

Assessment of ControlLyo® Technology for Improved Cake Morphology and Cycle Efficiency

1. Introduction

Lyophilisation is a widely used technique in pharmaceutical and biotechnological industries to preserve aqueous products. Traditional freeze-drying cycles suffer from prolonged drying times, often exceeding 72 hours, which can be partly due to random ice nucleation leading to heterogeneous crystal sizes. The inconsistency in ice crystal formation can also result in defects such as cake collapse, bubble entrapment, and increased residual moisture, all of which impact product stability and efficacy. Such extended drying times are suboptimal for pharmaceutical manufacturing, as they lead to higher energy consumption, reduced equipment availability, and, consequently, limited production throughput. While there is no universally fixed 'ideal' drying time, reducing the duration as much as possible - without compromising critical product attributes such as cake morphology and residual moisture - is essential for both efficiency and product quality. ControlLyo® technology has been proposed to induce uniform ice nucleation, potentially reducing drying time and improving product consistency. This study aimed to evaluate the effectiveness of controlled nucleation at different temperatures within the cycle and analyse the impact on product characteristics, focusing on optimising parameters for large-scale pharmaceutical manufacturing. While the primary focus is on pharmaceutical applications, the findings may also have broader relevance for other industries relying on freeze-drying - such as diagnostics and food technology - where consistent drying and product stability are critical.

2. Materials and Methods

2.1 Lyophilisation Cycle Parameters

The product under investigation is an aqueous formulation filled into 6R vials with a 1 mL fill volume, with a very low solid content of <1.5 % w/v. The primary drying time in the existing freeze-drying cycle is 78 hours without ControlLyo® and the collapse temperature is -38.3°C.

2.2 Controlled Nucleation Integration

The ControlLyo® nucleation step was incorporated at different temperatures to assess the impact on Cake morphology and drying efficiency. The experiments were conducted with controlled nucleation at -4°C, -8°C, -15°C, and -20°C, with varying hold times to determine the optimal conditions.

To ensure a valid comparison, the following parameters were held constant across all experimental cycles: Final Freezing Temperature, Primary Drying Shelf Temperature and Ramp Rate, Chamber Pressure, and Secondary Drying parameters.

Prior to the nucleation, a 60-minute equilibration hold was

maintained to ensure the product reached thermal stability. Product probes placed at multiple locations confirmed that the vials reached a steady-state plateau near the shelf set point. We observed that the offset between the probe and the shelf was minimized at lower nucleation temperatures (e.g., -8°C, -15°C and -20°C).

After we triggered the nucleation, we tested different hold times before moving to the final freezing temperature of -45°C.

3. Observations

3.1 Effect of Nucleation at -4°C

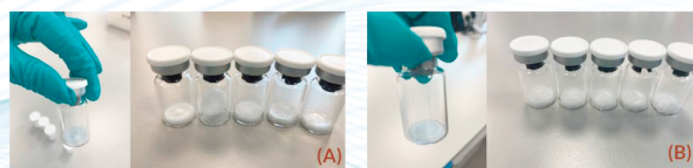


Figure 1 (A) Controlled Nucleation at -4°C with a hold time of 20 minutes. (B) Controlled Nucleation at -4°C with hold time of 180 min. In both conditions, cakes were partially collapsed, and crystallised particles were visible. The appearance did not meet the acceptable criteria

To ensure a valid comparison, the following parameters were held constant across all experimental cycles: Final Freezing Temperature, Primary Drying Shelf Temperature and Ramp Rate, Chamber Pressure, and Secondary Drying parameters.

3.2 Effect of Nucleation at -8°C



Figure 2 Controlled Nucleation at -8°C with a hold time of 20 minutes. Large bubbles were observed on the cake surface, indicating poor cake structure. The appearance did not meet the acceptance criteria.

In this cycle, controlled nucleation was initiated at a shelf temperature of -8°C. The vials were held at this temperature for 60 minutes to ensure thermal equilibration before the pressure/depressurisation was triggered. Following nucleation, an additional hold was performed—20 minutes. After this hold, the shelf was ramped down to the final freezing temperature at a rate of 1°C/min.

Assessment of ControLyo® Technology for Improved Cake Morphology and Cycle Efficiency

3.3 Effect of Nucleation at -15°C



Figure 3 Controlled Nucleation at -15 °C with a hold time of 20 minutes. White to off-white cake with no bubbles meeting the predefined acceptance criteria, suggesting that the applied process parameters led to improved cake structure and stability

In this cycle, controlled nucleation was initiated at a shelf temperature of -15°C. The vials were held at this temperature for 60 minutes to ensure thermal equilibration before the pressure/depressurisation was triggered. Following nucleation, an additional hold was performed for 20 minutes. After this hold, the shelf was ramped down to the final freezing temperature at a rate of 2°C/min. This higher ramp rate was deliberately selected for these later cycles to rapidly 'lock in' the ice structure and prevent Ostwald ripening which can happen the higher temperature based on the literature.

3.4 Effect of Nucleation at -20°C



Figure 4 Controlled Nucleation at -20°C with a hold time of 20 minutes. White to off-white cake with no bubbles, smooth and glossy appearance, meeting the predefined acceptance criteria, suggesting that the applied process parameters led to improved cake structure and stability.

In this cycle, controlled nucleation was initiated at a shelf temperature of -20°C. The vials were held at this temperature for 60 minutes to ensure thermal equilibration before the pressure/depressurisation was triggered. Following nucleation, an additional hold was performed for 20 minutes. After this hold, the shelf was ramped down to the final freezing temperature at a rate of 2°C/min. This higher ramp rate was deliberately selected for these later cycles to rapidly 'lock in' the ice structure and prevent Ostwald ripening which can happen the higher temperature.

4. Summary

Table 1 Summary of drying performance

Nucleation Temperature	Hold Time	Cake Appearance	Primary Drying Time (hrs) - PG/CM*	Moisture content of cake (%) as determined by KF (Acceptable target <3%)
Uncontrolled Nucleation	----	Uniform, no bubbles. Complies to specification	78 hours	Conservative cycles
-4°C	20 min	Appearance not meeting the specification	42 hours	Not tested**
-4°C	180 min	Appearance not meeting the specification	39 hours	Not tested**
-8°C	20 min	Appearance not meeting the specification	40 hours	Not tested**
-15°C	20 min	Uniform, no bubbles. Complies	54 hours	1.4%
-20°C	20 min	Glossy, stable cake. Complies	49 hours	1.2%

**End point of primary drying is determined by Comparative pressure measurement (i.e., Pirani Gauge (PG) vs. capacitance manometer (CM))*

***Testing was not continued as the appearance was not acceptable*

While the initial study aimed to explore both nucleation temperature and hold time, the results showed that temperature had a more pronounced effect on cake morphology. As a result, subsequent experiments focused on temperature, with plans to re-evaluate hold time under favourable conditions. In addition, future experiments will also examine cooling ramp rate after ControLyo® to address future scalability of this thermal treatment at production scale.

Assessment of ControLyo® Technology for Improved Cake Morphology and Cycle Efficiency

5. Conclusion

In this study, we have shown that, by integrating a ControLyo® nucleation step into the freezing stage, we can successfully reduce the primary drying time of a conventional cycle from 78 hours to 54 hours (31% reduction in primary drying time), demonstrating a significant process improvement for pharmaceutical manufacturing. Although shorter drying times were observed at higher nucleation temperatures (-4 °C, -8 °C), these conditions produced unacceptable cake structures. In contrast, lower nucleation temperatures (-15 °C to -20 °C) provided both efficient drying and robust cake morphology, with final moisture content consistently below 3%.

The observations in this study contrast with the existing literature, which generally suggests that higher controlled nucleation temperatures (-5°C to -8°C) facilitate faster sublimation by forming larger ice crystals, thereby reducing mass transfer resistance and improving drying kinetics (Rambhatla et al., 2004). However, in this case, lower nucleation temperatures (-15°C to -20°C) were found to be more effective in achieving better cake stability and preventing collapse. This outcome suggests that, despite the drying efficiency, higher nucleation temperatures may compromise product quality in certain formulations. Therefore, lower nucleation temperatures appear preferable in this specific case, prioritising cake stability over drying speed. One potential explanation is the low solid content and high water content in the formulation may have caused excessive ice crystal growth at higher nucleation temperatures, leading to poor cake structure. This discrepancy highlights the need for further investigation into the underlying mechanisms responsible for such differences, particularly in relation to formulation properties such as solid content.

It is important to note that these significant time reductions were achieved using a standard, non-optimised cycle. Notably, nucleation at -15 °C appeared to provide an optimal balance between drying efficiency and cake quality in this study. Nucleation at -20 °C gave comparable results, with a slightly shorter primary drying time (49 hours), suggesting that lowering the nucleation temperature beyond -15 °C may offer limited incremental advantage for this formulation. However, this observation may be formulation-dependent and warrants further investigation. Therefore, we propose additional studies to investigate nucleation robustness across different processes and conditions. Future investigations could explore whether adjusting the equilibration time before nucleation or extending hold times at lower temperatures could yield even better results. Furthermore, ongoing studies aim to assess whether comparable cycle time reductions can be attained through process optimisation alone, or if applying ControLyo® to an already optimised cycle could lead to even more significant gains in efficiency.

References

1. Rambhatla, S., Ramot, R., Bhugra, C., & Pikal, M. J. (2004). Heat and mass transfer scale-up issues during freeze-drying II. Control and characterization of the degree of supercooling. *AAPS PharmSciTech*, 5(4), 54–62