HEALTH

Lyophilization of Complex Drug Products: Formulation Challenges and Scale-Up

Introduction and Uses of Lyophilization

Lyophilization (also known as freeze drying) is a process in which a liquid is converted to a solid through sublimation and desorption of water or other solvents. The process consists of three highly interconnected stages: Freezing, Primary Drying (Sublimation), and Secondary Drying (Desorption). Lyophilization is often used to stabilize active pharmaceutical ingredients (APIs) and formulations that are not stable in liquid or frozen form. Because lyophilization does not require heat, it is an ideal drying method for thermally sensitive APIs and biologics, such as proteins and peptides.

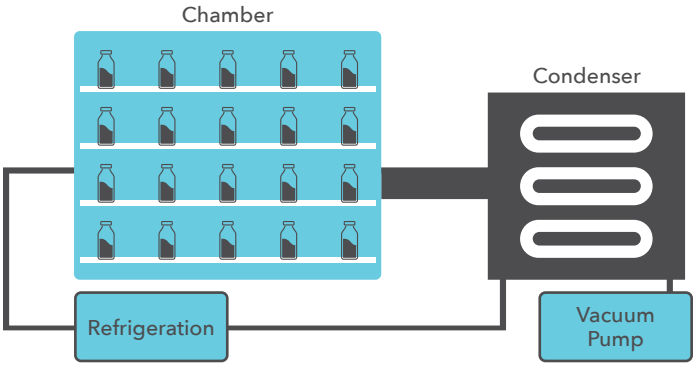
When lyophilization is used to manufacture parenteral drug products, the resulting powder is sealed within a vial, cartridge, or syringe. Prior to administration, the lyophilized powder is reconstituted, or combined with a liquid diluent, to form a homogenous solution or suspension for injection. The high surface area of lyophilized powder allows for fast reconstitution (i.e., rehydration) and injection at the bedside, which is especially useful for emergency products. These products are highly stable, often with shelf lives of more than two years. Lyophilization may also be used to produce intermediate powders that are further processed into final dosage forms. For example, a powder with high residual solvent content and thermal sensitivity may be lyophilized to drive off solvent before additional processing takes place. Lyophilization can also be used to produce a stable, flowable powder for milling or direct compression. In powder fills where a very small fill volume is desired, dissolving the powder in a liquid and lyophilizing can help with weight control, since it is easier to control the volume of a liquid fill.

Perhaps the most important feature of lyophilization is its compatibility with aseptic operations, making it a trusted option in parenteral dosage from development. From 2013 to 2015, half of new injectable and infusible drug approvals were lyophilized products, up from only 10% of these products from 1990 to 1981. This includes the multi-billion-dollar small molecule drug Alimta® as well as blockbuster biologics such as Lupron Depot®, Keytruda®, and Herceptin®. Growth of lyophilized drug products is only expected to continue as complex formulations and biologics with poor aqueous stability become more common.

Lyophilization Equipment and Components

Pharmaceutical lyophilization typically takes place in a tray or shelf lyophilizer, which contains a series of temperature-controlled shelves that hold product during the freeze-drying process. Inside a lyophilizer, there are various components that allow for precise control over temperature and pressure and drive the steps of the process. A basic diagram of a lyophilizer is shown in Figure 1.

Figure 1: Schematic Diagram of a Lyophilizer



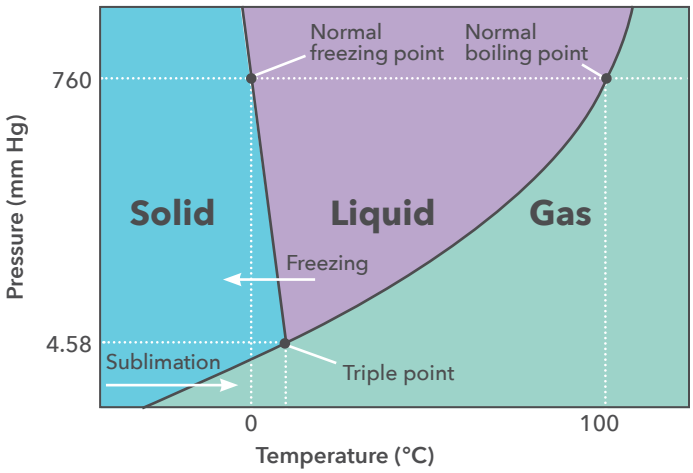
Lubrizol Life Science Health

Lyophilizers come in a range of sizes, including benchtop units for R&D, pilot scale units for process development and scale up, and larger production-sized units with 100+ ft^2 chambers for commercial supply. Production units are installed in ISO 5 clean room spaces to allow for seamless flow between sterile fill operations and lyophilization. These larger units typically come equipped with clean-in-place (CIP) and steam-in-place (SIP) capabilities for ease of cleaning and sterilization.

The Lyophilization Process

The main principle behind lyophilization is sublimation, a phase transition (Figure 2) wherein a solvent (almost always water) goes directly from solid to gas without becoming a liquid. During lyophilization, it is critical that frozen material not thaw out because it will irreversibly liquefy, preventing the formation of a stable solid. Lyophilization is generally broken down into three steps: Freezing, Primary Drying (Sublimation), and Secondary Drying (Desorption). Each step involves careful manipulation of temperature and pressure that is highly dependent on the specific API or formulation being lyophilized. There is no one set of guidelines to lyophilization. It requires a thorough understanding of the phase diagram of water and the ways in which formulation components affect phase transitions between the solid, liquid, and gaseous states.

Figure 2: Phase Diagram of Water



Lubrizol Life Science Health

Freezing

During the Freezing step in a shelf lyophilizer, the water in a product is gradually frozen by cooled shelves. This creates ice crystals that are separated from the drug product and more easily removed by sublimation. Freezing is the most important step of the lyophilization process because the crystal structure that is established affects all remaining activities. For example, a slower freezing rate may create smaller ice crystals and protect a drug product from damage caused by crystal growth, but it will likely lead to longer Primary and Secondary Drying cycles [2-4]. A valuable tool when establishing and optimizing a freezing process is Low Temperature Thermal Analysis, which includes methods such as Freeze-Drying Microscopy and Low Temperature Differential Scanning Calorimetry. These analytical methods allow one to evaluate how a material responds to changes in thermal energy and identify the conditions under which phase transitions (e.g., melting, freezing, sublimation) occur.

Primary Drying

Once a material is frozen, the material is ready to be dried in two phases—Primary and Secondary Drying. The goal of these steps is to facilitate drying while maintaining the crystal structure established during the Freezing process. During Primary Drying, pressure is manipulated to convert water directly from solid to gas via sublimation, and the resulting water vapor is collected on a condenser. In this process, a vacuum pump is used to reduce the pressure within the lyophilization chamber below the vapor pressure of ice, which can be determined using reference tables. Heat is also introduced to the product through the shelves of the lyophilizer to counteract the cooling effects of sublimation and maintain a consistent product temperature. Because the system is under vacuum, conduction between the shelf and the product container is the primary mode

Primary Drying (continued)

of heat transfer. Consistent contact between surfaces is critical to achieving consistent heating, so many lyophilized products are packaged in tubing vials which have a flat bottom to maximize contact area. However, radiative and convective heating cannot be completely disregarded during lyophilization. For example, pre-filled syringes and cartridges may not even touch the shelf during a lyophilization process, so heating must occur through radiation and convection. The endpoint of Primary Drying occurs when the product temperature equals the shelf temperature or when the pressure differential between a Pirani gauge and a capacitance manometer is below a defined value that indicates water content is at an acceptable level (see Process Development and Scale-Up of a Lyophilization Process).

Temperature Control During Primary Drying

A thorough understanding of a product's response to temperature leads to more efficient Primary Drying cycles. Higher temperatures can reduce the time needed for drying, and an increase of as little as 1°C can reduce Primary Drying time by as much as 13%! However, increased temperatures can also have detrimental effects. During Primary Drying, the temperature in the lyophilization chamber must never exceed the **Critical Collapse Temperature (CCT)** of the product, which is the eutectic point at which melting occurs in crystalline materials or a glass transition occurs in amorphous solids. If the temperature exceeds the CCT and the material liquefies, the flow of liquid leaves behind air pockets that result in shrinkage or complete collapse of the cake. Low Temperature Thermal Analysis can determine the CCT for a given material, and the process temperature during Primary Drying is typically set 3 – 5°C below the CCT (and as much as 10°C below the CCT) to avoid collapse.

Figure 3: Lyophilized Cakes in Vials

Secondary Drying

After Primary Drying is complete, there may still be residual moisture in the product. To drive off water that is bound to the product, a Secondary Drying process known as Isothermal Desorption is employed. During this process, the shelf temperature in the lyophilizer is gradually raised under low pressure, with careful consideration given to ensure the temperature doesn't exceed values at which product components are degraded or changed (this is especially important for thermally sensitive products like biologics). The rate and range of temperature increase is highly dependent on the product being lyophilized, especially because the CCT of a product changes as a product dries[5]. An optimized Secondary Drying cycle raises the temperature such that the chamber is always just below the CCT—maximizing drying without inducing collapse of the product. The end temperature in Secondary Drying is determined with a desorption isotherm, which looks at decreases in water content over a range over temperatures and determines when drying is essentially complete. Ultimately, the goal of Secondary Drying is typically to achieve a moisture content of 0.5 to 3%, depending on the product. For many products, a residual moisture content below 1% is ideal because lower moisture content results in longer shelf life. However, some biologics demonstrate optimum stability at a moisture content of 1 to 3%[6]. As a result, the end of Secondary Drying must be tailored to the product.

Formulating Products for Lyophilization

While cycle development is critical for lyophilization, formulation and excipient selection play an equally important role in ensuring a successful product is obtained. In pharmaceutical development, the goal of any lyophilization process is to obtain a stable drug product, but API characteristics and the desired route of administration can affect how that is achieved. Additionally, lyophilization is often applied to already complex formulations such as microparticles and liposomes, each of which bring their own set of development challenges.

Excipient Selection

The first step in formulating a lyophilized product is conducting studies on the physical and chemical stability of the API in question. Understanding how an API responds to changes in properties such as pH and ionic strength can inform excipient selection down the line. Pre-formulation studies often involve measuring solubility under a range of conditions to determine the excipient options and

Excipient Selection (continued)

concentrations in a formulation. From there, excipient screening can be performed to evaluate materials that are generally recognized as safe (GRAS) for the intended route of administration. Categories of excipients for lyophilized drug products include:

- **Bulking Agents (sugars, amino acids, polymers[7]):**
  Incorporating these excipients contributes to the physical structure of the lyophilized cake and improves the appearance of the final drug product. Bulking agents are particularly important for formulations with low solids content, such as highly potent compounds where drug content is relatively low. Crystalline bulking agents such as mannitol provide good appearance and cake structure but may not be compatible with complex formulations such as emulsions or liposomal products. In these cases, amorphous bulking agents such as disaccharides may be more appropriate. Regardless of which bulking agents are selected, characterization of crystalline structure must be performed to detect changes during processing, including polymorphic transitions and the formation of API-excipient complexes that could hinder product performance.

- **Buffers (Tris, salts, acids, bases[7]):**
  These excipients are introduced to stabilize pH during processing, storage, and reconstitution. Pre-formulation studies provide an understanding of how an API responds to changes in pH, which provides a target pH and informs buffer selection. The ideal buffer has a high glass transition temperature, low volatility, and causes a minimal shift in product pH. Buffers that crystallize during the freeze-drying process may significantly alter the pH, which can be especially detrimental to pH-sensitive compounds like proteins. As a result, amorphous buffers are often preferred.

- **Lyoprotectants and Cryoprotectants (disaccharides, polyols, amino acids[8]):**
  During the lyophilization process, formulations are subjected to both thermal and physical stresses. Therefore, excipients are selected to protect the API through stabilizing interactions. Lyoprotectants and cryoprotectants act as stabilizers to ensure that drugs or biologics are not degraded. For example, lyoprotectants form hydrogen bonds with proteins as water is removed during lyophilization, helping to maintain their structure and biological activity. Stabilizers also prevent re-aggregation of complex formulations such as nanosuspensions and lipid-based systems during lyophilization and reconstitution. Many bulking agents, such as disaccharides, also serve as stabilizers in lyophilized formulations.

- **Tonicity Modifiers (dextrose):**
  For parenteral administration, an isotonic final product is required. However, the addition of tonicity modifiers such as dextrose can reduce the CCT of the formulation and increase primary drying times. As a result, some formulations incorporate tonicity modifiers into the diluent instead of the lyophilized cake.

- **Others:**
  Antioxidants, co-solvents, complexing agents, surfactants, dispersing agents, antimicrobial agents, and CCT modifiers are other excipients used in lyophilized products. However, these excipients are not a "magic fix" and careful consideration should be given before adding them to a formulation. New excipients often change the properties of a product and may impact the steps of a lyophilization process. Process design and formulation development must go hand-in-hand to achieve a successful product.

Complex Formulation Considerations

The most common candidates for lyophilization are complex formulations and APIs that suffer from poor stability, aggregation, or drug leakage in an aqueous state, such as polymeric nano- and microparticles, liposomes, and biologics. These products introduce unique formulation considerations that must be optimized to ensure the product can be lyophilized while maintaining performance.

Biologics

The therapeutic effect, or bioactivity, of biologics (e.g., proteins, peptides, oligonucleotides) is highly dependent on the physical structure of the molecule. However, large molecules are easily denatured by both physical and chemical stresses during manufacturing. Changes in temperature, physical agitation, pH changes, and interfacial interactions are all common sources of instability for proteins and peptides[5,9]. And while the removal of water often imparts stability to pharmaceutical formulations, the structure of a protein is dependent on interactions with surrounding water. As a result, successful lyophilization of biologics relies on protecting molecules from these stresses through optimization of the surrounding environment and proper stabilizer selection. Sugars that can exist in an amorphous state (e.g., sucrose and trehalose) are particularly important stabilizers for biologics, as they interact with proteins throughout the lyophilization process. These sugars can hydrogen bond to protein molecules to counteract the loss of water during drying, and their high

Biologics (continued)

high glass transition temperatures may help stabilize molecules by forming a glassy solid. Adjustment of process parameters or formulation components may be needed to ensure these stabilizers maintain protection throughout the freezing and drying process. Surfactants are also often employed to protect against surface-induced denaturation, which can occur at multiple interfaces—air/water, water/container, and ice/solution. Finally, buffers are included with biologics to impart stability in the aqueous state. The bioactivity of large molecules is highly dependent on the pH of the surrounding environment, so ensuring a consistent pH pre- and post-lyophilization is critical.

Polymeric Nano- and Microparticles

Bioabsorbable polymeric nano- and microparticles such as those based on PLGA are developed with a defined particle size distribution and drug loading/release profile. Extensive formulation studies and specialized knowledge go into determining the ideal concentration of excipients and API to achieve these properties. Developing particulate-based products often requires collaboration between groups with different skill sets across formulation and lyophilization. Successful lyophilization of a nanoparticle or microparticle product results in a cake that can be reconstituted to obtain the same particle size distribution and drug loading as the original aqueous formulation. As a result, extensive characterization is needed to ensure the physical and chemical properties of a product are unchanged pre- and post-lyophilization. Measurements including particle size distribution, zeta potential, crystallinity, and assay/related substances are used to ensure particles can withstand lyophilization. And while analytical tools such as HPLC are common among drug developers, not all pharmaceutical companies will possess the physical characterization tools needed to characterize these products. As a result, many companies turn to contract development and manufacturing organizations (CDMOs) who have intentionally invested in the equipment and expertise to formulate, lyophilize, and characterize polymeric nano- and microparticle-based products.

Process Development and Scale-Up of a Lyophilization Process

Lyophilization Cycle Development

Lyophilization cycle development typically begins on R&D and pilot-scale freeze dryers. These smaller systems allow for process parameters such as shelf temperature, chamber pressure, process time, and heating/cooling rate to be tested and optimized through an iterative process. The process of testing different combinations of temperature and pressure allows a formulator to create a "design space," which defines the temperature and pressure conditions that create the most efficient path to optimal primary drying. From here, Target Studies are performed to ensure uniform batches of product can be made consistently at the shelf temperatures, chamber pressures, and process durations within the design space. Finally, Boundary Studies are performed in which shelf temperature and chamber pressure are set at the extremes of the design space to establish a proven acceptable range for these variables. Lyophilization cycle development, Target Studies, and Boundary Studies rely upon accurate, consistent measurement and control of temperature and pressure within the product chamber (Figure 4).

Temperature: Both the temperature of the product and the shelves of a lyophilizer are monitored during cycle development. These measurements are performed with thermocouples placed at the bottom of product containers, distributed across the surface of product shelves, at the inlet/outlet for thermal fluid, and on the condenser unit.

Pressure: Both the pressure of the chamber and the condenser of a lyophilizer are monitored during cycle development. Chamber and condenser pressure may be measured with a Pirani gauge (AKA a thermal conductivity gauge); however, these may not perform well in clean-in-place or steam-in-place lyophilizers. In these cases, a capacitance manometer may be used to measure chamber pressure. In practice, Pirani gauges and capacitance manometers are often used in tandem to create a complete picture of pressure within a lyophilizer.

Figure 4: Key Measurements in a Lyophilization Process

Process Step	Manipulated Variable	Key Measurements
Freezing	Temperature	Shelf temperature (value and cooling rate)
Primary Drying	Pressure	Chamber pressure Shelf temperature Condenser termperature
Secondary Drying	Temperature	Shelf temperature (value and heating rate)

During cycle development, excipient selection is also optimized, with the goal of creating a stable formulation that can be easily and consistently lyophilized. Most considerations for excipient selection relate to the functional characteristics and quality of the drug product—creating an isotonic solution, maintaining a reasonable dose size, GRAS-approvals, microbiological

control– but they may also impact the lyophilization process. For example, some excipients can lower the phase transition temperature of a formulation, making the lyophilization cycle more difficult to run. Formulators generally seek excipients that maintain a higher phase transition temperature to ease processing.

In general, lyophilization cycle development relies on a series of tests supported with accurate pressure and temperature measurements. This data helps create a design space and ensure lyophilization is being performed in an efficient and consistent manner.

Scale-Up and Tech Transfer of a Lyophilization Cycle

Lyophilized products often go through multiple technology transfers during their development. These may occur due to transfers between development and manufacturing sites or to accommodate scale-up from R&D batches to clinical/pilot-scale and, eventually, commercial manufacturing. While defining a design space and proven acceptable range for process variables is critical during development, even a well-designed cycle will change with different batch sizes and lyophilizers. Common issues during scale-up and tech transfer activities include:

- **Increased Loading:**
  Higher batch sizes in production mean that heating on each shelf occurs over a larger number of vials. This may result in slower primary and secondary drying times or slower temperature ramps. Scaling up a lyophilization cycle may require longer drying times than at lab-scale. Loading may also take place over a larger number of shelves, which can demonstrate a temperature gradient. Vials loaded on lower shelves may be exposed to lower temperatures throughout each phase of the lyophilization cycle, and this effect must be monitored.

- **Wall/Edge Effects:**
  Vials that are not surrounded by other vials are considered "edge" vials (as they reside on the edge of shelves). These containers are exposed to radiation from the door, windows, and walls of the lyophilizer, meaning their temperature profile varies from product closer to the center of a shelf. Wall/edge effects can even be measured multiple rows into a shelf. As lyophilizers increase in size, these effects are reduced and the temperature profile of product across the shelf may change. This needs to be accounted for during scale-up activities.

- **Environmental Effects:**
  Scale-up for clinical and commercial production often requires the lyophilization process to move from an open lab environment to a Class 100 cleanroom with minimal particulate matter. Particles serve as sites for the nucleation of ice, so lyophilizing product in a cleanroom often results in smaller ice crystals and differences in freezing and drying times. Filtration of samples in an open lab environment and the use of a laminar flow hood during R&D batch preparation can minimize the impact of moving into a cleanroom during scale-up.

Despite these common issues, effective scale-up of a lyophilization cycle can be managed with a well-characterized design space that allows for adjustments to process variables without compromising product quality. As a result, maintaining accurate control and monitoring of the process remains crucial from lab-scale through commercial production.

Conclusion

Lyophilization provides a critical route to stabilization of complex drug products and biologics, which are two of the fastest growing segments of the pharmaceutical industry. To date, several lyophilized products have achieved both therapeutic and commercial success, and that number will only rise as novel and generic/biosimilar products continue to utilize freeze-drying technology.

The process of lyophilization is well-understood, and thoughtful cycle design enables drug product developers to design robust freeze-drying processes that are amenable to scale-up operations. However, lyophilization is just one component of a complete development effort. Design and optimization of a lyophilization cycle must be accompanied by careful selection of excipients and consideration of how complex formulation components such as microparticles may be affected by the freeze-drying process. And while lyophilization is attractive due to its compatibility with aseptic manufacturing, producing sterile lyophilized products requires the proper facilities, personnel, and environmental monitoring to execute.

Finding an experienced partner with expertise in complex drug product formulation, lyophilization cycle development, and sterile/aseptic manufacturing helps to mitigate risk by closing knowledge gaps and maximizing efficiency between project milestones. As lyophilization becomes more prevalent in an increasingly complex pharmaceutical industry, the combination of these skill sets will enable more effective drug products and better patient care.

References

1. **Arduini M.** *Freeze Drying Market Analysis. Amherst*, New York; 2016.

2. **Bedu-Addo FK.** Understanding Lyophilization Formulation Development. *Pharmaceutical Technology: Lyophilization.* 2004:11-18.

3. **Barley J. Freeze Drying / Lyophilization Information: Basic Principles.** SP Scientific. https://www.spscientific.com/freeze-drying-lyophilization-basics/. Published 2009.

4. **Franzé S, Selmin F, Samaritani E, Minghetti P, Cilurzo F. Lyophilization of liposomal formulations: Still necessary, still challenging.** *Pharmaceutics*. 2018;10(3). doi:10.3390/pharmaceutics10030139

5. **Rey Louis, May JC.** *Freeze Drying/Lyophilization of Pharmaceutical and Biological Products.* Informa Healthcare; 2010.

6. **Schneid SC, Gieseler H, Kessler WJ, Luthra SA, Pikal MJ.** Optimization of the secondary drying step in freeze drying using TDLAS technology. *AAPS PharmSciTech*. 2011;12(1):379-387. doi:10.1208/s12249-011-9600-7

7. **Baheti A, Lokesh K, Bansal A.** Excipients used in lyophilization of small molecules. *Journal of Excipients and Food Chemicals.* 2010;1.

8. **Arsiccio A, Paladini A, Pattarino F, Pisano R.** Designing the Optimal Formulation for Biopharmaceuticals: A New Approach Combining Molecular Dynamics and Experiments. *Journal of Pharmaceutical Sciences.* 2019;108(1):431-438. doi:10.1016/j.xphs.2018.09.002

9. **Challener CA.** For Lyophilization, Excipients Really Do Matter. *BioPharm International.* 30(1):2017.

LUBRIZOL LIFE SCIENCE
9911 Brecksville Road
Cleveland, OH 44141-3201 USA

For more information, visit lubrizolcdmo.com
or call us toll free at +1 610-861-4701

19-216284