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Effect of Freezing on Lyophilization Process Performance and Drug Product Cake Appearance

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ABSTRACT

This study highlights the significance of the freezing step and the critical role it can play in modulating process performance and product quality during freeze-drying. For the model protein formulation evaluated, the mechanism of freezing had a significant impact on cake appearance, a potential critical product quality attribute for a lyophilized drug product. Contrary to common knowledge, a freezing step with annealing resulted in 20% increase in primary drying time compared to without annealing. In addition, annealing resulted in poor cake appearance with shrinkage, cracks, and formation of a distinct skin at the top surface of the cake. Finally, higher product resistance (7.5 cm^2.Torr.hr/g) was observed in the case of annealing compared to when annealing was not included (5 cm^2.Torr.hr/g), which explains the longer primary drying time due to reduced sublimation rates. An alternative freezing option using controlled ice nucleation resulted in reduced primary drying time (i.e., 30% reduction compared to annealing) and a more homogenous batch with elegant uniform (i.e., significantly improved) cake appearance. Here, a mechanistic understanding of the distinct differences in cake appearance as a function of freezing mechanism is proposed within the context of ice nucleation temperature, ice crystal growth, and presumed solute distribution within the frozen matrix.

Introduction

Freeze-drying is a commonly used unit operation to achieve acceptable shelf life for an otherwise unstable molecule. Freeze-drying is usually carried out in three steps: freezing (i.e., conversion of water into ice), primary drying (i.e., removal of ice by sublimation at low temperature and pressure), and secondary drying (i.e., removal of unfrozen water). Typically, water content at the end of the secondary drying step is $\leq$1%.

The ice nucleation temperature (or degree of supercooling) is a potential critical process parameter during the freezing step.[1] The pore structure in the dried matrix is a reflectance of how the solution was frozen with every 1°C increase in ice nucleation temperature resulting in a 1%-3% reduction in primary drying time.[2] A higher degree of supercooling results in smaller ice crystals, which results in higher product resistance and longer primary drying time but shorter secondary drying (due to higher specific surface area [SSA]). Thus, the freezing step affects not only primary drying but also secondary drying. The freezing step of the freeze-drying process has received significant attention in the last decade because this first step of the process governs not only the performance of the subsequent steps (i.e., primary and secondary drying) but also the product quality (i.e., physical stability, cake appearance, residual moisture, reconstitution time).[3] The ice nucleation temperature, random or stochastic in nature, is also an important process development and scale-up issue. Vials within a batch may vary in ice nucleation temperature from as high as −2°C to as low as −18°C. Thus, there is heterogeneity in ice nucleation temperature not only within a batch but also from batch to batch. An additional consideration is the clean room (Class 100) environment in manufacturing compared to laboratory bench preparation for a lab-scale dryer, resulting in differences in ice nucleation temperature.[2]

Annealing during freezing is often a common approach to mitigate heterogeneity in ice nucleation temperature, reduce primary drying time,[3] and crystallize any crystallizing excipients in the formulation.[4] During annealing, product temperature is maintained, for a certain period of time, above the glass transition temperature (Tg') but below the melting temperature of the formulation matrix, resulting in Ostwald ripening (i.e., growth of bigger ice crystals at the expense of smaller ones). Although optimization of annealing time and temperature is critical, annealing

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may not always be suitable from a product quality standpoint due to conformational changes in protein structure or amorphous-amorphous phase separation.[5,6] In addition, for formulation matrices wherein crystallization of solute is involved, annealing may not always result in shorter primary drying time (i.e., lower dry layer resistance).[7] Nonetheless, annealing during freezing is a way to mitigate the heterogeneity in ice nucleation temperature. Other alternative options have become available in the recent past to control ice nucleation temperature.[8-11] The effect of ice nucleation temperature on process performance (i.e., primary drying time) using controlled ice nucleation techniques is well established.[1,3,12,13] The SSA of the dried matrix is a good surrogate of the initial freezing characteristics of a solution. Higher ice nucleation temperature results in lower SSA and hence shorter primary drying time. Developing an understanding of the effect of controlled ice nucleation on product quality is now becoming critical to enable controlled ice nucleation techniques as process analytical tools to control ice nucleation temperature in clinical and commercial batches.

This work, presented as an industry case study, assesses the negative effect of annealing on cake appearance and demonstrates how controlled ice nucleation results in significant improvement in cake appearance as well as batch homogeneity. Cake appearance is a potential critical product quality attribute with a specification both for lot release and stability. The finished product is visually inspected (100%) for cake appearance because a change or variability in cake appearance from what is anticipated is likely an indication of change in product quality. Although the acceptance criterion for cake appearance is outside the scope of this work, the study here clearly demonstrates the significance of the freezing step and its impact on cake appearance.

Materials and Methods

Materials

The model protein (referred as Protein X) was produced and purified at MedImmune (Gaithersburg, MD). Protein X is a glycoprotein with a size of ~75 kDa, formulated at 10 mg/mL in a citrate-based buffer containing trehalose dihydrate, an arginine/arginine-HCl mixture, and polysorbate 80 (PS80) as excipients. All excipients used were of compendial pharmaceutical grade.

Lyophilization Process Parameters

For all freeze-drying experiments, 3.5 mL of 0.22-μm filtered Protein X formulation was filled aseptically into 10-mL vials and partially stoppered with 20-mm stoppers (both the vials and stoppers were autoclaved and sufficiently dried before use). The freeze-drying cycles were performed on a Lyostar 3 freeze-dryer (SP Scientific, Stone Ridge, NY) at 20% of total lyophilizer shelf load. A 15-min equilibration at 5°C was used before initiation of the freeze-drying cycle. Although different freezing methods were used (Table 1), the primary drying (−30°C, 100 mTorr) and secondary drying (40°C, 100 mTorr) steps were kept identical for all the cycles tested. The pressure was controlled using a capacitance manometer gauge. The duration of the secondary drying step was fixed at 6 h in all the freeze-drying cycles whereas the duration/end of the primary drying step was determined by a comparative pressure measurement wherein the Pirani gauge pressure measurement converges, within 10 mT, with that of the capacitance manometer chamber pressure.[14,15] The product temperature during freeze-drying was monitored using thermocouples (TCs) and also determined via Manometric temperature measurement (MTM). A total of 4 TCs were placed at the bottom center of selected vials located at the edge (2 vials) and center (2 vials) of each shelf. The TCs were distributed across the shelf such that any variation in product temperature as a function of shelf location was monitored.

To evaluate the impact of the freezing step on overall process efficiency and product quality, the freeze-drying cycles were designed to differ in the freezing step. One of the freeze-drying cycles included an annealing step with a temperature set point of −15°C (referred to as Anneal), whereas the other cycle used the ControLyo™ Technology[10] for a controlled ice nucleation step (referred as CN) at a temperature set point of −8°C in place of annealing. Finally, a third cycle without either annealing or controlled ice nucleation was evaluated (referred to as No Anneal). The freeze-drying process parameters for all three cycles are summarized in Table 1.

Determination of Product Resistance and Product Temperature Using MTM Measurement

MTM involved closing of the valve connecting the chamber and the condenser for a brief period (~25 s) and recording the pressure rise as a function of time. The pressure rise data were fitted to the MTM equation to determine product temperature at the sublimation interface, product resistance, and vial heat transfer coefficient.[16-19]

Differential Scanning Calorimetry

Differential Scanning Calorimeter Q2000 series from TA instruments (New Castle, DE) was used to determine the glass transition temperature (Tg'). For the Tg' measurement, 20 μL of liquid sample was added into an aluminum pan and sealed hermetically. An empty pan and lid were used as the reference pan. The sample was frozen to −60°C and then heated to 25°C at a rate of 5°C/min. The Tg' value was determined using Universal Analysis software (TA instruments, New Castle, DE) and reported as the midpoint of the glass transition.[20]

Table 1
Lyophilization Process Parameters for the Anneal, No Anneal, and Controlled Ice Nucleation (CN) Cycle

Process Parameter/Cycle	Anneal Cycle	No Anneal Cycle	CN Cycle
Ramp to freezing	1°C/min	1°C/min	1°C/min
Freezing temperature/hold time	−40°C/4 h	−40°C/4 h	−40°C/4 h
Annealing temperature/hold time	−15°C/2 h	NA	NA
Controlled ice nucleation temperature/hold time	NA	NA	−8°C/4 hr
Ramp to primary drying	0.1°C/min	0.1°C/min	0.1°C/min
Primary drying temperature/pressure/hold time	−30°C/100 mTorr/Pirani	−30°C/100 mTorr/Pirani	−30°C/100 mTorr/Pirani
Ramp to secondary drying	0.3°C/min	0.3°C/min	0.3°C/min
Secondary drying temperature/pressure/hold time	40°C/100 mTorr/6 h	40°C/100 mTorr/6 h	40°C/100 mTorr/6 h