

# CRYSTALLIZATION OF LYOPROTECTANT IN THE FROZEN SYSTEM AND ITS PHASE TRANSITION DURING DRYING

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**Background.** Lyoprotectants are stabilizers used to prevent denaturation of proteins during freeze-drying and subsequent storage. In order to be effective, the lyoprotectants must be retained amorphous not only during processing but also during the entire shelf-life of the product. The physical state of the lyoprotectant is usually characterized by subjecting the final lyophile to powder X-ray diffractometry. We demonstrate that lyoprotectant crystallization *during* freeze-drying may not become evident from characterizing the final lyophile.

**Purpose.** (i) To study the crystallization of trehalose in the frozen solution by X-ray diffractometry and (ii) to understand the phase transitions during the entire freeze-drying cycle.

**Method.** Aqueous trehalose solution was cooled from room temperature to  $-30\text{ }^{\circ}\text{C}$  at  $0.5^{\circ}\text{C}/\text{min}$  in a custom designed sample holder. The frozen solution was warmed to  $-18^{\circ}\text{C}$  and annealed with or without seeding. The XRD patterns were collected both during cooling and annealing. The annealed sample was subjected to primary drying, in the sample chamber of the diffractometer, and XRD patterns were continuously collected. The phase crystallized was identified by comparing the XRD patterns with Powder Diffraction Files of International Centre for Diffraction Data (ICDD).

**Results.** After cooling, hexagonal ice was the only crystalline phase observed. However, upon annealing, both in seeded and unseeded systems, crystallization of trehalose dihydrate was evident. Seeding, as expected, accelerated the solute crystallization. Thus phase separation of the lyoprotectant was observed in frozen solutions. During drying, dehydration of trehalose dihydrate yielded a substantially amorphous anhydrous trehalose.

**Conclusions.** (i) Crystallization of trehalose, as trehalose dihydrate, was observed in frozen solutions. (ii) The dehydration of the crystalline trehalose dihydrate to substantially amorphous anhydrate occurred during drying. Therefore, analyzing the final lyophile will not reveal crystallization of the lyoprotectant during freeze-drying. (iii) The lyoprotectant crystallization can only become evident by continuous monitoring of the system during the entire freeze-drying cycle.