# Evaluating the Impact of Controlled Nucleation on Finished Product Attributes of Bulk Lyophilized Material


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## Introduction

Nucleation On-Demand Technology (NODT) is an innovative technology that has been applied to vials to improve the uniformity of nucleation of ice during freezing within the lyophilizer. NODT uses pressurization and depressurization of the chamber with an inert gas to initiate this uniform nucleation. Pressurization occurs after loading is complete, and depressurization occurs during cooling of the product to yield instantaneous, homogenous ice formation.

Investigations with bulk processing of model crystalline and amorphous systems were performed in an pilot size lyophilizer. Lyophilization cycles were executed for both model systems in bulk trays using either NODT or a conventional freezing method. The bulk material from both processes was then dried using the same primary and secondary drying temperatures, pressures, and times.

After the nucleation of ice by NODT, there was a prolonged hold at -5°C. This allowed for slower ice crystal growth and larger ice crystal size in the frozen matrix. Larger ice crystal sizes leaves larger pores which can reduce the time required for Primary Drying. During sublimation the larger pores result in reduced resistance in water vapor mass transport through the dried layer above the sublimation front, which results in increased sublimation rates.

The data presented offers insight into the effects of uniformity across a tray on freezing, drying behavior, and finished product attributes of processed bulk material characteristics using NODT compared to the conventional freezing method.

## Processing Materials and Methods

- 8 square-foot lyophilizer (Hull Model 8FS15C), 4 Shelves – internal condenser
- Nucleation On-Demand Technology
- Crystalline (Mannitol, USP) and Amorphous (Sucrose, NF) model formulation systems
- 12” x 24” x 2” Stainless Steel Bulk Trays (Four, 1 per shelf)
- 22 Calibrated Type T Thermocouples for temperature data collection (see Figures 1 and 2 below)

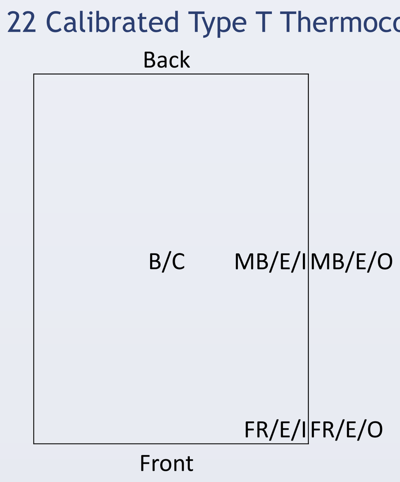



Figure 1: Thermocouples placed at the bottom on the inside and outside of the bulk trays on shelves 1 and 4 (Top view)

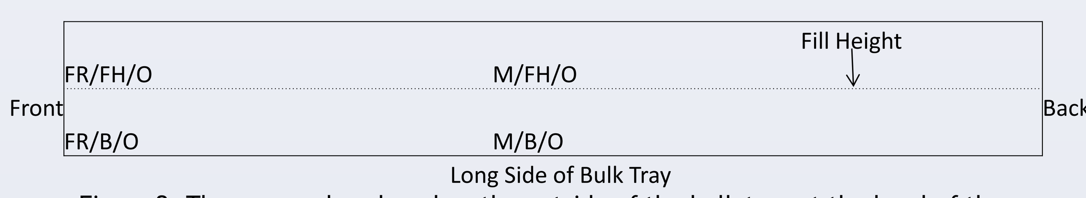



Figure 2: Thermocouples placed on the outside of the bulk tray at the level of the fill height and at the bottom on shelves 2 and 3 (Side view)

Table 1: Legend of the abbreviations used in the Figures and Graphs

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| M- | Mannitol | MB/E/I | Middle Bottom/Edge/Inside | FR/E/O | Front Right/Edge/Outside | FR/FH/O | Front Right/Fill Height/Outside |
| S- | Sucrose | MB/E/O | Middle Bottom/Edge/Outside | M/FH/O | Middle/Fill Height/Outside | FR/B/O | Front Right/Bottom/Outside |
| B/C | Bottom/Center | FR/E/I | Front Right/Edge/Inside | M/B/O | Middle/Bottom/Outside | | |

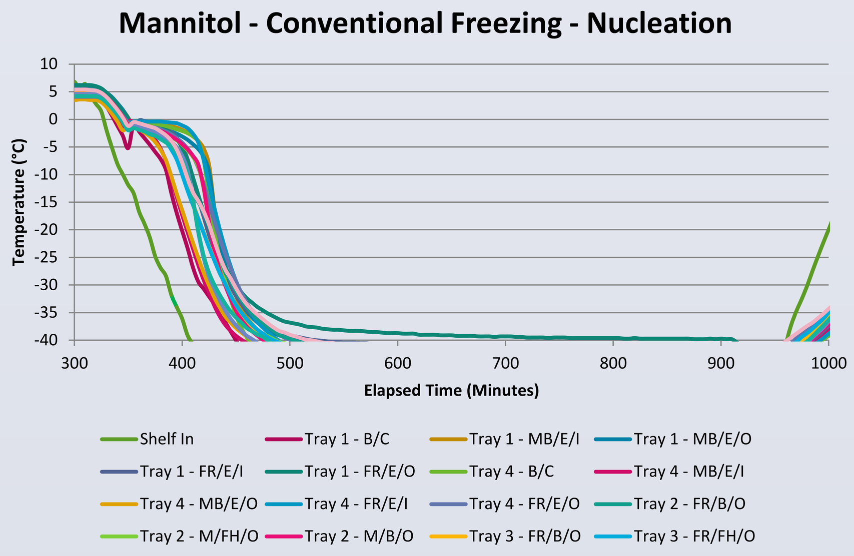
Procedure:

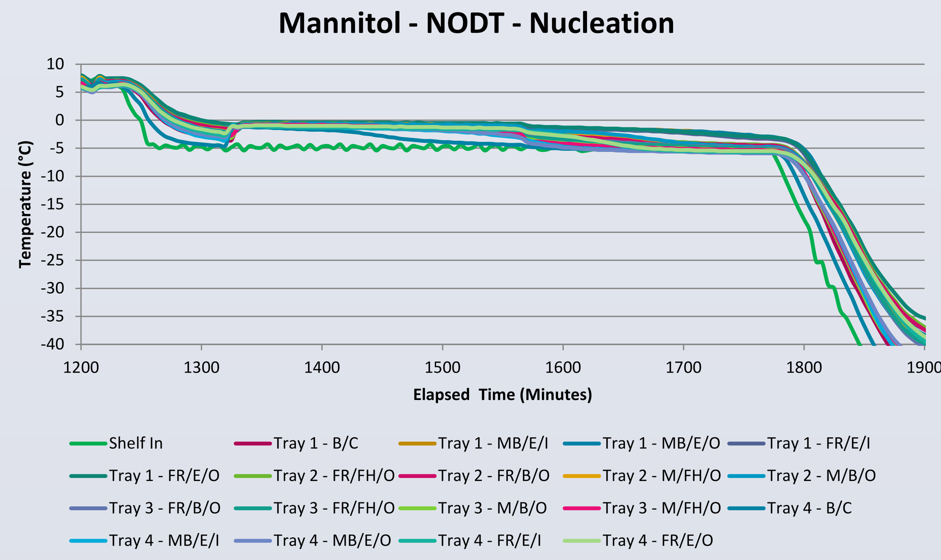
- Formulate Mannitol and Sucrose bulk solutions at 40 mg/mL and 0.22µm filter bulk solutions in a class 100 cleanroom.
- Dispense 2L of bulk solution into each tray.
- Place thermocouples in various locations inside and outside each stainless steel bulk tray. For thermocouples attached to outside of the bulk tray, place a small piece of insulation tape over the weld to minimize the influence of the chamber environment on the temperature measurement.
- Perform separate lyophilization cycles for each system with and without controlled nucleation.

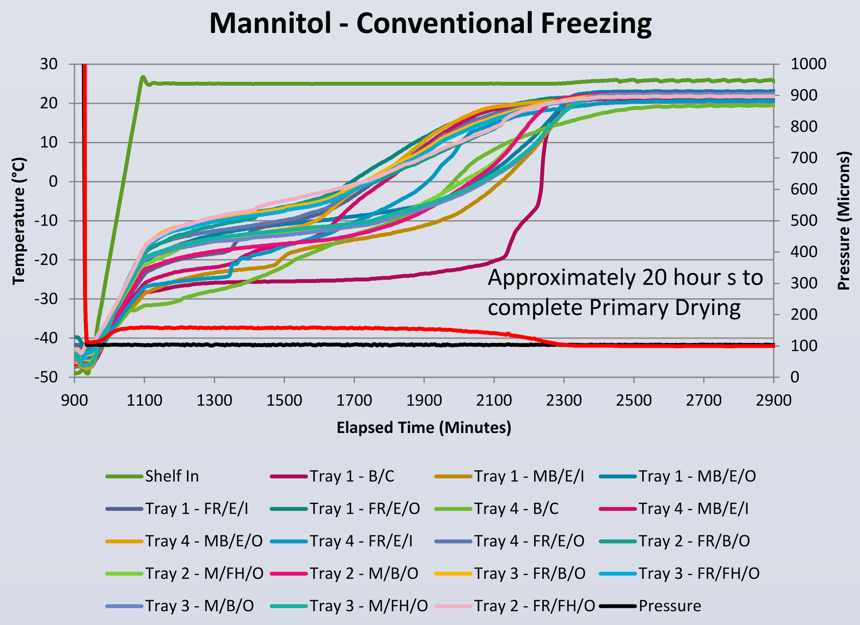
Table 2: NODT Parameters

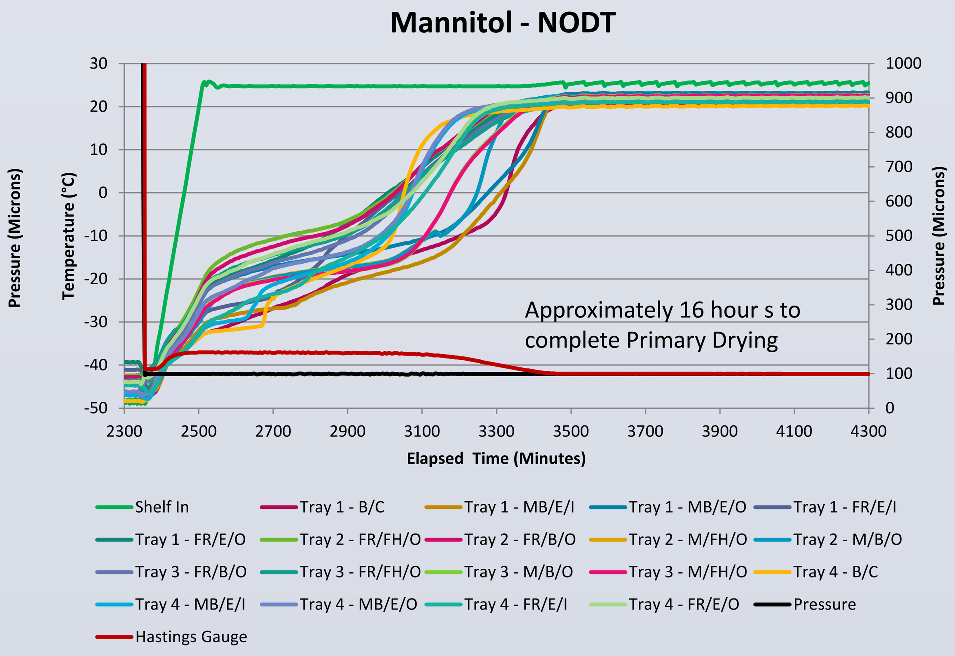
| Step | Shelf Temperature (°C) | Pressure (PSIG) | Hold Time (Hours) |
|---|---|---|---|
| Loading | 5 | 1 atm | n/a |
| Purge 1 | 5 | 12.5 | n/a |
| | | 2.5 | |
| Purge 2 | 5 | 12.5 | n/a |
| | | 2.5 | |
| Equilibration | -5 | 18.5 | 4 |
| Nucleation | -5 | 1.5 | n/a |
| Ice Crystal Growth | -5 | 1.5 | 7 |

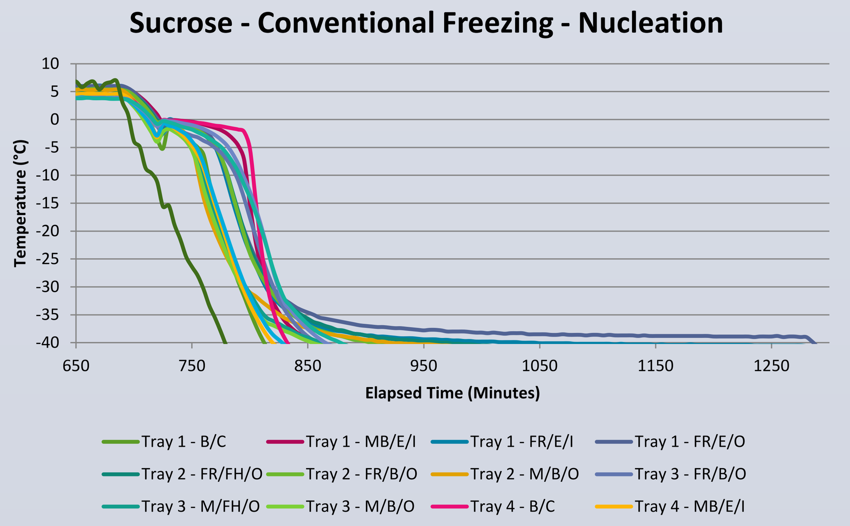
## Results

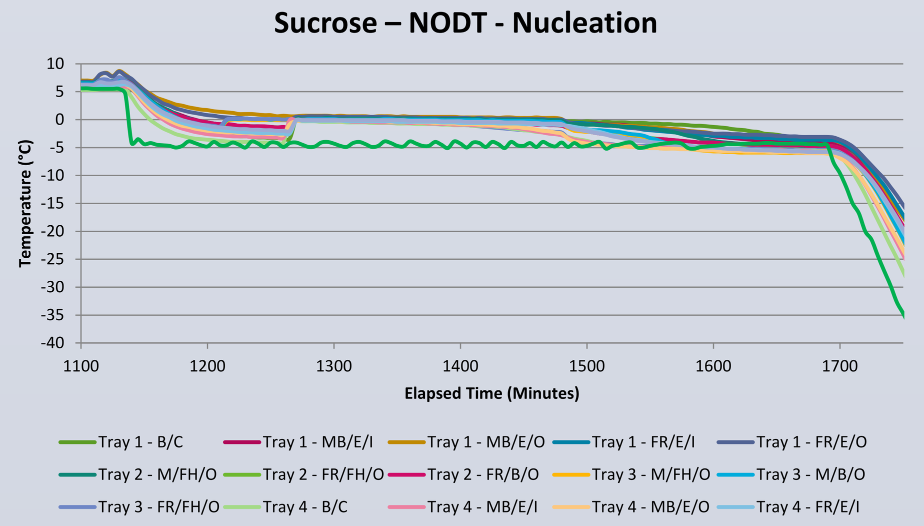


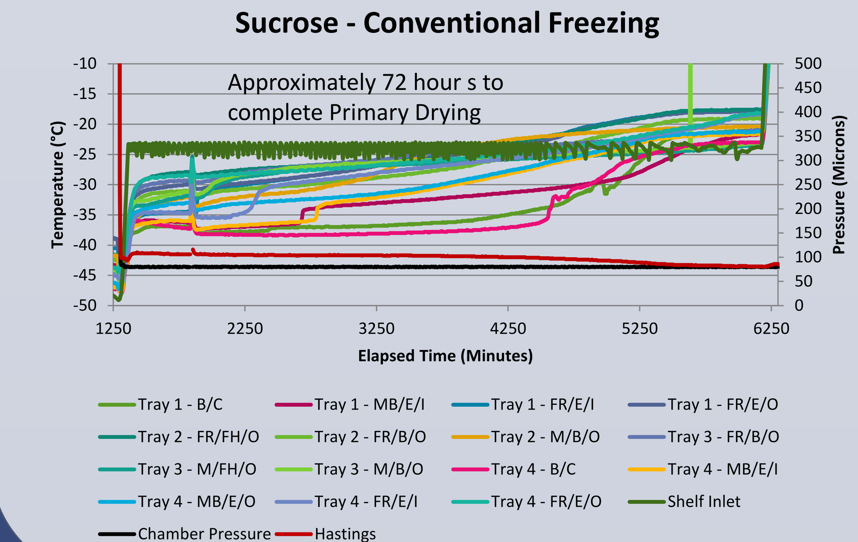



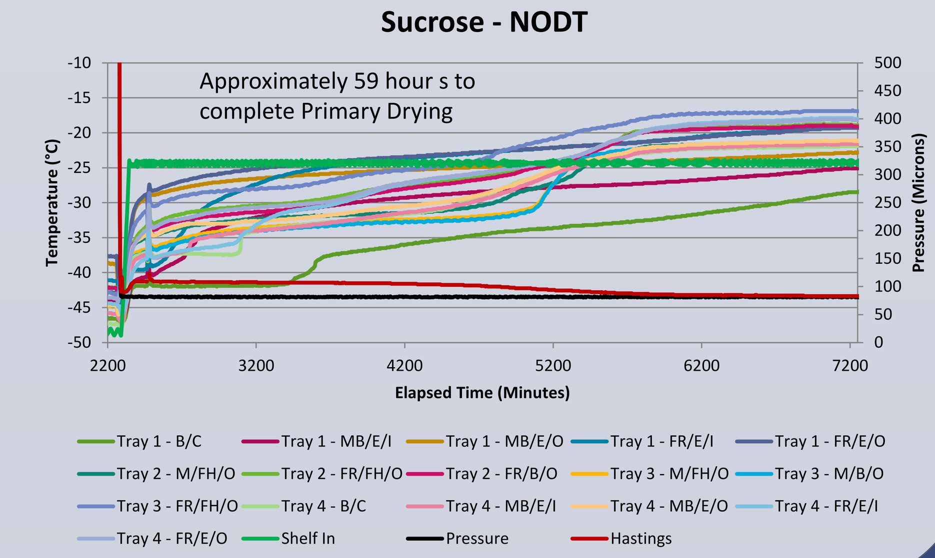




## Finished Product Testing Methods

After processing, the bulk trays were removed from the lyophilizer into a Nitrogen, NF purged isolator with a relative humidity controlled to approximately 10% to 15% to limit the amount of moisture uptake from the environment. The physical appearance was noted and pictures were taken of the bulk material. Only the material from Tray 2 was tested. The bulk material from Tray 2 was divided into 5 sections, the 4 corners and the center. Each section was then placed into a screw top HDPE container. The container was placed into a foil pouch, heat sealed, and then removed from the isolator. To perform residual moisture by coulometric Karl Fischer titration utilizing a solvent extraction method, 100 mg of material was aliquoted in a Nitrogen, NF purged glove box into cleaned and dried vials for each section of the tray. To perform high temperature differential scanning calorimetry material from each section of the bulk tray was sampled into non-hermetic DSC pan in a Nitrogen, NF purged glove box.

## Results

### *Physical Appearance and Pictures*

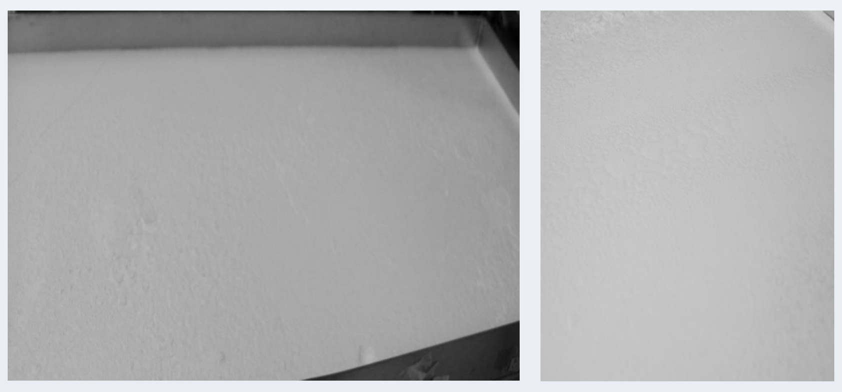


Figure 4: Mannitol Bulk Conventional Freezing

Figure 5: Sucrose Bulk Conventional Freezing

Figure 6: Mannitol Bulk NODT

Figure 7: Sucrose Bulk NODT

### *Residual Moisture by Coulometric Karl Fischer Titration*

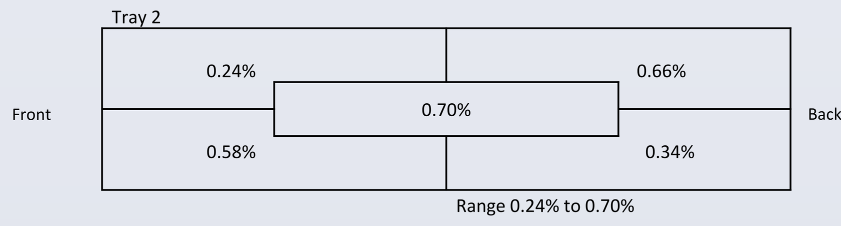



Figure 8: Mannitol – Standard Freezing

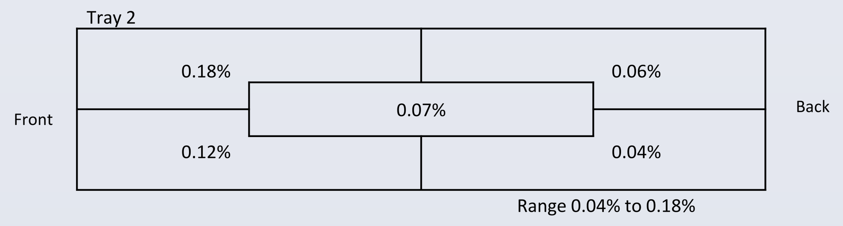



Figure 9: Mannitol – NODT

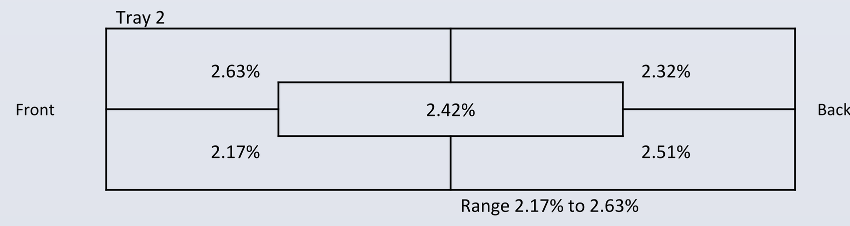



Figure 10: Sucrose – Standard Freezing

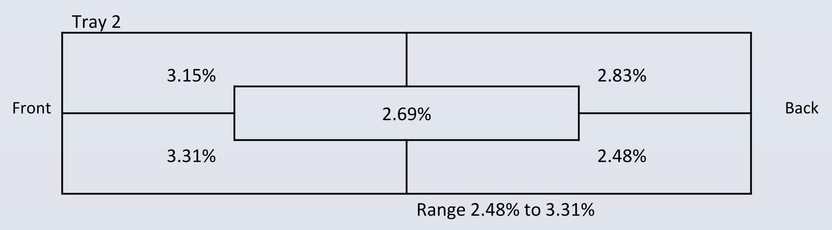



Figure 11: Sucrose – NODT

### *High Temperature Differential Scanning Calorimetry*

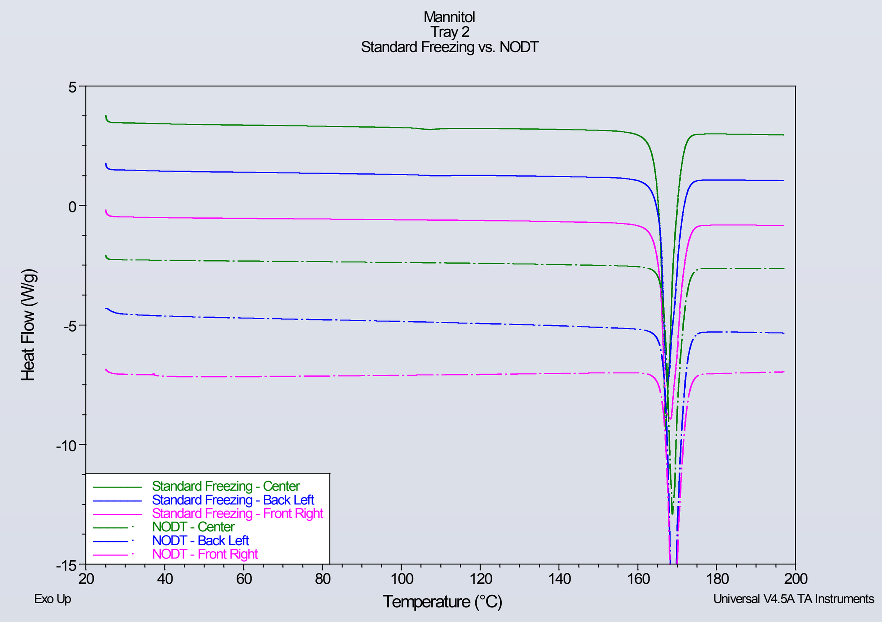



Figure 12: Mannitol – Overlay of DSC Results

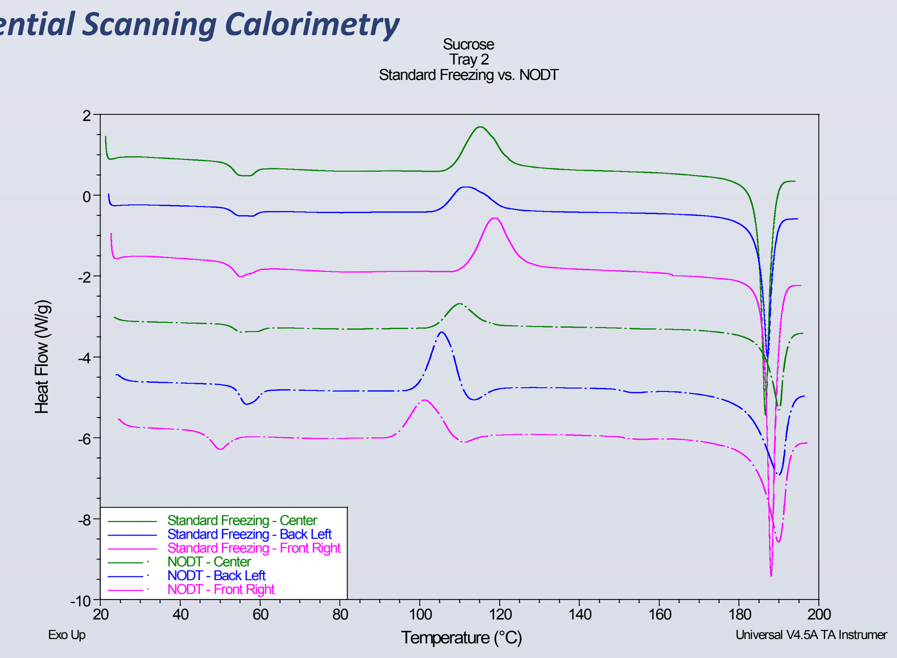



Figure 13: Sucrose – Overlay of DSC Results

## Summary of Results

- Sucrose, when processed after NODT, completed the sublimation of ice and reached the shelf temperature in approximately 59 hours. Sucrose, processed with a conventional freezing ramp, completed the sublimation of ice and reached the shelf temperature in approximately 72 hours. NODT resulted in faster rates of sublimation, most likely attributed to larger ice crystal size.
- Mannitol, when processed after NODT, completed the sublimation of ice and reached the shelf temperature in approximately 16 hours. Mannitol, processed with a conventional freezing ramp, completed the sublimation of ice and reached the shelf temperature in approximately 20 hours. NODT resulted in slightly faster rates of sublimation, most likely attributed to larger ice crystal size.
- Physical appearance of Mannitol processed with a standard freezing ramp revealed a slightly textured top surface with no cracks (Figure 4). Physical appearance of Mannitol processed with NODT revealed a textured top surface with cracks (Figure 6).
- Physical appearance of Sucrose when processed with a Standard Freezing ramp revealed a sheen top surface with numerous large cracks (Figure 5). Physical appearance of Sucrose processed with NODT revealed a sheen top surface with numerous cracks and a transparent skin (Figure 7).
- Residual Moisture results revealed a lower moisture content for Mannitol when processed with NODT compared to a standard freezing ramp (Figures 8 and 9). Sucrose did not follow the same trend as the moisture content when processed with NODT was higher then the material processed with a standard freezing ramp (Figures 10 and 11).
- HT-DSC results for Mannitol revealed consistent reproducible transitions regardless of the processing method (Figure 12).
- HT-DSC results from Sucrose revealed consistent reproducible transitions however the temperatures of the transitions where slightly lower for the material processed by NODT, most likely attributed to the higher residual moisture content (Figure 13).

## Conclusion

- When using aggressive critical processing parameters there appears to be no benefit of employing NODT as the time to complete Primary Drying was similar compared to conventional freezing. However, when using conservative critical processing parameters there was a significant reduction in the time to complete Primary Drying when employing NODT compared to conventional freezing.
- For products that would behave similar to Mannitol, the results revealed lower residual moisture content and a decreased range in the results. For products that would behave like Sucrose, there was an increase in residual moisture content and greater range in the results.
- The potential benefits of using NODT would have to be evaluated for each product. The effects of NODT on finished product attributes is not straightforward and the benefits will depend on the composition of the formulation.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