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# Freezing step controls the mannitol phase composition heterogeneity

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A B S T R A C T

Freeze-dried samples were prepared from D-mannitol solution by selected freezing conditions. Crystalline structures of prepared samples were determined by XRD analysis, and distributions of the various crystal structures of mannitol were obtained for each sample. It was found that the amount of each polymorph was quite well correlated to the freezing conditions, namely the ice crystal nucleation temperature and the cooling rate. In case of samples prepared at fast cooling rates, the samples where the ice crystals nucleated at higher temperature contained much more stable form than the samples nucleated at lower temperature. Besides, the samples prepared at slow cooling rates predominantly contained stable crystalline forms despite of the variation of nucleation temperatures. Moreover, the experimental results also indicated that the various polymorphs were also distributed vertically through the sample along the direction of the heat flux during freezing. The tendency of the polymorph distribution through the freeze-dried cake was also elucidated by using the simulated temperature profiles during freezing. Thus, the profiles of mannitol polymorphs after the freezing derived from the temperature distributions could predict the global tendency of polymorphism behaviour, and, consequently, would be useful to achieve quality control of freeze-dried formulations.



*Keywords:* Freeze-drying; Polymorph; Ice; Crystal growth; Freezing model; Mannitol

## 1. Introduction

Freeze-drying is a commonly used manufacturing process for pharmaceutical protein based products, especially in the preservation of their therapeutic or biological activity. When aqueous pharmaceutical formulations are lyophilized, cryo-protectants (or stabilizers) play a key role to protect them from irreversible damages like structural denaturations and activity losses. In general, sugars, polyols, polymers and amino acids are added to the formulation to achieve this stabilizing effect. A rational guidance has been reported by Carpenter et al. (1997) to set up the optimal formulation. Indeed, it appears that structural parameters of different polymorphs of selected cryo-protectant can greatly influence the various required quality factors of a freeze-dried matrix. This is why the way of controlling these structures was investigated in the present study.

Suzuki et al. (1999) reported that hydrogen bonding between sucrose (cryo-protectant) and protein has the main effect to protect the protein structure. From their point of view, the best stabilizer maximizes the number of hydrogen bonding depending on each type of pharmaceutical protein. It was also observed that an amorphous structure of the cryo-protectant is also a key parameter to fix the stability of many therapeutic proteins. Thus, it would be useful to find a freeze-drying condition that leads to amorphous structures in the final freeze-dried matrix. It is empirically known that a freezing protocol influences on the appearance of amorphous and/or crystalline form in freeze-dried matrices. For example, Cavatur and Suryanaraynan (1998) have investigated this influence with *in situ* X-ray powder diffractometry. Chang et al. (1996) observed that slow cooling rates were able to prepare stable drugs by lyophilization. Moreover, Strambini and Gabellieri (1996) reported that the increase of ice crys-

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**Nomenclature**

| | |
|---|---|
| $C_p$ | heat capacity (J/kg K) |
| $\Delta H_f$ | latent heat of crystallization (J/kg) |
| $h$ | convective heat transfer coefficient (W/m$^2$ K) |
| $k$ | thermal conductivity (W/m K) |
| $k_i$ | nucleation rate constant (kg/m$^3$ s K) |
| $Q_c$ | latent heat of crystallization (W/m$^3$) |
| $Q_n$ | latent heat of nucleation (W/m$^3$) |
| $s$ | thickness of undercooled zone (m) |
| $T$ | temperature (°C) |
| $t$ | time (s) |
| $T^*$ | temperature in supercooled liquid (°C) |
| $T^o$ | homogeneous undercooled temperature (°C) |
| $T_f$ | freezing front temperature (°C) |
| $T_n$ | nucleation temperature (°C) |
| $X_{ice}$ | ice fraction |
| $\rho$ | density (kg/m$^3$) |

*Subscripts*

| | |
|---|---|
| apparent | apparent |
| liquid | liquid zone |
| ice | frozen zone |

tals interfaces area could induce protein structural loss and/or aggregate formation so that freezing conditions that reduce ice crystal interfacial surface area – i.e. slow cooling conditions – could correspond to better freezing conditions. It was also reported by Izutsu and Kojima (2002) that crystallization of a buffer and/or a stabilizer often leads to lose stability or activity of lyophilized pharmaceutical proteins. Nevertheless, it seems that slow cooling conditions during freezing are not always adequate to avoid unfavourable crystallizations of buffer and/or stabilizer even if the crystallization of the active principle ingredient (API) is generally important to enhance its stability (Pikal et al., 1977, 1978). Thus, from the literature data, it is critical to identify freezing conditions that lead to an optimal ice crystal size in the frozen matrix and an optimal cryo-protectant phase in the freeze-dried cake. Dealing with crystalline forms of solid matrix, polymorphism is recognized as an issue that should not be ignored (Pikal, 1999). However, it is still difficult to predict the polymorphic behaviour in a crystallizing system, especially in the case of crystallization of complex pharmaceutical formulations. As pointed out by several researchers, it is worth trying to investigate the relationships between polymorphism phenomena and freezing or freeze-drying operating conditions (Kim et al., 1998; Cannon and Trappler, 2000; Hottot et al., 2008).

Concerning these topics, Nakagawa et al. (2006, 2007) reported a clear correlation between freezing condition (i.e. cooling rate during freezing, ice crystal nucleation temperature) and the ice crystals mean sizes during vial freeze-drying. The temperature profiles were fairly simulated for selected cooling rates and ice nucleation temperatures and the ice crystal size distributions were estimated from these temperature profiles. Thus, in the continuation of this work, we decided to investigate the relationships between freezing conditions and phase composition of the frozen cryo-protectant. For that purpose, freeze-dried samples were prepared from D-mannitol solutions at selected freezing conditions and fixed sublimation conditions. Crystalline structures of prepared samples were analyzed by X-ray powder diffractometry and the distributions of different mannitol polymorphs were experimentally determined. Finally, we attempted to elucidate the crystallization behaviour in a frozen solution using simulated temperature profiles during freezing with a physical model.

## 2. Materials and methods

### 2.1. *Materials*

10% (wt) mannitol solution prepared from distilled water and D-mannitol powder (Fluka Chemie AG) was selected as a simplified pharmaceutical formulation.

### 2.2. *Freezing and freeze-drying procedures*

A glass tube of 3 mL (diameter $D$ = 13.5 mm) was used in this work. As shown in Fig. 1A, a glass tube was fixed on a heat exchanger and sample solution (1.8 mL) was filled in the tube (height $h$ = 14 mm in frozen state). The sample temperatures were monitored by very thin type K thermocouples. A plate shaped thermocouple was attached on the exterior surface of the glass tube at a fixed position i.e. at about 3 mm from the vial bottom (Fig. 1B). By this external non-invasive fixing, the nucleation phenomena were not submitted to any artifact that usually results from the introduction of thermocouples inside the vial. Besides, an adequate thermal paste was used for improving thermal contact between thermocouple sensitive part and external surface of the vial glass wall. Then, sample

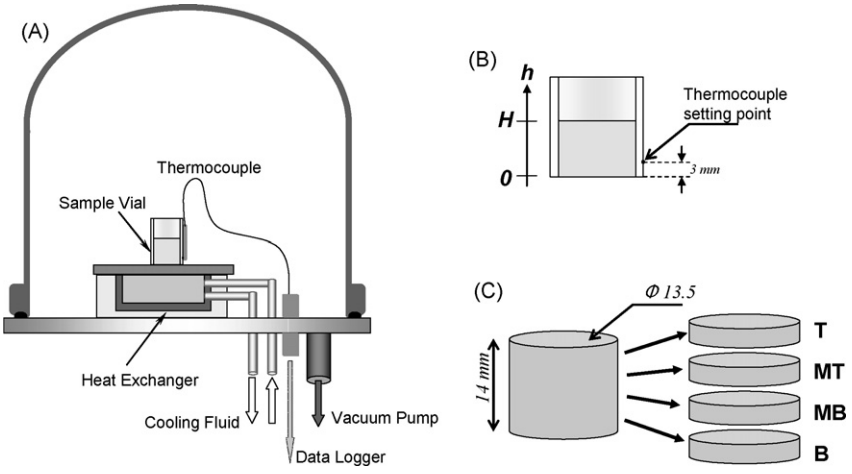


**Fig. 1 – Experimental schemes: (A) freeze-dryer; (B) thermocouple setting point; (C) sampling position for XRD observation.**