Freeze-drying Process Development for Protein Pharmaceuticals

BYEONG S. CHANG
Integrity Biosolution, Newbury Park, CA

SUGUNAKAR Y. PATRO
Amgen Inc., Thousand Oaks, CA

Abstract

This chapter provides an overview of the impact of protein formulation variables on different stages of the protein freeze-drying process and offers guidance for selection of critical process parameters during protein freeze-drying cycle development, optimization, and scale-up. There is particular focus on practical considerations including large-scale process development, scale-up challenges, and issues with less conventional freeze-drying delivery systems, such as dual-chamber syringes.

Introduction

The development of protein pharmaceuticals after the discovery of their biological activity is often considered to be easier than the development of conventional pharmaceuticals since most such proteins are found in the human body and therefore pose few safety concerns beyond the biological activity. On the other hand, development of the formulation and fill-finish process for proteins has been much more challenging, as they have only marginal stability during routine pharmaceutical handling conditions. For more information, the reader is referred to Chapter 11 of this volume.

In addition, proteins are difficult to deliver systemically by noninvasive routes due to their poor bioavailability. Proteins’ marginal stability and the strong preference of a parenteral delivery route make freeze-drying a common means for manufacturing protein products. Even when nonparenteral routes are being considered for proteins, dehydration via lyophilization, spray-drying, or spray freeze-drying may be a preferred processing method (e.g., see Chapter 13 of this volume).

In spite of these advantages, freeze-drying is considered to be an expensive process due to the significant capital investment required to purchase lyophilizer(s), their power consumption, and the lengthy drying times that freeze-drying processes generally entail. For these reasons, it is not uncommon to find that the lyophilization process becomes a bottleneck. Consequently, most companies producing freeze-dried products wish to find more efficient drying processes. Since some key process parameters of freeze-drying cycles (e.g., product temperature during primary drying) are primarily determined by the
physicochemical characteristics of formulations, such as the formulation collapse temperature, the importance of developing good formulations cannot be overemphasized. Formulations that are developed without considering optimal drying process conditions (e.g., those that have a low collapse temperature) may not only necessitate the use of a costly and laborious lyophilization cycle, but also may expose the product to greater risks from undesirable manufacturing process deviations due to marginal room for process errors.

Optimization of the drying cycle for a given formulation requires a balanced understanding of the fundamental science of freeze-drying, formulation characteristics, equipment capabilities, and practical risks associated with process parameters. Ultimately, the optimized drying cycle should be efficient and robust without introducing significantly great manufacturing risks or compromising the pharmaceutical quality of the product.

In this chapter, we will provide an overview of the effect of formulation variables on various segments of the freeze-drying process and provide guidelines for process parameter selection in each stage of lyophilization, along with some practical scale-up considerations. In addition, practical issues in developing the freeze-drying cycle will be discussed, such as scale-up and the challenges presented by drying less common delivery systems (e.g., dual-chamber syringes). In providing these guidelines, focus will be placed on more practical considerations, while the underlying theory and equations are described in more detail in Chapter 3 of this book.

**Description of the Freeze-drying Process**

The lyophilization process primarily consists of three stages.\(^6,7,10,11\) The first stage is freezing, which involves freezing the product and creating a solid matrix suitable for drying. This step impacts the drying characteristics of the next two stages. The second stage is primary drying, sometimes preceded by an additional step called annealing or thermal cycling. Primary drying involves the removal of ice through sublimation by reducing the pressure of the product environment while maintaining the product temperature at a low target level. The third stage in the process is called secondary drying, in which bound water is removed until the residual moisture content reaches its targeted level.

A generic description of industrial freeze-drying process is as follows: After vials are loaded to the shelf, they are cooled down to around 5°C by lowering the freeze-dryer shelf temperature. The shelf temperature is further decreased to –5°C to –10°C and held for approximately thirty minutes to ensure uniform supercooling across the batch. The freezing step is initiated by quickly cooling the shelves to the desired freezing temperature and holding the temperature constant for equilibration. To help crystallize the bulking agent, an annealing step is sometimes included whereby the shelf temperature is raised to near or above the formulation’s glass transition temperature in the frozen state.
Following freezing, primary drying is initiated by introducing vacuum in the chamber. Chamber pressure is reduced below the saturated vapor pressure of ice at the frozen product temperature. The difference between the vapor pressure of ice and the chamber pressure provides the driving force for sublimation. By maintaining the chamber under vacuum, the chamber pressure is constantly maintained below the saturated vapor pressure of ice and sublimation continues. When all frozen bulk water is removed via sublimation, primary drying is complete. At this point, there is still some bound unfrozen water remaining in the product that can be removed by desorption at higher temperatures experienced during secondary drying. Therefore, the shelf temperature is typically raised to ambient or higher temperatures at this stage and held until the desired residual moisture is achieved. At that point, secondary drying is also complete, and the freeze dryer has the provision to stopper the vials inside the chamber. The chamber is then aerated to partially break the vacuum prior to the stoppering of the vials. After the stoppering, the chamber vacuum is fully released before vials are unloaded. Note that the above description is generic and some equipment design variations are available.

**Description of Freeze Dryers**

At its most basic level, a freeze dryer consists of a chamber with shelves onto which the filled vials are loaded for lyophilization, a condenser for capturing the sublimed water vapor as ice, a refrigeration system that facilitates temperature control, and a vacuum pump that can reduce chamber pressure to subatmospheric values. The freeze-dryer chamber is usually manufactured with AISI 316 L stainless steel or its equivalent. Chamber pressure is maintained at its setpoint by introducing an inert, dry bleed gas in a controlled manner (normally nitrogen gas). In most cases, the chamber is separated from the condenser via a main valve. The product is loaded onto the stainless steel shelves, whose temperature is controlled via a heat transfer fluid (e.g., silicone oil) that circulates through them. The temperature of the heat transfer fluid is controlled via the refrigeration system. The freeze-drying equipment also has provisions for defrosting the condenser, clean-in-place (CIP) and steam-in-place (SIP) of the freeze dryer, and computer interface to input, monitor, and control the cycle parameters via a Programmable Logic Control (PLC). For greater details regarding freeze-drying design, operation, and validation, the reader is referred to Chapters 1 and 2 of this volume.

**Influence of Formulation Variables on the Freeze-drying Process**

Maintaining the biological activity and stability of each protein during routine manufacturing, distribution, storage, and delivery processes should be the priority when optimizing formulations. This requires a fine balance of formulation components (e.g., pH, buffering agent, stabilizer(s), and/or tonicity modifier). As every protein is designed to have different structural features for its biological function, no single formulation can satisfy all proteins. Consideration of the freeze-drying process goes beyond these complex formulation requirements. Selecting appropriate functional ingredients that have good drying characteristics is the key to successful development of freeze-dried formulations (i.e., each additive should be selected and optimized for an efficient freeze-drying cycle).
Successful freeze-dried protein formulations generally meet the following criteria. The formulations should provide sufficient stability in solution so that bulk proteins can be formulated, filtered, and filled for freeze-drying. Obviously, formulations should also support the stability of proteins during the freeze-drying process and have sufficient expiry in the lyophilized form at desired storage conditions. In addition, it is desirable that dried formulations should show no sign of obvious sample collapse or meltback during freeze-drying. For the remainder of this section, we will discuss the phenomena of collapse and meltback and how careful selection of formulation excipients can aid in developing a process free from these problems.

Collapse

Collapse is defined as the process by which the structure created during freeze-drying is annihilated with the passage of the subliming interface. Besides the unacceptable appearance, various undesirable properties result from the collapse of the cake during freeze-drying (e.g., by clogging the paths where water can escape, collapse can significantly reduce the rate of sublimation). As a result, the final product tends to retain higher moisture content than a product dried without collapse, and the residual water may be distributed unevenly through the sample. Also, slower reconstitution is generally experienced with the collapsed product due to the loss of porosity and resultant reduction in powder surface area. A long reconstitution time can pose a significant marketing challenge. Finally, there has been some speculation that cake collapse may perturb protein structure, but no supporting data are available yet.

As ice is sublimed during primary drying, collapse is prevented by maintaining the structural integrity of the maximally freeze-concentrated amorphous phase that surrounds the ice crystals. The physicochemical and mechanical properties of this phase are temperature dependent and characteristic of the formulation composition. Below its glass transition temperature (defined as $T_g'$), this amorphous phase exists as a "glass", which is a hard and brittle material with negligible mobility on practical timescales. The characteristic temperature at which motion begins, the $T_g'$, has been found to correlate strongly with the temperature at which the frozen cake undergoes collapse. Therefore, it is critical during primary drying to maintain the sample temperature below the $T_g'$ of the formulation to prevent collapse of the cake.

From the above discussion, it is clear that it is desirable to have formulations with as high a $T_g'$ as possible so that the product temperature requirement is not too low. Some lyophilizers may not be able to achieve the required product temperature and/or chamber pressure if the $T_g'$ is too low. A typical processing strategy to maintain a frozen product below its $T_g'$ is to choose a primary drying chamber pressure such that ice in equilibrium at this pressure will have a temperature below $T_g'$. The relationship between ice vapor pressure and temperature is depicted in Table 1. If collapse temperature of a formulation is $-50^\circ$C, for example, then the product temperature needs to be maintained below $-50^\circ$C during primary drying. To accomplish this, chamber pressure should be below the vapor pressure of ice at $-50^\circ$C, which is 29.6 microns (see Table 1). Keeping chamber pressure below 29.6 microns is difficult to achieve and control for most commercial lyophilizers.
Even if the chamber pressure can be maintained below 29.6 microns, the overall drying time will be long due to a slow sublimation rate at that low pressure.

Table 1. Data for the vapor pressure of ice at sub-zero temperatures.

<table>
<thead>
<tr>
<th>Temperature (ºC)</th>
<th>Vapor Pressure (µm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>4579</td>
</tr>
<tr>
<td>–10</td>
<td>1950</td>
</tr>
<tr>
<td>–20</td>
<td>776</td>
</tr>
<tr>
<td>–30</td>
<td>286</td>
</tr>
<tr>
<td>–40</td>
<td>96.6</td>
</tr>
<tr>
<td>–50</td>
<td>29.6</td>
</tr>
<tr>
<td>–60</td>
<td>8.08</td>
</tr>
<tr>
<td>–70</td>
<td>1.94</td>
</tr>
</tbody>
</table>

The Tg’ that is characteristic for the formulation is based on the mass-averaged Tg’ values of each of the amorphous components. Therefore, the Tg’ of a formulation can be increased by increasing the weight fraction of excipients with higher Tg’s or by including additional excipients that have higher Tg’s.13,15,16

Another approach for achieving good cake is to include additives that crystallize and give the cake a rigid macroscopic structure. In such a cake, collapse of the amorphous fraction will only be manifested on a microscopic scale in the regions between crystals. This approach is only recommended in cases where all other options fail because even microscopic collapse can lead to the adverse effects noted above. Moreover, the crystallization of bulking agent(s) can cause vial breakage due to expansion of the frozen phase.17 Also, the interface generated by the phase separation of excipient(s) can damage proteins.18-21 Therefore, a careful evaluation of protein stability needs to be performed when introducing crystallizing additive(s) to the formulation or when using an annealing process to force the crystallization of some additives.

Meltback

In contrast to collapse, meltback is due to the eutectic melting of crystalline agents in the frozen formulation. Additionally, while collapse occurs as the freeze-drying front proceeds through the sample, eutectic melting occurs throughout the frozen portion.10 To prevent meltback, the product temperature must be kept below the eutectic melting temperature (Te) of the crystalline component(s) of the formulation during primary drying. A more practical issue concerning these eutectic additives may be the complex kinetic inhibition that can be caused by the presence of other additives. For example, amorphous solutes, including proteins, can inhibit the crystallization process in the frozen, dried, and wetted states.16,22-25 In addition, the eutectic melting temperature can further decrease if there is another additive to form a ternary or higher-level eutectic mixture.26 Sodium chloride has a eutectic melting temperature of –21.1 ºC, but it has a tendency to remain in an amorphous form that has low collapse temperature.12,27
Analytical Techniques for Formulation Characterization

A number of subambient analytical methods have been introduced to characterize the complex physical changes occurring in frozen formulations. These include differential scanning calorimetry,\textsuperscript{16,28} differential thermal analysis,\textsuperscript{10} thermomechanical analysis,\textsuperscript{16} electrical resistance,\textsuperscript{29,30} and freeze-drying microscopy.\textsuperscript{31,32} Pikal and Shah\textsuperscript{14} have reviewed the characteristics of each method and compared how the results that they generate can be used to determine the rate of water removal during lyophilization.

Examples showing different types of differential scanning calorimetry results are shown in Figure 1.\textsuperscript{16} Type A excipients show only glass transition signal(s), suggesting that they remain amorphous during freezing and subsequent warming processes. Commonly used Type A excipients include sugars such as sucrose and trehalose, polymers such as dextran, starch, and proteins, and ionic species such as NaH$_2$PO$_4$, citrate, and histidine. As these additives will not crystallize under routine practices, the freeze-drying process will have to be designed to prevent their collapse. Type B excipients have a strong tendency to crystallize and show eutectic melting signals instead of glass transition signals. Typical examples of Type B excipients include Na$_2$HPO$_4$, Na$_2$SO$_4$, (NH$_4$)$_2$SO$_4$, imidazole, and Na$_2$CO$_3$. The freeze-drying process will have to be designed to prevent the eutectic melting of these excipients. Type C excipients not only show both glass transition signal(s) and a eutectic melting signal, but also show a devitrification signal located between them. The devitrification signal appears when an excipient forms a metastable glass, or doubly unstable glass, during the freezing process, but crystallizes during a subsequent annealing process.\textsuperscript{16,33-35}

![Figure 1. Examples showing different types of additives with their respective differential scanning calorimetry results.](image-url)
Optimization of the Freezing Step

Physical Changes upon Freezing

The complex physical changes occurring during the freezing process can contribute to the denaturation of proteins. Evaluating the susceptibility of individual proteins to each stress is important for optimizing the formulation and drying cycle. Also, an understanding of known cryoprotectants’ contributions to each stress factor will make the formulation much more efficient. For example, some additives crystallize during the freezing process and may affect the stability of proteins. One of these, sodium phosphate buffer, was used in many earlier freeze–denaturation studies. In those studies, the denaturation of proteins seen during freezing was primarily caused by pH shifts that resulted from the selective crystallization of buffer components (e.g., pH decreased as low as 3.5 when disodium phosphate crystallized, while mono-basic phosphate remained amorphous). In addition, several reports indicate that protein denaturation during the formation of ice crystals can be a significant factor. Optimization of the freezing process should therefore take the effect of the ice surface area into account since it can contribute to freeze-induced protein denaturation.

Freezing Methods and Their Impact

The manner in which a sample is frozen greatly affects the size and shape of the ice crystals it forms and, hence, the morphology of the final cake and the capacity to remove water from the frozen sample once a vacuum is applied. As the balance between crystal growth and ice nucleation determines the number, shapes, and sizes of ice crystals, the temperature where ice nucleation takes place is an important factor. The nucleation temperature is determined by the treatment samples received during the freezing process, which begins after they are placed on the lyophilizer shelf.

The samples are then frozen by either slowly cooling them from ambient temperature by a gradual reduction in shelf temperature or rapidly cooling them on a prechilled shelf. While some supercooling is observed in both methods, the former treatment leads to more supercooling and results in relatively homogeneous ice crystals. The advantage of having some degree of supercooling is the consistency of product throughout the vials, a consistency that includes moisture content, crystallinity of excipients, and distribution of product. Supercooling can be achieved by equilibrating the products near their equilibrium freezing temperature, followed by a rapid cooling below this point.

An alternative approach is to freeze the product so that growth of ice crystals proceeds faster than nucleation. For example, vials can be frozen by loading onto a shelf that has been prechilled (e.g., –50°C). Ice crystal growth starts at the colder bottom of container and progresses to the warmer top of the container. The principle underlying this approach is to freeze under conditions in which ice nucleation is favored in the lowest portion of the sample by quenching that fraction of the product to a very low temperature while the rest of the product is being cooled to a temperature range in which crystal growth is favored over nucleation. This freezing approach leads to larger ice crystals
than those seen with the method in which the vials are loaded at 2–8°C. Large ice crystals provide better paths for ice sublimation and therefore increase its rate. However, in practice, this rapid cooling approach is harder to scale-up as temperature control is more difficult and batch-to-batch variation is greater at larger-scale lyophilization. In addition, loading on prechilled shelves leads to frost build-up and can be a challenge with automated loading systems at commercial scale.

It should also be noted that freezing products by immersing in liquid nitrogen could lead to irregularly shaped ice crystals with a very large ice surface area. The large surface area can improve both sublimation rate during primary drying and desorption rate during secondary drying. In addition, this increased ice surface area may affect the stability of proteins.\textsuperscript{36,43,44}

Figure 2 shows scanning electron microscope (SEM) pictures of a lyophilized product that was frozen by different processes. While the sample frozen with supercooling shows relatively homogeneous cavities, the sample frozen with liquid nitrogen shows irregular cavities with larger surface areas.

![SEM picture of dextran samples that were lyophilized with different freezing processes. Picture A shows the supercooled sample, and picture B shows the sample frozen by a liquid nitrogen quench. Cake B showed larger surface area than cake A.](image)

**Key Process Parameter Selection for Freezing**

Key process parameters during the freezing stage are the \textit{minimum freezing temperature}, the rate at which this temperature is reached (the \textit{freezing rate}), and the \textit{hold time at the freezing temperature}. As explained above, the freezing temperature chosen should be below the glass transition temperature of the formulation, as well as sufficiently low to initiate nucleation of the bulking agent. These values are normally determined with small-scale and pilot-scale freeze dryers. However, when selecting a minimum freezing temperature, the capabilities of the large-scale commercial equipment also need to be considered. While commercial equipment capabilities can vary widely depending on their design specifications and their age, temperatures below –45°C to –50°C are generally
hard to achieve consistently in very large-scale lyophilizers. Similarly, small-scale lyophilizers are capable of achieving much higher freezing rates than are normally feasible at larger scales (e.g., 0.5 to 1.0°C/min), and this should also be taken into consideration while choosing freezing rates. As mentioned above, there is an optimum freezing rate range for a given formulation based on protein degradation considerations and ice crystal size, and it should be established that this range can be achieved comfortably and consistently at commercial scales. The hold time once that freezing temperature is achieved should be sufficiently long to ensure that all the vials in commercial scale batches are equilibrated with the shelf temperature.

Optimization of Presublimation Annealing

Annealing and Its Benefits

After freezing is completed, the frozen product can be thermally treated to allow the crystallization of some excipients, such as mannitol and glycine, and/or to increase the size of ice crystals.\(^{16,45}\) This additional step can significantly increase the primary drying rate and improve the homogeneity of the cake and appearance of the final product. Completing the crystallization of these excipients during the freezing process may be beneficial as the storage stability of the product can be compromised if residual amorphous or hydrated forms crystallize during storage.\(^{46,47}\) When an excipient converts from an amorphous and/or hydrated form to a crystalline form during storage, the moisture associated with it may be released to the protein product, thereby creating potential stability issues. As discussed earlier, the annealing process should be implemented with protein stability in mind as crystallization can affect the stability of proteins. In addition, the crystallization of bulking agent(s) can cause vial breakage due to the expansion of frozen phase.\(^{17}\)

Annealing can also be applied to increase the size of ice crystals. In this approach, the shelf temperature is increased to allow smaller ice crystals to melt. As this annealing generally happens around the temperature where ice crystal growth rate is greater than the ice nucleation rate, subsequent cooling of shelf temperature will allow amorphous water to crystallize on existing larger ice crystals. This annealing process often presents numerous advantages, such as a more rugged cake structure and an increased T\(_g\)' due to crystallization of excipients.\(^{45}\)

Process Parameter Selection During Annealing

The key parameters during annealing are the annealing temperature and the annealing time. The annealing temperature is chosen on the basis of the devitrification temperature, which is determined by DSC or freeze-drying microscopy. The selected temperature should be about 10°C higher than the devitrification temperature since higher annealing temperatures normally produce a greater degree of crystallinity and require less time to completely achieve the percent crystallinity that is feasible at that temperature. However, it is important to avoid raising the annealing temperature so high that eutectic melting occurs. It should be noted that the collapse temperature may vary according to the product’s annealing history as the amorphous fraction of any metastable excipient(s) will
contribute to the collapse temperature. Temperature selection should take into consideration commercial equipment variability from batch to batch and any deterioration in equipment capabilities that may occur over time.

After annealing, the shelf-temperature is changed to the desired operating temperature for primary drying, which is the next stage.

**Optimization of Primary Drying**

**Rationale for Process Parameter Selection**

Primary drying, wherein the majority of water is removed from the product, is initiated by introducing vacuum in the chamber. Primary drying represents the costliest portion of the freeze-drying process in terms of time and energy consumed. Therefore, optimization of the lyophilization cycle is generally focused on minimizing the primary drying time. The main objective of this optimization is to dry as fast as possible while maintaining the integrity of the cake.

Only two process parameters can be controlled during the primary drying step: *shelf temperature* and *chamber pressure*. There are other parameters that are not controllable from a process point of view, but require careful attention nonetheless as they significantly affect the primary drying process (e.g., container, formulation, fill volume, stopper, total product load, condenser capacity, condenser temperature, location of condenser, intra- and intershelft temperature variability). The relative degree of each controllable parameter’s contribution to overall drying performance varies. For example, shelf temperature is an important parameter because it affects the sublimation rate by controlling product temperature through heat transfer and/or by providing necessary energy for sublimation. If the shelf temperature is too low, then there may not be sufficient sublimation due to a minimal difference between the vapor pressure of ice at the product temperature and the chamber pressure. On the other hand, the condenser may not be able to handle all of the sublimed water vapor if the shelf temperature is set to too high. Chamber pressure also affects product temperature by facilitating sublimation and controlling the thermal transfer between vials and the shelf.

Assuming that the condenser has sufficient capacity, the following equation has been used to represent the drying rate during primary drying:

\[
\frac{dm}{dt} = \frac{(P_0 - P_c)}{(R_s + R_p)}
\]  

(1)

where

- \( \frac{dm}{dt} \) = rate of sublimation, g/cm²/hr
- \( P_0 \) = vapor pressure of ice at the product temperature, mm Hg
- \( P_c \) = chamber pressure, mm Hg
- \( R_p \) = product resistance (cm² mm Hg hr g⁻¹)
- \( R_s \) = stopper resistance (cm² mm Hg hr g⁻¹)
To maximize the sublimation rate, the combination of the highest allowable product temperature and the lowest possible chamber pressure should be used, thereby maximizing \((P_0 - P_C)\) in Equation (1).

The following set of basic equations can help to better understand the interdependence of shelf temperature, chamber pressure, product temperature, and sublimation rate. Rate of heat input to the system, \(\frac{dQ}{dt}\), is given by the following equation:

\[
\frac{dQ}{dt} = A_v \cdot K_v \cdot (T_s - T_p)
\]  

(2)

where

\(A_v\) = the heat transfer area of the vial  
\(K_v\) = the heat transfer coefficient of the vial  
\(T_s\) = the shelf temperature  
\(T_p\) = the product temperature.

A heat balance in the system dictates that the rate of heat input be equal to the rate of heat output. In other words, rate of heat input = rate of heat required for sublimation + rate of heat utilized in heating the product without phase change + rate of heat losses.

Rate of heat required for sublimation = \(\frac{dm}{dt} \Delta H_s\)  

(3)

where

\(\Delta H_s\) = the heat of sublimation, approximately 650 cal/gm.

Rate of heat utilized to increase product temperature = \(\frac{dm}{dt} \cdot C_p \cdot \Delta T_p\)  

(4)

where

\(C_p\) = the specific heat capacity of the product  
\(\Delta T_p\) = the increase in product temperature.

Neglecting heat losses and assuming a negligible increase in product temperature during primary drying, we can combine Equations 1, 2, 3 and 4 as

\[
A_v \cdot K_v \cdot (T_s - T_p) = \frac{(P_0 - P_C)}{(R_s + R_f)} \Delta H_s
\]

(5)
Equations (1) and (5) constitute the simplest mathematical representation of the interplay between shelf temperature, chamber pressure, product temperature, and sublimation rate. It should be noted that $K_v$ is a strong function of chamber pressure and $P_0$ is a function of $T_p$.

The relationships between chamber pressure, shelf temperature, product temperature, and sublimation rate are well illustrated as a schematic in Figure 3. This figure shows the general increase in the sublimation rate as chamber pressure increases at a constant shelf temperature (see points B, A, and C). Likewise, it shows the increase in sublimation rate as shelf temperature increases at a constant chamber pressure (see points B and D). By examining isothermal curves of product temperature, it can be seen that the combination of high shelf temperature and low chamber pressure provides a higher sublimation rate than the combination of low shelf temperature and high chamber pressure. In addition, it shows that going from point B to point C leads to an increase in product temperature that may not be desirable if the product temperature at point B can not be exceeded from a collapse prevention standpoint. On the other hand, if the product temperature at point C is tolerable, then point D provides a greater sublimation rate than point C, and it is advisable to go to from point B to point D rather than to point C. Thus, the most efficient drying can be achieved when the shelf temperature is set as high as possible within the allowances of the system and product stability and when the product temperature is maintained below the maximum allowable temperature by reducing chamber pressure.

![Figure 3. The relationships between chamber pressure, shelf temperature, product temperature, and sublimation rate.](image)
In summary, the objective of primary drying is to remove the bulk water by drying the product below its collapse temperature. Therefore, the target product temperature can be established to be a few degrees below the collapse temperature of a given formulation. Even after finalizing the target product temperature, there are multiple combinations of shelf temperature and chamber pressure that can lead to the target product temperature, and the key to developing a lyophilization cycle lies in knowing which combination to choose.

Typically, a combination with higher shelf temperature and lower chamber pressure is better because it leads to a higher sublimation rate than a combination of lower shelf temperature and higher chamber pressure. As a general rule, a chamber pressure that is approximately 10–30% of the vapor pressure of ice at the target product temperature should be chosen. To avoid vial-to-vial inconsistencies in heat transfer, chamber pressures in the range of 100–200 mTorr are preferred.48,50 After fixing the chamber pressure, an appropriate value for the shelf temperature is chosen so that the combination of chamber pressure and shelf temperature leads to the desired product temperature while ensuring that the resulting sublimation rates are not so high as to overload the condenser at commercial scale. Therefore, knowledge of the sublimation rates and product temperatures associated with multiple combinations of shelf temperature and chamber pressure, as well as knowledge of commercial equipment capabilities, is critical for the selection of appropriate process parameters.

**Determination of the End of Primary Drying**

An accurate determination of the completion of primary drying is important because a premature transition to secondary drying may result in a partial collapse of the cake. Conversely, maintaining primary drying conditions after sublimation is completed does not provide further drying of the product and is a needless waste of time and energy. The completion of primary drying can be monitored in a number of ways at pilot scale: (1) The product temperature starts to increase to match that of the shelf temperature as the sublimation of ice, and the resultant product cooling, comes to an end. (2) There is a significant reduction in the fluctuation of chamber pressure, which is due to the decrease in water vapor leaving samples during sublimation. (3) A chamber pressure rise test can be employed. The chamber is temporarily isolated from the vacuum pump by closing the main valve separating the chamber and the condenser. If sublimation is not complete, there will be a rise in chamber pressure due to subliming water vapor. (4) Vials may be withdrawn from the drying chamber without disturbing the vacuum and weighed to determine the completion of sublimation. (5) The vapor pressure of moisture in the drying chamber can be monitored by analyzing gas composition with mass spectrometry. A rapid drop in moisture content indicates the completion of primary drying.

At production scale, the end of primary drying is normally determined either by temperature or pressure measurement. As mentioned in option (1) above, since sublimation takes the latent heat for phase change from the product, the product remains colder than the shelf temperature during sublimation; at the end of sublimation, the temperature of the vial contents approaches that of the shelf. Therefore, by having temperature sensors (such as thermocouples) in some vials, the end of sublimation (for
that vial) can be detected when the vial temperature reaches the shelf temperature. However, the majority of vials in the batch do not contain thermocouples, and their behavior can be significantly different from that of the vials that do. The non-thermocouple-containing vials freeze later, have smaller pores, have greater product resistance to vapor flow, dry at a higher temperature, and take longer to dry compared with the thermocouple-containing vials. Therefore, with this method of detection, a delay time of 10–15% of the primary drying time is usually used as "soak time" before secondary drying can be initiated. To accurately determine primary drying time by this method, performing experiments at production scale is recommended. Such a cycle is normally termed a “time-based” cycle for the primary drying portion.

The second viable production-scale method for the determination of primary drying completion is the pressure-rise method. With automated loading/unloading operations, placement of thermocouples is harder and may not be accurate. A transition detected by pressure measurement is more accurate and is therefore preferred over temperature measurement via thermocouples. Chamber pressure is normally controlled by the capacitance manometer reading. The limit for pressure rise is calculated by taking into account the batch size and sublimation rate expected at the end of the primary drying. Some lyophilizers have an additional device, the Pirani gauge, which is sensitive to gas composition. During primary drying, most of the chamber is filled with water vapor. On the other hand, the chamber is essentially filled with nitrogen gas at the end of primary drying. Therefore, during primary drying, the Pirani gauge gives a different pressure reading (because of the water vapor presence) than the capacitance manometer; toward the end of primary drying, the Pirani gauge reading converges with that of the capacitance manometer. This is an accurate indication of the completion of primary drying.

**Optimization of the Transition to Secondary Drying**

At the completion of primary drying, the product temperature needs to be increased to initiate secondary drying. Even after primary drying has been completed, the product will still contain sufficient moisture to easily collapse the cake if the product temperature is increased too fast without appropriate evaporation of moisture. Therefore, an important factor for a successful transition to secondary drying is the determination of the optimal product heating rate to reach the secondary drying temperature. The safest way to do this is to simply increase the product temperature as slowly as possible. However, an unnecessarily slow transition will delay the drying process. Since the collapse temperature of partially dried product is affected by its moisture content, the Tg' will increase as moisture content decreases during the drying process. The ideal heating rate matches the rate of the increase of Tg' of the product during the drying process.

The most commonly used method to raise the product temperature for the transition to secondary drying is to increase the shelf temperature at the completion of sublimation. In addition to the product stability considerations mentioned above, production scale equipment capabilities and limitations also need to be considered in recommending this ramp rate.
Another approach for the transition is to allow the rate of sublimation to control the product temperature. In this approach, the shelf temperature is set to the secondary drying level during primary drying. The product temperature during the primary drying step is maintained below the maximum allowable value by properly setting the chamber pressure. Heat loss from the sublimation of ice keeps the product frozen during primary drying. As sublimation finishes and heat loss at the sublimation front diminishes, the product temperature will naturally equilibrate to the shelf temperature, where secondary drying begins.

There are numerous advantages to the approach discussed above. The combination of high shelf temperature and low chamber pressure provides for faster sublimation, as explained earlier. There is less chance of failure associated with a premature transition to secondary drying because the start of secondary drying is triggered by the completion of primary drying. For the same reason, it is not necessary to wait for the last vial to complete primary drying before starting secondary drying, which eliminates the need for an external control of the transition. Since the product temperature is primarily controlled not by shelf temperature but by chamber pressure, this approach is useful for containers that have very little thermal transfer from the shelf (e.g., prefilled syringes). A precaution for this single-step drying cycle is that some products may collapse during the transition if the warming rate is too fast. It should also be noted that, with many formulations, it is not possible to set the shelf temperature to the secondary drying level while maintaining a sufficiently low product temperature by decreasing chamber pressure. Also, the extent to which pressure affects product temperature can be dependent upon various system properties such as the contact resistance to heat transfer, the capacity of the condenser, and the rate of mass transfer.

**Optimization of Secondary Drying**

In this phase, removal of moisture from the product is continued by evaporation. Both the final product temperature and the duration of secondary drying are important factors because they determine the final moisture content of the product. The final product temperature, which is primarily determined by the shelf temperature, is considered to be more critical for the determination of moisture content.

An additional process step that can be included in secondary drying is annealing the dried powder to complete the crystallization of metastable glass. During storage, the metastable glass of some additives (e.g., mannitol) will crystallize and release associated moisture, which may significantly affect the storage stability of proteins. Such metastable glasses can be crystallized by raising the shelf temperature before the completion of secondary drying. Most dried proteins do not denature or precipitate during such thermal treatment. By forcing the metastable glass to complete crystallization, storage stability can be further improved.

Proteins are known to denature when they are excessively dried in the absence of stabilizer(s). When optimizing secondary drying-process conditions, the process should be designed to maintain the critical moisture level for formulations
lacking in stabilizers. Results reported by Carpenter et al.\textsuperscript{53-57} support the contention that sugars protect labile proteins during drying by hydrogen bonding to polar and charged groups as water is removed and thus prevent drying-induced denaturation of the protein. Formulations containing sufficient stabilizer(s) can be dried to moisture contents below 1\% (w/w).

**Process Parameter Selection for Secondary Drying**

Knowledge of the residual moisture levels in the product as a function of shelf temperature and hold time is critical for successful secondary drying parameter selection. The allowable level of residual moisture content has to be predetermined during formulation development based on stability data. A common starting point for shelf temperature during secondary drying is approximately 25–30°C for proteins. A lower shelf temperature requires longer drying times, but will improve the uniformity of moisture level in the product batch.\textsuperscript{6} On the other hand, some products may not dry to the required moisture levels without using a high shelf temperature. Normally, the focus is on the shelf temperature at this stage since it has been generally accepted that the drying rates for protein pharmaceuticals during secondary drying are independent of chamber pressure up to values of at least 200 mTorr.\textsuperscript{6}

Secondary drying is complete when residual moisture levels in the product reach the desired, low level. At that point, the vials are stoppered inside the chamber (typically under a partial vacuum of nitrogen gas). The chamber is aerated and the shelf temperature is maintained at around 4°C until the vials are unloaded.

**Other Practical Considerations and Challenges with Less Common Systems**

Successful lyophilization scale-up is critical to meet the clinical and market demand for the drug in a timely manner. In the Process Parameter Selection sections, we discussed some scale-up considerations that are due to equipment differences between small- and large-scale freeze dryers. In this section, we will examine some additional practical considerations that are important to successful lyophilization scale-up.

**Equipment Capability Differences Between Pilot Scale and Commercial Scale**

One of the common pitfalls in lyophilization scale-up is to assume that a laboratory scale freeze dryer containing a few hundred vials behaves in an identical manner to a production-scale freeze dryer containing many tens of thousands of vials (some as high as 100,000 vials are not uncommon). As mentioned in the Process Parameter Selection sections, differences in small- and large-scale equipment capabilities need to be factored in while selecting process parameters for drug product batches lyophilized at large scales. In addition, a production-scale freeze dryer may have more inertia due to the large batch sizes, and subsequently some of the steps may take longer to complete at production scales. Also, radiation effects can vary between small- and large-scale equipment. These differences should also be factored in and studied during developmental runs in the production-scale freeze dryer.
Matching Product Resistance During Developmental Runs

For large-scale development runs, it is necessary to match product resistance with that of small-scale runs. However, while small-scale experiments are usually performed with sufficient active material, material limitations are quite common in large-scale runs, necessitating the use of a mimic solution (such as HSA or BSA) in place of the protein component. Use of mere placebo for process characterization can lead to questionable results since placebo normally has a lower resistance than the product and consequentially dries at a lower product temperature and faster rate than the active protein. Use of a mimic solution can help mitigate these differences.

Impact of Vials and Stoppers on Lyophilization

Both the vial type and stopper type impact the lyophilization process. The vial type influences the heat transfer properties, while the stopper type influences those of mass transfer. Therefore, it is highly recommended to use the same type of vials and stoppers for full-scale practice runs and commercial purposes. For instance, if a lyophilization cycle is running on the edge of condenser overload, changing to a vial that causes an increase in the sublimation rate can result in condenser overload and batch failure. In addition to using the same vials and stoppers during pilot- and production-scale runs, using the same vial and stopper processing conditions is also recommended so that there is consistency between pilot and production scale in factors such as the extractable profiles of components and the initial stopper moisture content.

Lyophilization Cycle Characterization

Details of the characterization approach are not part of the scope of this document. However, the authors would like to point out the criticality of recommending a well-characterized lyophilization cycle—one whose boundaries of failure are well understood, and one that is capable of being validated per current Good Manufacturing Practices (cGMP) requirements. For further details, see Chapter 2 of this book.

Characterization needs to occur at pilot scale with broader tolerances around critical parameters and at production scale with reduced tolerances. The resultant information provides ranges for critical parameters over which the product is demonstrated to be stable. It should be noted that these ranges could be influenced by equipment changes and capabilities. As a part of characterization, the lyophilized product needs to be tested for all critical quality attributes via stability-indicating assays. Performing biophysical characterization as well to test for any potential conformational changes is also recommended. Characterization efforts should be thorough enough to ensure success in validation efforts in terms of inter- and intrabatch consistency for all critical quality attributes.

Lyophilization of Dual-chamber Prefilled Syringes

The use of dual-chamber prefilled syringes for lyophilization can significantly reduce the amount of handling that patients and clinicians must perform to reconstitute and
administer drugs. While these systems are very convenient from the end-user perspective, they do provide unique challenges for process development and the scale-up of lyophilization processes. For instance, in a dual-chamber syringe system, the liquid that needs to be lyophilized resides in the middle of the syringe barrel (on a stopper) rather than at the bottom of a container with reasonably good heat transfer, as in the case of a vial. Consequently, there is a greater than usual lag between shelf temperature and product temperature in these systems. Depending on the targeted shelf temperature, this lag can normally vary between 5°C and 15°C. To compensate for the lack of good contact with the shelf, these systems normally require much higher chamber pressure.

The key to lyophilization cycle development in dual-chamber syringes is to ensure that the product experiences approximately the same product temperature profile as it would during a successful vial lyophilization process. In other words, the principles governing the target product temperature, target annealing temperature, and target secondary drying temperature are still applicable to freeze-drying in dual-chamber syringes. However, because of the temperature lags mentioned, shelf temperature and chamber pressure setpoints actually used in the dual-chamber lyophilization cycle can be quite different from those used for vial freeze-drying. Pilot-scale experiments should be performed in the intended commercial system configuration to determine shelf temperature and chamber pressure setpoints that lead to the desired product temperature profile. Most scale-up considerations discussed under scale-up of vial lyophilization are applicable here as well, although the magnitude of heat and mass transfer varies due to differences in configurations between vials and dual-chamber prefilled syringes.

Given that the process of freeze-drying proceeds from the top to the bottom of the frozen material and that the freeze-dried material at the top offers additional resistance to the mass transfer of water vapor subliming from the bottom portion of the frozen material, it is in general harder to lyophilize taller cakes consistently at large scales. In the case of vial lyophilization, this is normally addressed by using vials of larger diameter when appropriate. However, this approach is highly restrictive with dual-chamber syringes. Since dual-chamber syringe configurations are usually driven from the standpoint of drug delivery convenience, these systems have unique requirements in terms of the maximum size and diameter of syringes that can be used. This in turn may limit the volume of material that can be lyophilized consistently at large scales in dual-chamber syringes.

**List of Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA</td>
<td>Bovine Serum Albumin</td>
</tr>
<tr>
<td>cGMP</td>
<td>Current good manufacturing practices</td>
</tr>
<tr>
<td>CIP</td>
<td>Clean-in-place</td>
</tr>
<tr>
<td>DSC</td>
<td>Differential Scanning Calorimetry</td>
</tr>
<tr>
<td>HSA</td>
<td>Human Serum Albumin</td>
</tr>
<tr>
<td>PLC</td>
<td>Programmable logic control</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscope</td>
</tr>
<tr>
<td>SIP</td>
<td>Steam-in-place</td>
</tr>
</tbody>
</table>
References


34. MacFarlane DR. Devitrification in Glass-forming Aqueous Solutions. *Cryobiology* 1986; 23:230-244


39. van den Berg L. The Effect of Adding Sodium and Potassium Chloride to the Reciprocal System: KH₂PO₄-Na₂HPO₄-H₂O on pH and Composition During Freezing. *Arch Biochem Biophys* 1959; 84:305-315


45. Searles JA, Carpenter JF, and Randolph TW. Annealing to Optimize the Primary Drying Rate, Reduce Freezing-induced Drying Rate Heterogeneity, and Determine T(g)' in Pharmaceutical Lyophilization. *J Pharm Sci* 2001; 90:872-887


