Lyophilization Development of Pharmaceutical Products

Authors: Daniel Schenck, Joel Hoekstra, Chaoju Xiao, Arunya Usayapant, Amina Faham*

Development and Commercial Capabilities at Fresenius Kabi

Fresenius Kabi USA's research and development facility in Melrose Park, IL is well equipped to perform all activities required to develop lyophilized drug products at laboratory scale. Our scientists have many years of experience in lyophilized product development and scale-up, as evidenced by our portfolio of approved and marketed commercial lyophilized products. As an active research and development facility that houses Fresenius Kabi USA's formulation and analytical development teams, we are experts at preparing bulk product solutions in the laboratory prior to the lyophilization process. Our site has extensive experience in the preparation and characterization of such formulations based on the specific requirements of the drug product.

Our R&D facility is equipped to characterize bulk product solution to determine critical product temperatures for the optimization of the lyophilization cycle. We use freeze dry microscopy (FDM) and differential scanning calorimetry (DSC) to identify the critical temperatures prior to initial lyophilization trials. To perform lyophilization cycle development studies, Fresenius Kabi USA utilizes VirTis Genesis pilot-scale lyophilizers equipped with Pirani gauges to monitor and optimize primary drying efficacy during cycle development. These lyophilizers are also equipped with multi-shelf hydraulic stoppering systems and can be used to prepare hundreds of stoppered product vials in a single run.

Through the establishment of an appropriate design space, Fresenius Kabi USA can produce and characterize sufficient lab-scale batches to identify optimal and scalable freeze-dry cycle parameters for the product. The suitability of products within the cycle design space is assessed through extensive testing of lyophilized products using methods that can include assay %, impurity levels, moisture content, visual inspection, XRD, DSC, and polarized light microscopy, to name a few. Such testing is supported by Fresenius Kabi USA's in-house analytical development team.

Lastly, we at Fresenius Kabi USA's R&D site have experience in lyophilized product scale-up to our various manufacturing sites for both ICH (International Council of Harmonization of technical requirements for human use) stability batches and commercial product launches. We understand the challenges of lyophilized product scale-up and are well positioned to overcome any such issue that may arise.

Lyophilization of Pharmaceutical Drug Products

Lyophilization (freeze drying) is the process of removing water from a frozen solution under a vacuum while maintaining its solid structure. Lyophilization is a commonly used technique in the preparation of certain pharmaceutical drug products.

Why perform lyophilization?

In pharmaceuticals, lyophilization is performed to stabilize formulations that are unstable in solution due to hydrolysis, physical instability, or other degradation pathways. Lyophilization is a commonly used processing strategy in the production of parenteral drug products for therapeutic peptides, proteins, and other biologics which can be prone to such instabilities. Lyophilization can also be used to improve the stability of formulations which interact with their container closure systems, thereby reducing potential adsorption, glass delamination, or other unwanted interactions in some cases. Lyophilization produces a dry and stable powder of target formulations that can be more easily transported as a finished drug product. Lyophilization can also be used to produce stable intermediates for manufacturing of drug products.

* Senior VP of Global Research & Development, Fresenius Kabi LLC
Amina.Faham@fresenius-kabi.com

How do excipients play a role in lyophilization?

The choice of excipients in the drug product formulation is critical to ensure that lyophilization of the drug product meets requirements for visual appearance and physicochemical stability.

A common class of excipients used to prepare lyophilized formulations are bulking agents. A bulking agent adds mass to the formulation, especially when the drug concentration is low. It can provide structural support during lyophilization, improving the efficiency of primary and secondary drying, providing mechanical strength to the cake, and promoting the appearance of a pharmaceutically elegant product. Common bulking agents include mannitol, sucrose, trehalose, and glycine, to name a few. These excipients (among others) can also serve as cryoprotectant stabilizers in formulations which can improve physicochemical stability of the drug molecule during lyophilization. Other excipients in a product formulation can include buffers, tonicity modifiers, or surfactants. The specific composition will depend on the requirements and sensitivities of the drug product, but such excipients have been widely studied for use in the lyophilization of drug products.

What are the steps of lyophilization?

Filling of vials:

The product solution is formulated and filled into vials. Specially designed vented stoppers are then placed onto the vials which will allow the water to escape from the vial during the freeze-drying process. The vials are placed onto trays which are then loaded into the lyophilizer for the initiation of freeze drying.

Freezing step:

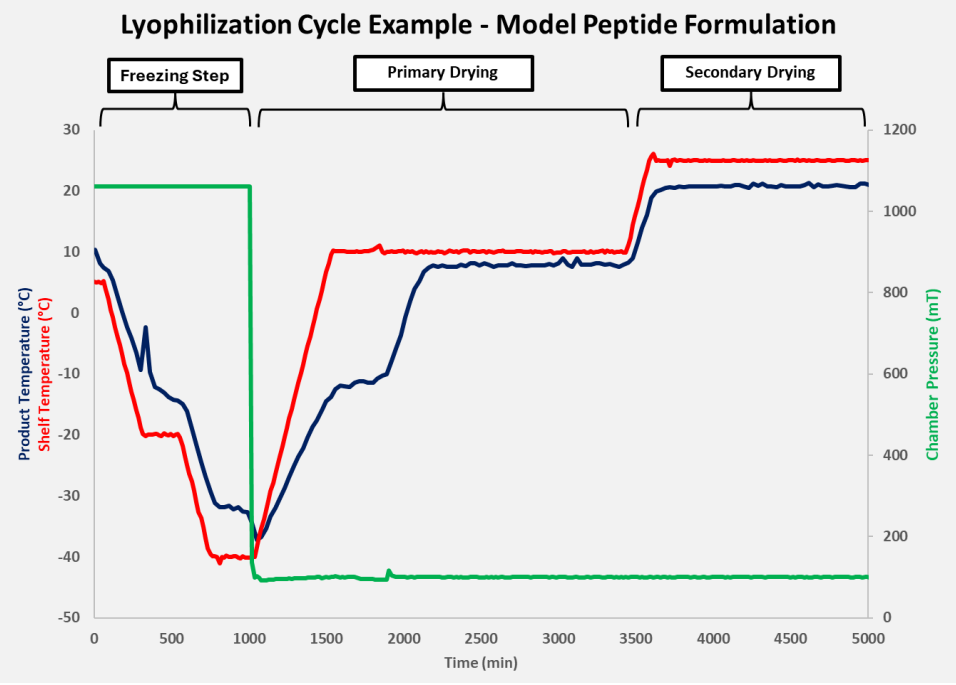
The first step in lyophilization is the freezing step, which will define the structure of the final lyophilized cake. The lyophilizer is sealed and the shelf temperature is reduced to a specified freezing temperature which allows for the crystallization of ice to occur. The rate of temperature drop, the freezing temperature, and the duration of freezing are product specific and can be optimized through lyophilization development trials.

Primary drying step:

Once the drug product is in a sufficiently frozen state, primary drying can commence. The frozen solution is subjected to a partial vacuum and a slightly higher shelf temperature to allow for the sublimation of ice to occur. During this primary drying phase, the temperature of the lyophilizer shelves remains low enough to maintain the solid structure in the product vials while the water vapor is slowly removed by vacuum through the vented stoppers.

Secondary drying step:

The final phase of lyophilization is the secondary drying, or desorption phase. This is performed by further raising the shelf temperatures under vacuum above their primary drying settings to remove the remaining bound water from the lyophilized cake.



This figure presents the different phases of the lyophilization cycle

Lyophilization Cycle Development – Determination of Critical Temperatures and Their Use for Cycle Optimization

With drug product formulation candidates identified, lyophilization cycle development is next required to determine the optimal parameters for the freezing, primary drying, and secondary drying steps of the process. The determination of the critical lyophilization temperatures is necessary to design an optimal cycle that produces a pharmaceutically elegant product and avoids melt-back or cake collapse.

Intact Cake *Collapsed Cake*

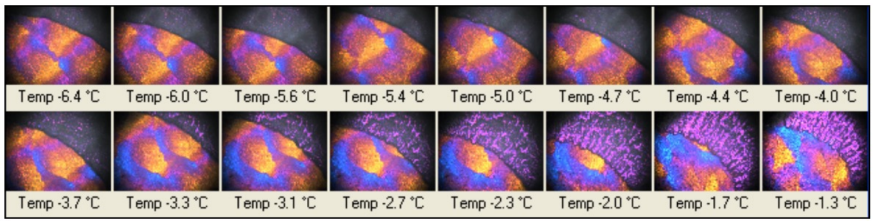
These critical temperatures are as follows in the table below:

Temperature	Characterization Method	Definition and usage
Glass Transition Temperature ($T_{g'}$)	Differential Scanning Calorimetry (DSC)	Temperature where product transitions from a brittle, glassy solid into a more rubbery state. ▪ Freezing step should target a temperature below $T_{g'}$ to ensure complete freezing. ▪ Lyophilization above the $T_{g'}$ is prone to cake collapse. ▪ Stabilizing excipients can prevent collapse from occurring for primary drying above $T_{g'}$.
Eutectic Melt Temperature (T_{EU})	Differential Scanning Calorimetry (DSC)	Temperature where product transitions from frozen crystalline solid to a liquid (melting). Product temperature must remain below T_{EU} during primary drying to prevent melt-back or cake collapse.
Collapse Temperature (T_c)	Freeze Dry Microscopy (FDM)	Temperature where structural integrity of frozen product is lost during primary drying. Related to T_{EU}. Cake collapse is seen if primary drying temperature exceeds T_c.

Differential Scanning Calorimetry for Lyophilization Cycle Development

Differential Scanning Calorimetry (DSC) is a powerful tool for the thermal characterization of materials. It is particularly useful for lyophilization cycle development through the determination of the glass transition temperature ($T_{g'}$) and the eutectic melt temperature (T_{EU}) of frozen solutions. Briefly, DSC measures the heat flow into (endothermic) or out of (exothermic) a sample as the system temperature is ramped up from freezing temperatures. Through comparison of the heat flow of sample pans to empty reference pans, the instrument can detect thermal events such as melting, crystallization, and glass transitions. Analysis of DSC thermograms provides the values of $T_{g'}$ and T_{EU}.

Freeze dry microscopy (FDM) is another important tool for the characterization of frozen product solutions in lyophilization cycle development. This is a visual method for the determination of the product collapse temperature (T_c) through microscopic imaging of the freeze-drying process. The system is able to freeze thin layers of sample solutions, mimicking the crystallization state of the frozen product during lyophilization. The system is then able to sublimate the frozen sample by producing a vacuum in a process similar to primary drying. The stage temperature is slowly raised and the resulting solid structure is observed through a polarized light microscope until visible structural collapse is observed. Careful analysis of this series of images can be used to pinpoint the collapse temperature (T_c).



Example: Collapse temperature (T_c) measurement with FDM of a lyophilized peptide product. Collapse can be observed at approximately -3.0°C

Critical Temperatures & Freezing Step Design

Freezing step design is an important aspect of lyophilization cycle development as the structure of the frozen product before primary drying will determine the morphology and visual appearance of the freeze-dried cake. Using the glass transition temperature ($T_{g'}$), the freezing steps of the lyophilization cycle can be designed and assessed. Selecting a freezing temperature below the $T_{g'}$ ensures that product solution reaches a rigid and solid frozen state before primary drying. The stability of the lyophilized product can also be improved through the process of annealing (controlled warming and cooling) at temperatures above the $T_{g'}$ but below the eutectic melt temperature (T_{EU}). Annealing can promote the growth of ice crystals during the freezing step prior to primary drying, resulting in faster primary drying and a more uniform and stable cake structure. Annealing can also help to crystallize stabilizing excipients in the drug product.

Differential Scanning Calorimeter

Critical Temperatures & Primary Drying Step Design

The design of the primary drying step is critical to ensure the frozen structure obtained in the freezing step is maintained during sublimation. For amorphous products, it is ideal to keep your product temperature below the glass transition temperature ($T_{g'}$) or the collapse temperature (T_c) during primary drying to maintain structural integrity. Primary drying of crystalline products can be performed at temperatures well above the $T_{g'}$ as long as the product temperature does not exceed the eutectic melt temperature (T_{EU}). In such crystalline systems (e.g., an amorphous peptide stabilized by mannitol), performing primary drying above the $T_{g'}$ will hasten sublimation and optimize your lyophilization cycle. The completion of primary drying can be observed through chamber pressure changes as monitored by a Pirani gauge and through thermocouple monitored product temperature.

Before Lyophilization *After Lyophilization*

Critical Temperatures & Secondary Drying Step Design

Secondary drying is a desorption process for the removal of bound water after sublimation (primary drying) is complete. As a result, $T_{g'}$, T_c, and T_{EU} are no longer relevant. The secondary drying temperature should therefore be defined by product specific stability requirements (e.g., degradation kinetics, moisture sensitivity).

Overall, a lyophilization cycle can be properly designed and optimized if the critical temperatures are first identified and understood. This information is crucial to help define a design space for lyophilization trials and to develop a robust and scalable cycle for a product.

Pilot Scale Lyophilizer

Fresenius Kabi AG
Three Corporate Drive
Lake Zurich, IL 60047
cmo.fresenius-kabi.com