

# Trehalose Crystallization During Freeze-Drying: Implications On Lyoprotection

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## MATERIALS AND METHODS

### Materials

Trehalose dihydrate ( $C_{12}H_{22}O_{11} \cdot 2H_2O$ ; Sigma, purity >99%) was used as received. Appropriate amount of trehalose dihydrate was dissolved in degassed deionized water to yield a 4% w/v trehalose ( $C_{12}H_{22}O_{11}$ ) solution. The deionized water was degassed by stirring at 70°C for 5 minutes, followed immediately by filtration (0.45  $\mu$ m membrane filter). The water was allowed to cool to room temperature (RT) in a closed container.

### Methods

#### X-ray diffractometry (XRD)

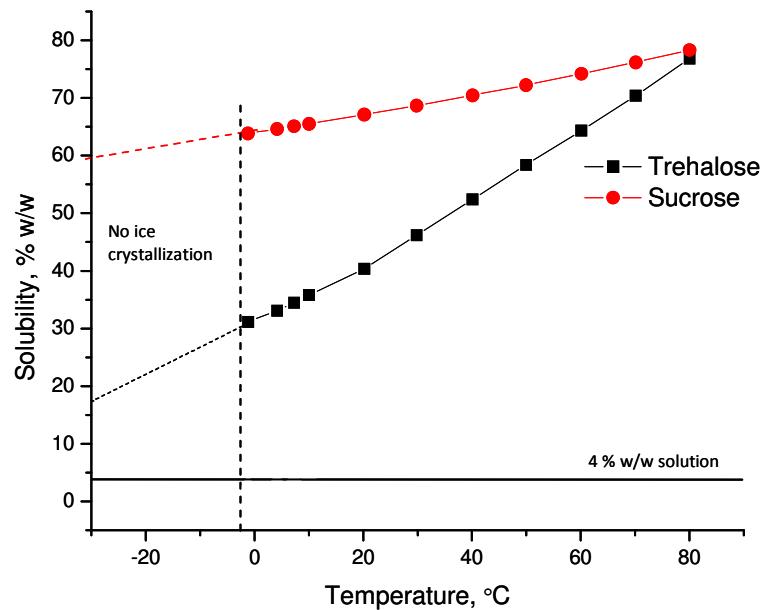
A powder X-ray diffractometer (Model XDS 2000, Scintag; Bragg-Brentano focusing geometry) with a variable temperature stage (High-Tran Cooling System, Micristar, Model 828D, R.G. Hansen & Associates; working temperature range: -190 to 300 °C) and a solid-state detector was used. The sample stage was cooled using liquid nitrogen while a vacuum pump was used to evacuate the chamber.

About 1 ml of the trehalose solution was filled into a custom designed sample holder. The sample chamber was sealed using a stainless steel cover. The beryllium window on the cover enabled X-ray diffraction studies *during* the freeze-drying. Thus the entire freeze-drying cycle was simulated in the sample chamber of the diffractometer, and the phase transitions were continuously monitored. Typically, the solutions were cooled, at 0.5 °C/min, from RT to -40 °C, held for 15 minutes, and warmed at 0.5 °C/min to the annealing temperature of -18 °C. While the primary drying was conducted for 3 hours at -25 °C (~ 200 mTorr), the secondary drying was performed at -10 °C for 1 hour, and subsequent drying was conducted at 0 and at 10 °C, for 30 minutes at each temperature. The sample was periodically exposed to Cu K $\alpha$  radiation (45 kV x 40 mA) over an angular range of 5 to 40° 2 $\theta$  with a step size of 0.05° and a dwell time of 1 second.

Selected experiments were performed at the synchrotron beamline 6-ID-B of the Midwest Universities Collaborative Team (MUCAT), at the Advanced Photon Source, Argonne National

Laboratory (Argonne, IL, USA). More details of the synchrotron XRD instrumental setup, experimental conditions, and data refinement are provided in our earlier publication.<sup>17</sup> The XRD patterns were compared with the published data in the Powder Diffraction Files (PDF) of the International Centre for Diffraction Data (ICDD).<sup>16</sup>

In an effort to accelerate solute crystallization, experiments were also preformed with seeding. The frozen solution was seeded with crystals of either succinic acid or trehalose dihydrate and annealed.



Aqueous solubility of trehalose and sucrose as the function of temperature. The data were taken from Miller et al., 1997.<sup>7</sup> The horizontal line represents the trehalose concentration in the prelyophilization solution.