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# Importance of Assessing Mannitol Crystallinity in Lyophilized Drug Products

WHITE PAPER EDITION

# Importance of Assessing Mannitol Crystallinity in Lyophilized Drug Products

## Introduction

Mannitol is a versatile excipient used in freeze-dried injectables in the pharmaceutical industry. Depending on the freezing and/or annealing conditions employed, mannitol is retained either as amorphous or crystalline. Crystalline mannitol is more prevalent in drug product formulations than amorphous mannitol. When crystalline, it provides an opportunity to conduct lyophilization cycles at aggressive conditions due to its high eutectic melting point (about -1.5°C). The resultant freeze-dried solids are pharmaceutically elegant in appearance with cake uniformity. Reconstitution of the dried solids is generally rapid and complete.

During lyophilization, mannitol either crystallizes spontaneously during the freezing step or an annealing step is included to encourage its crystallization. However, if mannitol remains amorphous or if crystallization is incomplete, it may crystallize during storage which may have serious consequences to the drug product stability due to changes in the residual moisture level associated with the conversion of amorphous to crystalline mannitol.

There are examples in the literature that demonstrate the impact of mannitol crystallization during storage and its detrimental effect on the stability of the product. Kreilgaard et. al. showed that solute (mannitol) crystallization during storage compromised stability of recombinant human factor XIII (Reference 1a), whereas amorphous solutes (sucrose and trehalose) provided the necessary protection of the protein. The authors observed increased aggregation in the presence of mannitol when it remained partially crystalline after lyophilization in their studies with Humicola lanuginose lipase (HLL). This was due to continued crystallization during storage, contributing additional

residual moisture to HLL for aggregation (Reference 1b).

An extensive study on the effect of amorphous mannitol on the solid-state stability of freeze-dried methylprednisolone sodium succinate was conducted by Herman et. al. at varying drug: excipient ratios (Reference 2). Stability of the freeze-dried solid was assessed during storage at 25 °C and 40 °C. When the API: mannitol ratio was 1:1, mannitol remained amorphous during freeze drying, which was ascertained using X-ray powder diffraction (Figure 1, A). But upon storage, crystallization of mannitol was observed, despite low residual moisture levels (1.0-1.5%) in the freeze-dried solids. Onset of crystallization of the δ-polymorph of mannitol was observed after 2 months of storage at 40 °C (Figure 1, B) and further crystallization after 6 months (Figure 1, C).

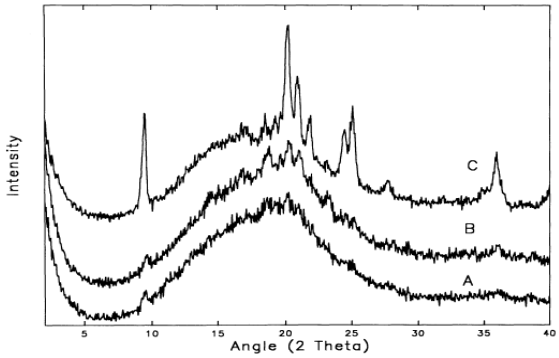


**Figure 1.** X-ray Powder Diffractograms of Formulation: (A) after freeze drying, (B) after 2 months of Storage at 40 °C, and (C) After 6 months of storage at 40 °C. Figure reproduced from Reference 4.

The importance of physical state of mannitol in the stability and aerosol performance of spray-dried recombinant humanized anti-IgE monoclonal antibody was investigated by Constantino and co-workers (Reference 3) for the treatment of allergic asthma via pulmonary delivery. Aqueous solutions of rhuMAbE25 containing various amounts of mannitol (0-40% w/w, dry basis) were spray dried. XRPD analysis indicated gradual crystallization of mannitol during storage at both 5°C and 30°C when varying concentrations of mannitol were used (Figure 2).

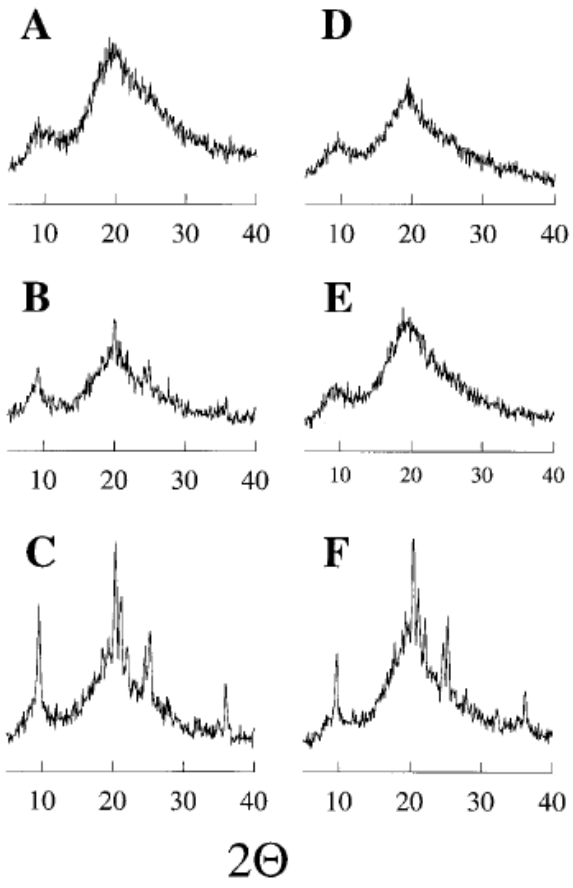


**Figure 2.** X-ray Powder Diffractograms of spray dried powders of rhuMAbE25 containing (A) 10%, (B) 20% and (C) 30% mannitol stored at 5 °C and (D) 10%, € 20%, and (F) 30% mannitol stored at 30 °C for 15 weeks. Data reproduced from Reference 5.

Mannitol was amorphous at both storage temperatures when used at 10% or 20% concentrations. At 30% concentration, crystallization was observed.

One of the consequences of mannitol crystallization was a decrease in the aerosol performance during storage, which was measured by Fine Particle Fraction (FPF) (Figure 3). The stability was assessed by soluble aggregate formation.

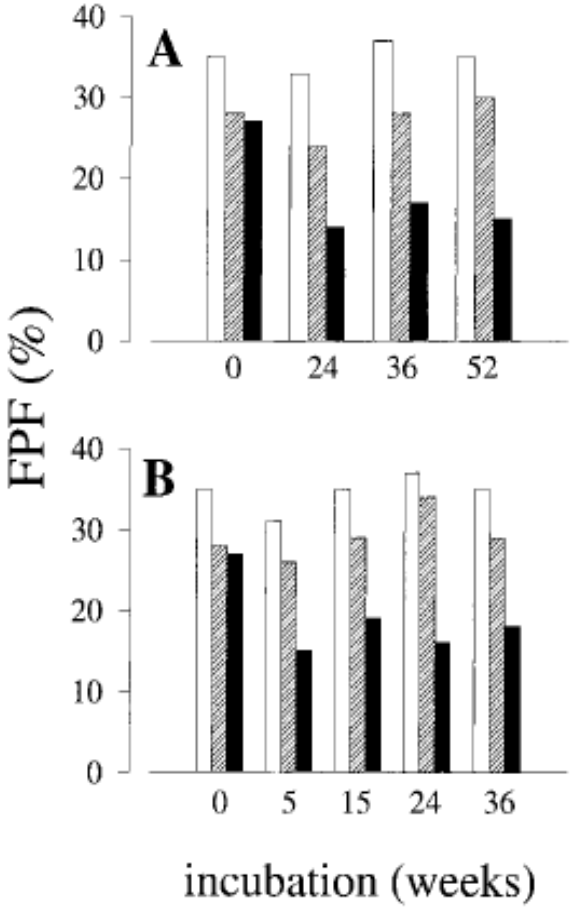


**Figure 3.** Aerosol performance of spray dried rhuMAbE25: mannitol stored as powder blends. (A) FPF at 5 °C of (open bars, 10%), (striped bars, 20%, and (filled bars, 30%) mannitol. (B) FPF at 30 °C of (open bars, 10%), (striped bars, 20%, and (filled bars, 30%) mannitol. FPF = Fine Particle Fraction. Data reproduced from Reference 5.

Initially, formulations containing 10-30% mannitol all exhibited a Fine Particle Fraction (FPF) of about 30%. The FPF was stable for samples containing 10%

and 20% mannitol during storage at both 5 °C and 30 °C, but a significant drop was observed in the 30% mannitol sample. Crystallization of mannitol likely increased the particle size and adversely impacted the aerosol performance. The authors also determined that the 30°C sample showed more aggregate formation than the 5°C samples.

One of the proprietary drugs that the R&D group at Baxter BioPharma Solutions conducted development studies for demonstrates an example of the influence of mannitol crystallization during storage on a lyophilized pharmaceutical product. The product contained mannitol, among other excipients. At insufficient concentration, mannitol crystallization was observed during storage at elevated temperatures when the residual moisture level was high (Figure 4). This resulted in the degradation of the drug product which was demonstrated by the formation of aggregates by Size Exclusion Chromatography (data not shown).

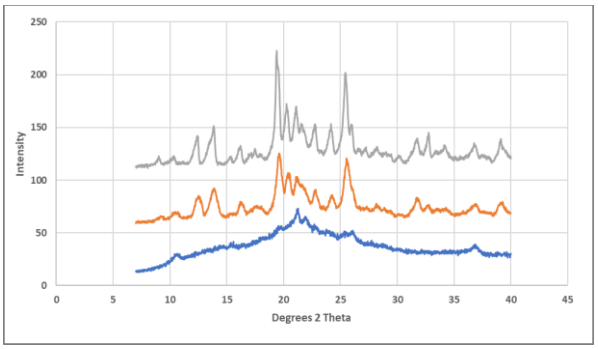


**Figure 4.** XRPD Diffractograms of a proprietary drug product: (a) initially, blue trace, (b after 2 months of storage at 40 °C, orange trace, and (c) after 4 months of storage at 40 °C, grey trace. Notice the characteristic mannitol crystallinity pattern in the stability samples.

There has been only one known product on the market where amorphous mannitol did not negatively impact the drug product stability. Dalvance® is a semi-synthetic lipoglycopeptide that is currently used for the treatment of acute bacterial skin and skin structure infections (ABSSSI) in adults. The formulation contains mannitol and API in the ratio of 1:3.9. The mannitol is present in the amorphous physical state in the freeze dried solid and the residual moisture is <0.5%. It remained amorphous during freezing and subsequently during storage and there has been no adverse effect of its crystallization reported in the literature.

## Practical Implication

The implication of mannitol crystallization during storage is the redistribution of residual moisture in the freeze-dried matrix and the availability of the residual moisture associated with the amorphous component to the drug. This may result in product destabilization. In addition, transfer of residual moisture from stopper to freeze-dried cake is a common phenomenon. Typically, this water is located in the amorphous component. If all of mannitol is crystallized, stopper moisture might transfer directly to the drug matrix causing chemical degradation.

The Research & Development group at Baxter BioPharma Solutions discourages the use of amorphous mannitol and recommends sucrose or trehalose for use as lyoprotectant as well as bulking agent. Our approach is to conduct low temperature thermal analyses using Differential Scanning Calorimetry (DSC) to assess mannitol crystallinity in a formulation. If mannitol crystallization is not observed, we include an annealing step to encourage its crystallization. Changes in its crystallinity, both as soon as the completion of the lyophilization cycle and during storage, is assessed using X-ray Powder Diffractogram (XRPD) to observe changes in the diffractogram pattern. We conduct these studies for small and large molecules as well as biologics routinely.

Our capabilities include a Q2000 DSC by TA Instruments, a Mettler Toledo DSC 3+, and a Bruker XRPD Instrument (Figure 5) to facilitate complete formulation development prior to lyophilization process development.


**Figure 5.** Bruker D2 Phaser Instrument housed in R&D of Baxter BioPharma Solutions.

## References


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