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Investigation of solid state changes in freeze-dried biomacromolecular samples: Process simulation by variable temperature X-ray powder diffraction

Hjalte Trnka , Holger Grohganz and Jukka Rantanen

Department of Pharmaceutics and Analytical Chemistry, Faculty of Pharmaceutical Sciences, University of Copenhagen

E-Mails: hjat@farma.ku.dk; hgr@farma.ku.dk; jtr@farma.ku.dk

Abstract: The solid form of mannitol, a common bulking agent in freeze-dried formulations, was investigate during lyophilization using in-situ variable temperature X-ray powder diffraction (VT-XRPD). The aim of the study was to investigate the influence of processing conditions on the solid form of mannitol during various stages of the freeze-drying process.

Keywords: Freeze-drying, Lyophilization, Mannitol, Proteins, In-situ, XRPD

1. Introduction

Biomacromolecules such as proteins and peptides have received increased focus in the pharmaceutical field during recent years. Several challenges however need to be overcome for these molecules to become effective pharmaceuticals. One of them is the low stability that hampers biomacromolecules. In order to prolong the shelf life of proteins formulations, they are commonly freeze-dried. Additives such as stabilizers and bulking agents are often needed in order to obtain a stable and pharmaceutically elegant product. One of the most common additives in freeze-dried formulations, the bulking agent mannitol, can exist in at least five different solid forms, namely α, β, δ, hemi-hydrate and amorphous [1-3]. Both formulation composition and processing conditions are reported to affect the solid state of mannitol after freeze-drying [4,5]. The solid form of mannitol has in turn been reported to affect the stability of freeze-dried proteins [6]. The aim of this study was to investigate the effect of changing freeze drying parameters, applying in-situ freeze-drying in a variable temperature X-ray powder diffractometer (VT-XRPD).

2. Results and Discussion

The freeze-drying process can be subdivided into four steps; freezing, annealing, primary drying and secondary drying. As very little change was seen in the solid state of mannitol during primary drying, only the remaining three stages of the process are discussed here.

2.1 Freezing

The first step in the freeze-drying cycle is freezing. To study the effect of freezing rate, the sample solutions were frozen a three different rates to -50 °C, and diffractograms measured. Figure 1 shows that intermediate freezing rates caused crystallization of mannitol. The main solid form of mannitol seen after intermediate freezing was the hemi-hydrate indicated by the reflection at 18°2θ, this finding is in good agreement with literature reports [2,7]. The reflection at 20.5°2θ indicates that some δ-mannitol was also formed. As seen in Figure 1 fast and slow freezing rates caused no apparent mannitol crystallization. The lack of crystallization during fast freezing confirms results from other authors and was explained by the short time available for crystallization between freezing of water and reaching temperatures below the glass transition temperature (T_g) at - 32°C where crystallization is stopped or at least substantially slowed [8,9]. The absence of crystallization at low freezing rates could be caused by differences in the freeze-concentrate created at different freezing rates. Slow freezing possibly removes enough water from the freeze-concentrate to inhibit the formation of hemi-hydrate seen during intermediate freezing. Another interesting phenomenon observed with the VT-XRPD in-situ freeze drying, was the formation of ice crystals seen at higher °2θ values, this subject is however outside the scope of this paper.

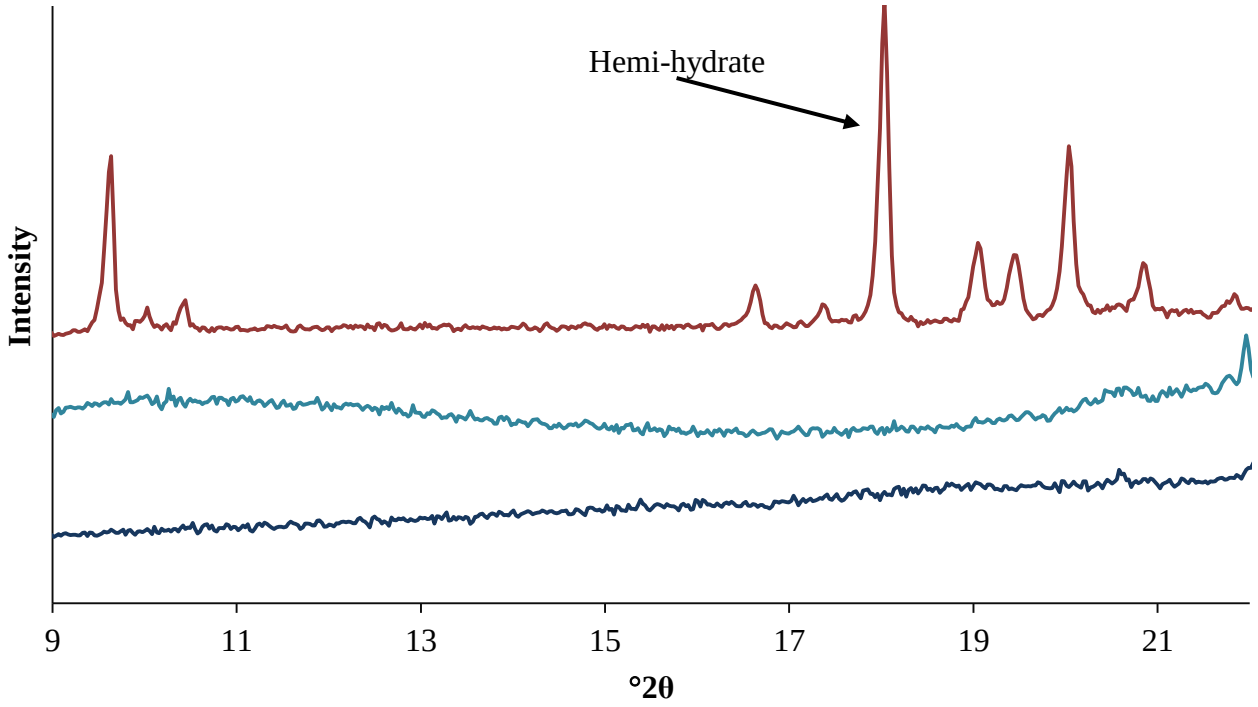

Figure 1. Effect of freezing the samples to - 50 °C at different freezing-rates, 10 °C/min(–), 0.5 °C/min(–) and 1 °C/min(–).

2.2 Annealing

After freezing an annealing step is sometimes included in the process to promote crystallization. To investigate the effect of annealing at different temperatures, the samples were heated from -50°C to -30 °C and -5°C and diffractograms were recorded for 90 minutes. The annealing step was in both cases found to result in the formation of δ-mannitol. The rate of crystallization was however found to be temperature dependent with higher temperatures resulting in faster crystallization. As seen in Figure 2 the δ-mannitol reflection at 20.5°2θ was not apparent after freezing to -50 °C. After heating to temperatures above T_g δ-mannitol was formed. The hemi-hydrate reflection at 18°2θ was however not reduced in intensity during 60 minutes of annealing. This observation indicates that formation of δ-mannitol during annealing is not a conversion of hemi-hydrate, like stated in the literature [4], but rather a crystallization process from amorphous mannitol.

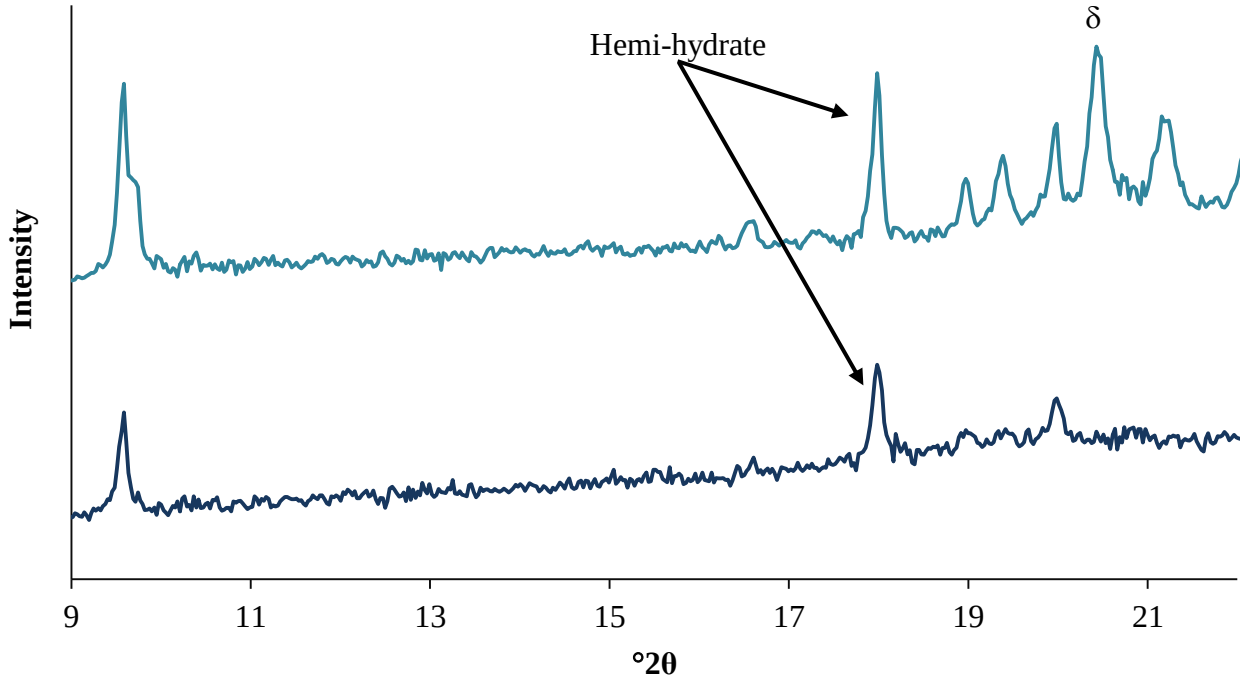

Figure 2. Sample frozen at 1 °C/min to -50°C (**–**) and the effect of annealing for 1 hour at – 30 °C (**–**). It is clearly seen that the hemi-hydrate reflection is not decreased in intensity after annealing.

2.3 Secondary drying

After primary drying, a secondary-drying step at higher temperatures is often incorporated in the freeze-drying cycle to minimize the residual humidity in the samples. Secondary drying at high temperatures is also reported to lead to dehydration of mannitol hemi-hydrate [10]. In this study, dehydration of mannitol hemi-hydrate was investigated at two different temperatures, 30°C and 40°C. Figure 3 shows that secondary drying at 30°C for four hours had little effect on mannitol hemi-hydrate. Secondary drying at 40°C for four hours lead to almost complete conversion of hemi-hydrate, indicating that if hemihydrate is unwanted in the samples, secondary drying should be performed above 30°C.

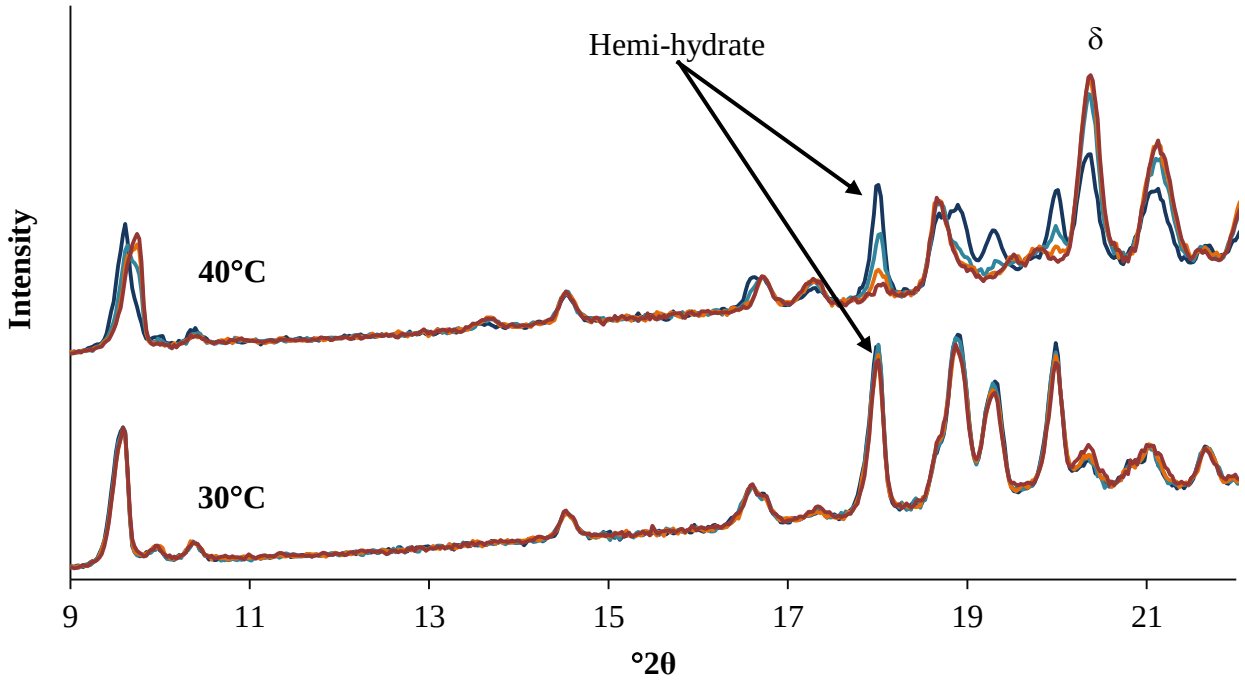

Figure 3. Effect of secondary drying for 1 hour (–), 2 hours (–), 3 hours (–) and 4 (–) hours.

3. Experimental

In-situ freeze-drying was carried out in the temperature stage of a PANalytical X'pert PRO (PANalytical B.V., Almelo, The Netherlands), connected to a vacuum pump. 100 µl of sample solution containing 100 mg/ml mannitol along with 3 mg/ml of the model protein ferritin was placed in the 0.2 mm deep sample holder. The samples were frozen to -50 °C at different rates from 0.5 °C/min to 10 °C/min. After the freezing the samples were annealed and dried under various conditions, applying vacuum to the temperature chamber. Diffractograms were continuously recorded during the process.

4. Conclusions

In-situ freeze-drying with the VT-XRPD was successfully used to provide insight into the solid state behavior of mannitol during the different stages of freeze-drying. Freezing experiments showed that the freezing rate had pronounced effect on the solid state of mannitol, with intermediate freezing causing crystallization of the hemihydrate form while fast and slow freezing resulted in amorphous mannitol. Annealing promoted crystallization of the δ from amorphous mannitol. Secondary drying caused conversion of mannitol hemi-hydrate to the δ form if conducted at 40°C for four hours.

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