo*-inositol in lyophilized solids, accompanied by variation in the protein-stabilizing effect, have limited use of *myo*-inositol in pharmaceutical formulations.[32] The structures of a dihydrate and two anhydrate (monoclinic and orthorhombic) *myo*-inositol crystals obtained by single-crystal X-ray diffraction have been reported.[33–36] Appropriate control of the physical properties can enable wider application of *myo*-inositol as an amorphous stabilizer or crystalline bulking agent in lyophilized formulations.[32,37] Here, for comparison, we also studied physical properties of *myo*-inositol solids prepared by cooling of its heat-melt.

MATERIALS AND METHODS

Materials

Myo-inositol and mono- or disodium hydrogen phosphate were purchased from Wako Pure Chemical Company (Osaka, Japan). Polyvinylpyrrolidone (PVP) 29,000 was obtained from Sigma–Aldrich Company (St. Louis, Missouri).

Preparation of Cooled-Melt and Freeze–Thawed Precipitate Solids

A differential scanning calorimeter (DSC Q-10; TA Instruments, New Castle, Delaware) was used to prepare cooled-melt *myo*-inositol solids. *Myo*-inositol powders (approximately 1 mg) in hermetic aluminum DSC cells were heated to 245°C under a nitrogen gas flow before slow cooling (10°C/min) on the DSC stage or quench-cooling by immersing the cells in liquid nitrogen (LN_2) outside the furnace. Open aluminum cells containing approximately 10 mg of *myo*-inositol powder were used to prepare larger amounts of the cooled-melt solids required for a powder X-ray diffraction (PXRD) analysis. Cooled-melt *myo*-inositol and D-sorbitol mixture solids were prepared by heating the physically mixed powder to 245°C and then quench-cooled by immersion into LN_2. *Myo*-inositol dihydrate crystals were prepared by recrystallization from aqueous solution containing PVP 29,000.[33] Some aqueous *myo*-inositol solutions (100 mg/mL, 2 mL) in glass vials were freeze–thawed (−15°C, overnight). The solid residue obtained by paper filtration was immediately applied for the PXRD analysis.

Freeze-Drying

A lyophilizer (Freezone-6; Labconco, Kansas City, Missouri) was used for the freeze-drying of aqueous *myo*-inositol solutions (0.5–2.0 mL each, 2.0 mL unless otherwise mentioned) in flat-bottom glass vials (21-mm diameter, SVF-10; Nichiden Rika Glass, Kobe, Japan) with half-closed rubber caps. After the vials were transferred, the lyophilizer shelves were cooled to −32°C at 0.5°C/min (shelf-ramp slow cooling) and then held at that temperature for 2 h to freeze the aqueous solutions. The primary drying segment was performed at −32°C (0.04 mbar, 30 h). The secondary drying segment was performed at 30°C (0.04 mbar, 4 h) after heating of the shelf at 0.2°C/min. The vials were closed with rubber stoppers under vacuum. Some solutions were frozen by immersing the vials in LN_2 immediately before the primary drying under vacuum at −32°C (rapid cooling).

Some shelf- and LN_2-frozen solutions were heat-treated at −10°C for 2 h before the shelf was cooled again down to −32°C for the primary drying (postfreeze heat treatment). Some solutions were dried after freezing by one of the "controlled nucleation" methods reported by Geidobler et al.[38] with some modifications. The lyophilizer shelf was cooled to −5°C and maintained at that temperature for 1 h. The chamber pressure was then reduced by vacuum pump down to 4 mbar. Ice nucleation was triggered by the quick release of the vacuum by induction of nitrogen gas. Primary drying was started after the shelves were cooled to −32°C (0.5°C/min) and held at that temperature for 2 h. All of the solutions were freeze-dried without apparent loss of the cylindrical cake structure. Some freeze-drying runs were terminated by closing the vial with rubber cap before the shelf heating for the secondary drying to obtain the "primary dried" solids.

Thermal Analysis

Thermal analysis of frozen aqueous solutions and solids were performed using the DSC Q-10 system. Aliquots of aqueous solutions (10 μL) in aluminum cells were cooled to −60°C at 10°C/min before a heating scan at 1°C/min. Heating scans of some frozen solutions were paused at a designated temperature (−50°C, −32°C, or −10°C), held at the temperature for 120 or 480 min, and then cooled to −60°C for the second heating scans to study the effect of heat treatment. The freeze-dried *myo*-inositol solids (1–2 mg) were placed in open aluminum cells for thermal scan from −10°C at 10°C/min. Exotherms of *myo*-inositol crystal transition were obtained at 70°C to 130°C. The glass transition temperatures (T_g) of quench-cooled heat-melt *myo*-inositol and D-sorbitol mixture solids (245°C, LN_2, approximately 10 mg) were obtained by heating scan from −50°C at 5°C/min. We conducted a thermogravimetric/differential thermal analysis (TG/DTA) using a Thermo Plus TG-8120 (Rigaku Company, Akishima, Tokyo). Open aluminum pans containing the samples were heated from 25°C to 300°C at 10°C/min under a nitrogen purge.

PXRDand PXRD–DSC Simultaneous Measurement

A powder X-ray diffractometer (D8 DISCOVER with GADDS; Bruker AXS, Karlsruhe, Germany) with Cu-Kα radiation (40 kV × 40 mA) was used for the PXRD analysis. Diffraction patterns obtained in the range of 5°–45° (2θ, 3-min total scan time) were recorded. Simultaneous PXRD and DSC analyses were performed using a XRD–DSC II system (Rigaku). Operating conditions of the XRD were 40 kV voltage, 40 mA current, and a Cu-Kα source (1.60 Å). The solids were heated up to 250°C at a scanning rate of 5°C/min under N_2 atmosphere.

Measurement of Residual Water

A Karl Fischer moisture meter (CA-200; Mitsubishi Chemical Analytech, Chigasaki, Japan) was used to measure residual water contents of freeze-dried *myo*-inositol solids suspended in methanol (3 mL). Residual water contents were less than 0.5% of the anticipated weights in all the solids studied (data not shown).

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