

Freeze-Drying Laboratory Guide

Illustrated Toolkit for General Users

BÜCHI

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1. Introduction



The term freeze-drying is rather evocative and implies a process where a sample is first frozen and then dried. Freeze-drying or lyophilization removes ice or frozen solvent through the physical principle of sublimation. One of the ultimate goals of freeze-drying is to produce a well-dried product with a nice cake appearance (without defects), short reconstitution time, long shelf life, and recovery of activity after rehydration, amongst other requirements.

Freeze-drying is used with many substances and materials in a wide range of applications such as:

· Removal of water for further processing.
· Preservation of a substance's characteristics.
· Preservation of the initial form.
· Packaging of materials.
· Chemical analysis.
· Others.

A typical lyophilization process comprises three main steps, which can take several days to complete – freezing (solidification), primary drying (ice sublimation), and secondary drying (moisture desorption – optional). The process is known to be rather expensive due to the slow drying rate and the high investment and operating costs.
By identifying the critical properties of the formulation, so that the process conditions can be specifically tailored to the formulation characteristics, the freeze-drying cycle can be optimized to use only the required amount of energy and time. Successful freeze-drying necessitates a good balance of several factors and can be a complicated process to manage. Despite improvements in process knowledge, many misconceptions persist. This booklet aims to counteract those misunderstandings through technical notes and answers to frequently asked questions.

The booklet is divided into four main parts. The first part focuses mainly on hardware. The second part offers an overview of the most important parameters and steps in sample preparation. The third part concentrates on the freeze-drying process itself by focusing on the most important process parameters. Finally, the last part helps to better understand how to perform freeze drying when solvents other than water are used, either pure or diluted.

2. How to choose the right instrument for your needs

2.1 Overview

Lyophilization is commonly used as a solution for sample preparation, sample formulation, or to improve storage properties. A wide variety of systems, with a range of sizes, capabilities, and configurations, is available on the market. No system will be suitable for every possibility, and it is important to define what the freeze dryer will be needed for, so that an appropriate instrument can be found.

Attention must be paid to how much moisture the freeze dryer must handle and what type of samples will be freeze-dried. Choosing an instrument with a collector that is too small will lead to a need for constant defrosting, while a unit that does not reach the correct temperature will not be able to collect the sample vapor. Selecting a robust vacuum pump that can reach low vacuum quickly and maintain it in a reliable way is also mandatory for a good freeze-drying process (more details page 16).

2.2 Water content

To avoid overloading the condenser capacity, it is important to know how much moisture will need to be removed. The more ice accumulates on the condenser coil, the more isolation will build up and the less efficient the collection will be, until no more ice can be trapped anymore.

Using a freeze dryer that does not have sufficient ice capacity can increase collector temperature due to ice isolation and poor vacuum in the system due to vapor accumulating in the drying chamber and spoiling the oil in the pump.

When comparing freeze dryers, it is important to inquire about the condenser capacity (how many kg of ice/liter of water can be contained on the coil) and the condensing capacity or freeze-drying rate (how many liters/kg per 24 hours can be trapped on the coil). The freeze-drying rates are usually measured on a manifold tree with shell-frozen water. Slant-frozen or bulk-frozen samples will have a slower sublimation rate (see page 25).

It is advisable to select a freeze dryer with a defrosting feature, either automatic or manual, to reduce the time needed to melt the entire ice buildup in the condenser. This process typically takes a few hours. By minimizing defrosting time, this feature allows for higher sample throughput, as the instrument is quickly ready for use again. Additionally, it helps protect the lab environment by limiting vapor formation and exposure.

2.3 Sample throughput

It is advisable to select a freeze dryer with a defrosting feature, either automatic or manual, to reduce the time needed to melt the entire ice buildup in the condenser. This process typically takes a few hours. By minimizing defrosting time, this feature allows for higher sample throughput, as the instrument is quickly ready for use again. Additionally, it helps protect the lab environment by limiting vapor formation and exposure.

At high sample throughput, the freeze dryer consumes the most electrical energy. A freeze dryer with an adaptive or smart cooling design can regulate electrical consumption based on factors such as sample quantity, sample volume, and drying progress. This feature contributes to reducing overall energy consumption and, ultimately, the process cost.

2.4 Sample type and product characteristics

Basic product characteristics, such as solvent type, the freezing temperature of the solvent, and critical temperature (temperature at which the sample only exists in the solid phase) of the formulation, are essential to determine the process parameters. A sample must remain completely frozen during all stages of freeze-drying. If the temperature of the sample exceeds its critical temperature during the process, it will begin to melt and collapse. The instrument must be able to reach the pressure required to maintain an adequate temperature in the sample during lyophilization.

To freeze-dry samples properly, a difference of at least 15–20 °C between sample and condenser temperatures is required. If the collector is not cold enough, the sample will bypass it and enter the vacuum pump, leading to extra maintenance and possible damage to the pump.

Most water-based samples can be used with a standard -55 °C freeze dryer. For other cases, a lower condenser temperature is recommended (see page 50).

2.5 Vials, bulk, flasks, tubes...?

Freeze-drying can be carried out in several formats, such as vials, ampules, bulk trays, flasks, Petri dishes, 96-well plates, blister packs, or syringes. Many accessories are available to customize the freeze dryer to specific needs, and it is important to make sure that they match the sample format.



Figure 1: Beaker flask (100–1200 mL) and ampule adapter for manifold applications.

For example, when vials need a stopper under vacuum, a stoppering tray and top are required (Figure 2). Flasks and ampules are ideal for use on a manifold tree but not on a shelf-style dryer.



Figure 2: Freeze drying in a vial. The system includes stoppering trays and a stoppering top.

2.6 Vacuum pump

Working at very low pressure is necessary to create an environment that encourages sublimation. A robust vacuum pump adapted to the solvents being used must be selected, as it can reach low vacuum quickly and maintain it reliably.

When selecting a vacuum pump, several options are available, and it is important to ensure that the pump meets the minimum specifications required. Freeze-drying can be carried out in several formats, for example, vials, ampules, bulk trays, flasks, Petri dishes, 96-well plates, blister packs, or syringes. There are many accessories for freeze-drying. To determine which pump to choose, it is important to consider whether the samples are in acid, water, organic solvent, or a mixture. Freeze-drying instruments are usually equipped with rotary vane pumps. Depending on the application, some gases may bypass the condenser and enter the vacuum pump. Regular oil changes are advised; however, this maintenance step may not be enough. Scroll pumps or hybrid pumps are then recommended (see page 17 and 18).



Figure 3: Up: Pfeiffer two-stage rotary vane pump, Down: Edwards Scroll pump.

3. The colder the condenser, the faster the process

3.1 Overview

A common misconception is to believe that a colder condenser will “evaporate the water out faster” and improve the freeze-drying process. Colder condensers are best used to process solvents with lower freezing points than water. Choosing a colder condenser will only increase the cost and complexity of the equipment, not speed and performance.

3.2 How does it work? Condenser parameters

Temperature alone does not affect the freeze-drying rate. The driving force of the sublimation process is the vapor pressure difference between the sublimation surface (sample) and the ice layer on the condenser. In a freeze-drying process, if the sample is not heated, its temperature will be defined by the pressure set in the chamber. The coil's temperature defines the ice vapor pressure over the condenser wall. Table 1 shows that to increase the pressure difference, increasing the product temperature is more efficient than reducing the condenser temperature.

This can be illustrated simply by calculating the difference in vapor pressure between -60 °C and -80 °C (0.0103 mbar) and between -60 °C and -40 °C (0.118 mbar). When reducing the temperature, the vapor pressure decreases quickly to reach a plateau. This effect can be observed when pressure and temperature are plotted together on a graph (Figure 4).

Table 1: Vapor pressure of ice.

Temperature [°C]	Pressure [mbar]
0	6.11
-5	4.02
-10	2.60
-20	1.04
-30	0.39
-40	0.13
-50	0.0395
-55	0.021
-60	0.0108
-70	0.0026
-80	0.00054
-100	0.0000133
-120	0.00000031

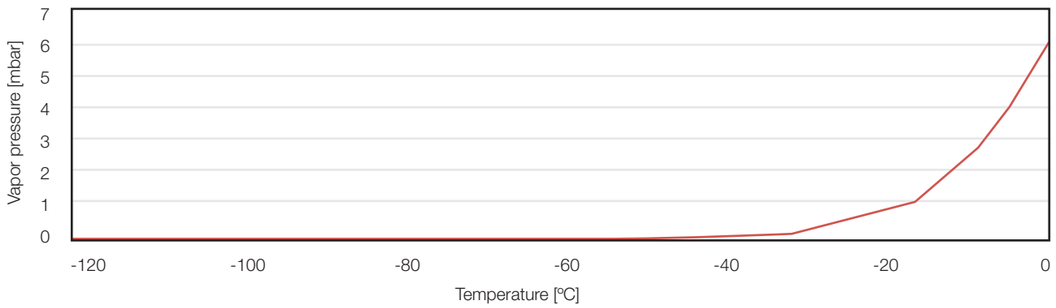


Figure 4: Relationship between ice temperature and the vapor pressure above it.

3.3 Conclusion

The optimal condenser temperature for a freeze-drying system should be chosen according to the critical temperature of the sample (see page 21) and the type of solvent (see page 50) being used. The condenser must be 15–20 °C colder than the sample for an optimal process. When working with aqueous samples, an instrument with a -55 °C condenser is adequate for most cases, and a colder condenser will not speed up the process. Extra cold condensers have not been designed to process aqueous samples but to process solvents with low freezing points.

4. Good maintenance is imperative – how to take care of the instrument

4.1 Overview

A freeze dryer is a combination of several components working together to provide the user with the desired results. Keeping these components in good working order is essential so that the equipment does not contribute to process failures or bad results. Routine maintenance can help extend the lifetime of your lyophilization unit. The longevity of your freeze dryer depends on your applications, the frequency of use, and how well the system is maintained.

Important components to take extra care of include:
· Instrumentation system
· Vacuum system
· Drying chamber

4.2 Sensor instrumentation system

The instrumentation system contains the temperature and pressure sensors on a freeze dryer. Those sensors should be checked regularly and recalibrated when possible. Please note that some sensors cannot be calibrated and must be changed, whereas others can only be calibrated by service engineers (check the operation manual).

Temperature sensors such as thermocouple product probes can be checked using ice water to assess their accuracy at 0 °C. In a system with both a Pirani gauge and a capacitance manometer, a simple check of the vacuum sensors can be performed by running the freeze dryer empty and dry, with a set point at 0 mbar. Once the freeze dryer is stabilized at relatively low pressure, both gauges should be in close agreement. It is important to know that even though Pirani gauges read erroneously high in the presence of water vapor, they are typically very stable once calibrated. If the sensors do not agree and the system is dry and fully evacuated, one of the pressure probes most likely needs recalibration.

4.3 Vacuum system and drying chamber

The vacuum system comprises the vacuum pump and the drying chamber.

Vacuum pumps are mainly either “wet”, oil-lubricated, two-stage rotary vane pumps or “dry”, oil-free scroll pumps (Figure 6).

Oil-lubricated pumps can reach very low vacuum and are quite inexpensive. It is, however, important to keep an eye on the quality of the oil and to perform oil changes regularly. Usually, it is recommended to change the oil every 2000 working hours; however, this number could become more flexible if applications with a solvent other than water are performed. Visual inspection of the oil should be done before and after each freeze-drying cycle. If the oil has darkened significantly (Figure 5) or looks cloudy, the oil needs to be changed.



Figure 5: Color scale for operating fluid P3 from Pfeiffer Vacuum. If discoloration reaches levels 4–5, the oil should be changed immediately. At a color level higher than 6, it is possible that the pump is already damaged.

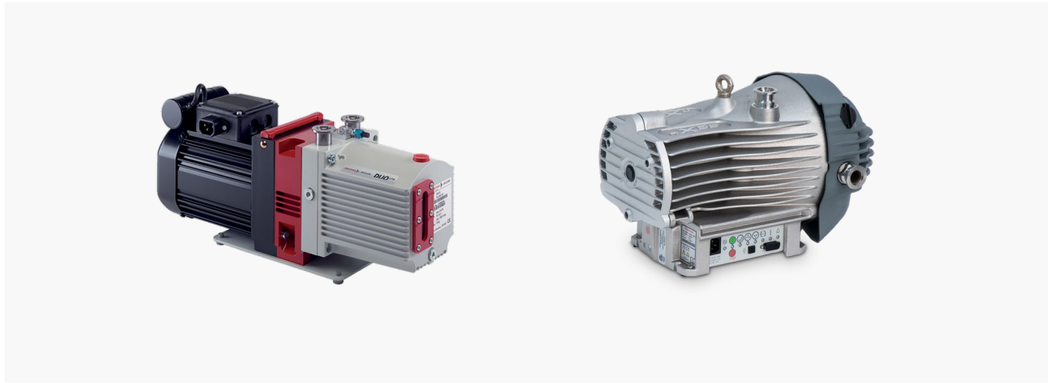


Figure 6: Left: Pfeiffer two-stage rotary vane pump, Right: Edwards Scroll pump.

Oil-free vacuum pumps do not require an oil change; however, the scrolls must be replaced every 40,000 working hours to ensure optimal vacuum.

O-rings and gaskets should be visually inspected for cleanliness, dryness, and cracks. They do not require lubrication unless they have become dry. O-rings can be cleaned from dust with a damp cloth impregnated with a mild solvent such as ethanol or water. If lubrication is needed, high vacuum grease should be used in very small amounts to lubricate O-rings and seals and should never be used to fill up cracks or splits on worn-out items. Damaged sealings should be replaced immediately. The gaskets in the drain valve might wear out fast since they are in direct contact with solvents during defrosting. Consider using chemically stable material such as FFKM.

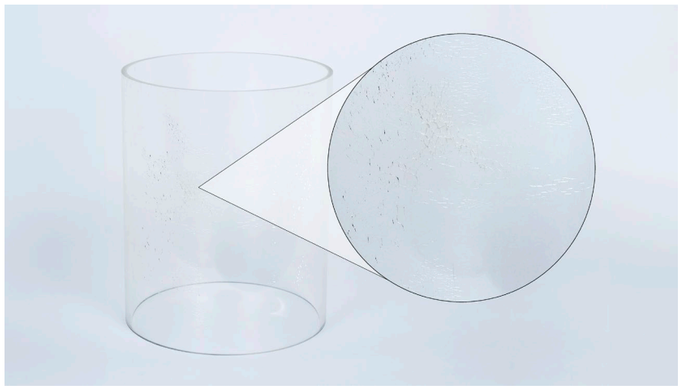


Figure 7: Etched acrylic drying chamber should be replaced.

Acrylic parts of the system should be inspected for cleanliness, cracks and etching, especially if organic or acidic solvents are being used (Figure 7).



Figure 8: Manifold valves and glassware need regular inspection.

Manifold valves should be examined and cleaned regularly, and glassware should be checked for cracks and scratches. Even a small scratch can cause a flask to implode when exposed to high vacuum (Figure 8).

The ultimate vacuum (0 mbar) should be tested in a clean and dry system with the condenser turned on. If it does not reach the technical specifications, there is either a leak in the system or the vacuum pump needs servicing.

A leak test can be performed to verify the integrity of the drying chamber and all the seals. Acceptable leak tests are based on the volume of the vacuum chamber. The leak test is passed on BUCHI's Lyovapor™ line if the leakage rate is less than 10.10 mbar*L/h. A vacuum test can be performed to test the pump's performance. A vacuum of 0.1 mbar or less should be reached in less than 10 min.

All items in the drying chamber should be kept clean. After each run, spilled product, broken glass, or vial stoppers should be removed. Stainless steel and acrylic surfaces should be cleaned. When acids or organic solvents are being used, extra care should be taken to prevent them from resting against the acrylic drying chamber or against any parts that can be damaged. Solvents should be drained immediately and disposed of properly. When acids are used, they should be neutralized to protect the instrument.

4.4 Conclusion

Following the instructions provided by your service engineer and the operation manual is the best method to determine the correct interval for system maintenance. Both the inside and outside of the instrument should be kept clean. Cleaning procedures should be incorporated into SOP.

5. Pump – what to choose and how to maintain it?

5.1 Overview

Even though it may seem like a small piece of the freeze dryer, the choice of a reliable vacuum pump and its maintenance are crucial. Freeze-drying processes must operate at very low pressures to create an environment that encourages sublimation. It is essential to select a robust vacuum pump adapted to the solvents used, which can reach low vacuum quickly and reliably maintain it.

5.2 Have the right vacuum pump

When it comes to selecting a vacuum pump, several options are available, and it is important to make sure that the pump meets the minimum specifications required for freeze-drying (min displacement capacity of 5 m3/h, capability to reach 0.1 mbar in less than 10 min and 0.05 mbar in less than 30 min). To determine which pump to choose, it is also important to consider whether the samples are acid, water, organic solvent, or a mixture of both.

5.3 Two-stage rotary vane pump

The most common pump for freeze-drying applications is a two-stage rotary vane vacuum pump (two rotary vane pumps in series). It can reach deep ultimate vacuum levels, has a high displacement capacity, is low on up-front costs, and is relatively small compared to other types of pumps.

Rotary vane pumps use oil to guarantee a tight seal, lubricate the working parts, and cool down the rotors. For the pump to be in optimal working order, paying attention to the oil quality and regularly checking the pump oil for discoloration, particles, or water droplets is mandatory. If the oil is dirty or cloudy, it is time to change it. The frequency of oil changes depends on the use of the freeze dryer and the solvents in use. It varies from often (every time the freeze dryer is used) to seldom (every few months). Regular oil changes (at least every 2000 working hours or earlier if needed) will keep the pump pulling at optimum vacuum during freeze-drying.

If the solvents bypass the condenser or the condenser is not cold enough, vapors can migrate towards the pump. Any solvent not trapped or recovered upstream of the pump will most probably condense in the pump oil, spoil the oil, and, in the worst case, damage the inside of the pump. This leads to shorter intervals between oil changes and shorter effective pump life.

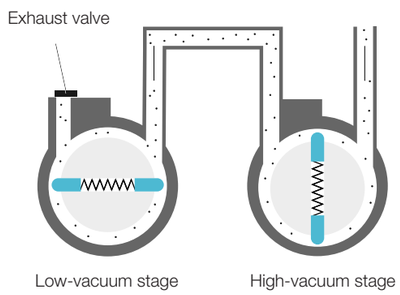


Figure 9: Two-stage rotary vane pump.

Opening the pump's gas ballast for 10–15 minutes after a freeze-drying run before switching off is considered good practice. This will enable the evacuation of some solvent vapors that have not been condensed, raise the pump temperature to degas, and clean the oil of some solvent contamination. The gas ballast must then be turned off to avoid oil carryover to the exhaust.

Rotary vane pumps are recommended for aqueous samples. Solvents with high boiling points whose vapors can be trapped in the condenser before reaching the pump could also be used carefully.

5.4 Scroll pump

Scroll vacuum pumps are dry pumps that use two spiral scrolls to displace and compress air and vapors toward the exhaust. They can achieve a deep vacuum and have a high displacement capacity.

Because they do not need oil to work, scroll pumps are more environmentally friendly, and their overall maintenance costs are high.

Scroll pumps undergo a lot of wear during operation, and the scrolls should be changed every 40,000 working hours to keep the pump pulling an optimal vacuum during freeze-drying. Moreover, it is mandatory to open the gas ballast (for half an hour to one hour daily).

Scroll pumps are recommended for freeze-drying aqueous and solvent samples. However, when handling acids above 20%, the metallic scrolls, even on chemical—or corrosion-resistant models, can be damaged faster than advertised..

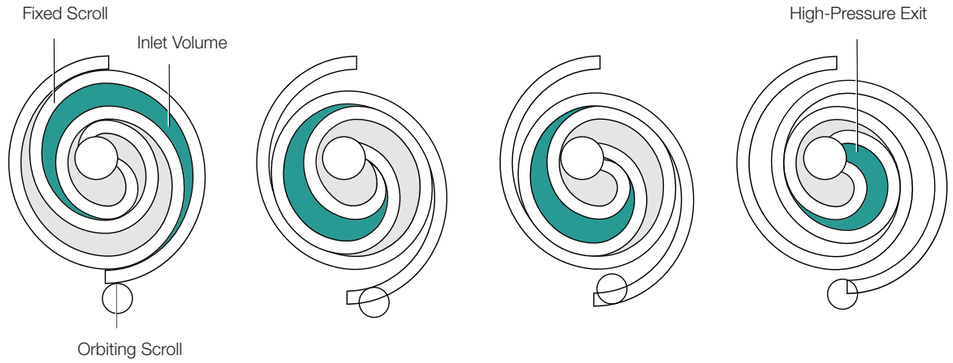


Figure 10: Schematic diagram of a scroll pump.

5.5 Hybrid pump

Hybrid pumps combine a rotary vane pump with a diaphragm pump. The diaphragm pump keeps the rotary vane pump's oil under negative pressure, reducing the vapors that pass through it and condensate in the oil. Compared to rotary vane pumps, hybrid pumps' oil remains cleaner and lasts up to 10 times longer between changes.

The ultimate vacuum level and displacement capacity of a hybrid pump are similar to those of rotary vane pumps. The incorporation of the diaphragm in the design makes these pumps better at handling acids and solvents than rotary vane pumps. Hybrid pumps are recommended for corrosive or volatile samples since they can be used with acids and harsh chemicals.

6. The importance of freezing

6.1 Overview

Freeze-drying or lyophilization is a process that comprises three steps (Figure 11): freezing, primary drying, and secondary drying. It is commonly used to create a dry product. The focus when developing a cycle is typically on optimizing the drying, mainly the primary drying step, which is the most time-consuming of the three.

When optimizing the process, less attention is paid to the freezing conditions and their possible effect on the primary and secondary drying or the characteristics of the final product. Differences in vapor diffusion, drying rate, and sample morphology depending on sample freezing conditions have been reported extensively. Freezing should be considered an essential step of the process.

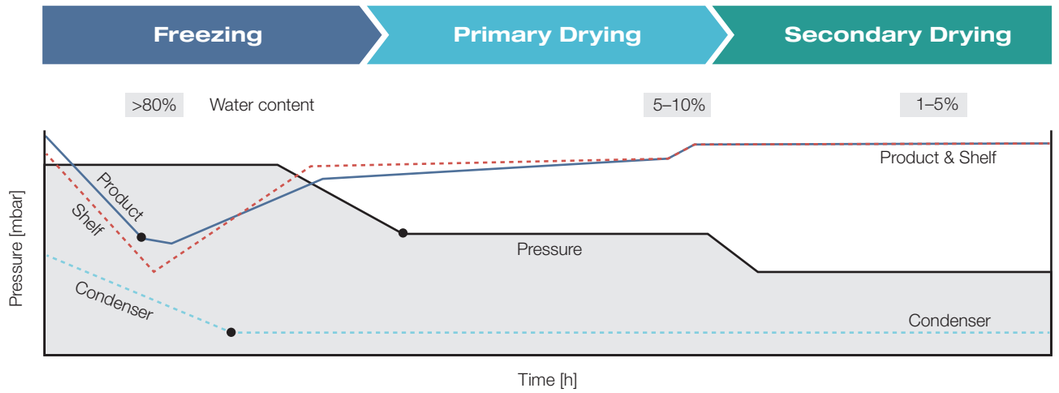


Figure 11: Main steps of a freeze-drying process – Freezing, primary drying, and secondary drying.

6.2 Why is it important to freeze adequately?

Many samples include elements that can create complex and unpredictable freezing and drying behavior. An analysis of the product's critical temperatures can ensure that no unforeseen events occur during the freeze-drying process.

Freezing is a critical step during which the microstructures of the dry product are usually established. The product must be frozen to a low enough temperature to solidify completely. Since freeze-drying works on the principle of sublimation, which is a phase change between solid and gaseous phases, materials to be freeze-dried must be properly solidified and, hence, frozen. If this fails to occur, the vacuum applied in primary drying will cause any unfrozen product to boil, resulting in defective samples. The method of freezing and the final temperature of the frozen product is, therefore, crucial in influencing the success of the freeze-drying process.

6.3 What are the effects of cooling speed?

The manner in whichway freezing is undertaken will affect how the drying process progresses. Most liquid products or formulations freeze by forming crystals. The size and shape of those crystals depend on the cooling speed. A rapid cooling will result in small ice crystals, whereas slower cooling will lead to larger crystals.

Large crystals create an open structure facilitating the drying process thanks to fewer restrictive channels in the matrix. Slow freezing can be damaging to biological products. Large crystals can damage cells, while solute

concentration and surface-induced denaturation can damage proteins. Small crystals are useful for preserving structures to be examined microscopically. However, small crystals also result in a product that is more difficult to freeze-dry due to narrower vapor paths.

Even though the ideal crystal matrix structure and the cooling rate vary from product to product, fast freezing (e.g., liquid nitrogen) generally creates small crystals, whereas slow freezing (e.g., deep freezer) creates large crystals.

6.4 How can you choose the right freezing temperature?

Most products subjected to freeze-drying consist of a solvent, for example, water, and some materials, such as the solute, dissolved or suspended in the water. The freezing temperature of such a formulation is defined by its characteristics and composition.

Formulations consisting of eutectic or amorphous mixtures typically freeze in different ways. Eutectic mixtures contain substances that freeze at lower temperatures than the surrounding water. When a eutectic mixture cools, water is the first component to separate and freeze into ice. The formulation may appear frozen, but the remaining substances are still liquid. They form concentrated areas that freeze eventually at temperatures below the freezing point of water. The temperature at which all components of the mixture are properly frozen is called the eutectic temperature. This is the critical temperature of the formulation and the maximum temperature the formulation can endure during the freeze-drying process. Applying vacuum to an incompletely frozen eutectic mixture may result in the destruction of the product as unfrozen components expand when placed under vacuum.

The other class of mixtures are amorphous and form glassy states when frozen. With decreasing temperature, the formulation becomes increasingly viscous and eventually freezes to a vitreous solid at the glass transition point. In the case of amorphous products, the critical point in terms of stability is called collapse temperature. The collapse temperature is typically slightly lower than the glass transition point. Generally, amorphous products are challenging to freeze-dry.

6.5 Conclusion

Freezing methods used before lyophilization can significantly influence the structure of the ice formed, affecting both the water-vapor flow during primary drying and the final product. Understanding and controlling how a solution freezes can lead to shorter freeze-drying times and more stable products.

7. Knowing the critical temperature of the sample helps avoid defects

7.1 Overview

To develop a safe and efficient freeze-drying cycle, it is vital to determine the thermal characteristics of a product. This will help to define the maximum permissible product temperature during the primary drying step of the freeze-drying process. Drying above this temperature will result in loss of cake structure. Both the glass transition temperature of the sample (freeze concentrate) (T_g) and the eutectic temperature (T_e) of the sample can be identified using differential scanning calorimetry (DSC) or a freeze-drying microscope. Frozen-state temperatures are important to find the collapse temperature (T_c) and to ensure that the cycle is right for the product. Dry-state temperatures are useful for understanding a freeze-dried product's stability and storage conditions, quality control, and submitting a product for regulatory approval.

7.2 What is the glass transition temperature (T_g)?

The glass transition temperature is the point where some materials turn from a hard or brittle "glassy" state (amorphous) into a viscous liquid. For crystalline samples, the equivalent is called the eutectic point. At values above this temperature, the sample melts and/or collapses. This range is called the collapse temperature. Collapsed samples often show changed properties, i.e., dissolution behavior and appearance. In some cases, sample collapse can even lead to activity loss of certain active pharmaceutical ingredients.

7.3 Freeze drying above and below the T_g

To investigate the influence of the freeze-drying temperature during primary drying, a sample was prepared with a defined T_g of -29 °C. To that end, a solution (200 mL) of bovine serum albumin (BSA) (20 mg/mL), sucrose (25 mg/mL), and phosphate-buffered saline (PBS) (6 mM) were prepared and frozen in a metal tray at -40 °C for 18 hours. The sample was then freeze-dried at two different chamber pressures, i.e., vapor pressures, which translates to different sample temperatures. Figure 12 shows the sample and shelf temperatures measured during the freeze-drying process.

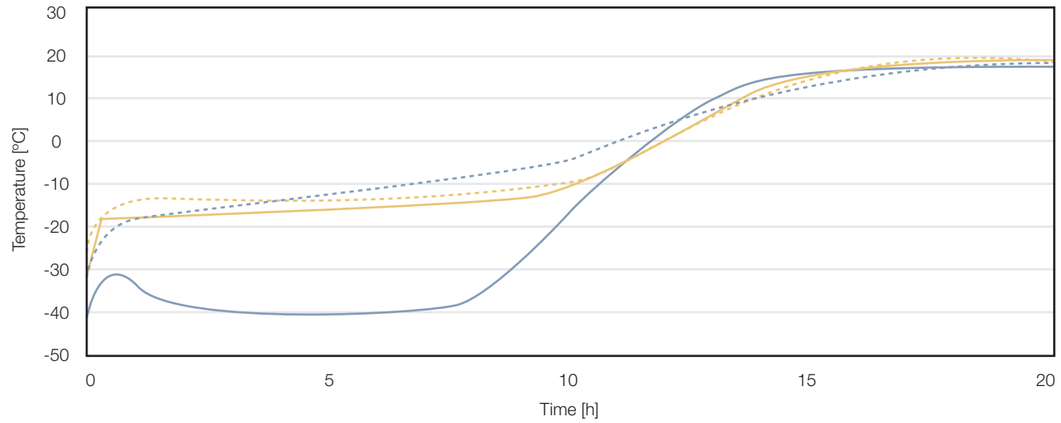


Figure 12: Sample and shelf temperature during a freeze-drying process above and below the T_g. Sample (full line) and shelf (dash line) temperature during process above (p= 1 mbar, green lines) and below (p=0,08 mbar, blue line) the T_g.

The sample freeze-dried at higher chamber pressure (1 mbar) stays above -20 °C during primary drying, whereas the sample freeze-dried at lower pressure (0.08 mbar) remains below the T_g at -38 °C. No significant difference in the time needed for full sublimation could be observed. Both samples were subsequently taken out after the process had reached its endpoint, i.e., sample and shelf temperature were equal, photographed, and imaged using SEM (Figure 13). SEM samples were taken by cutting a square out of the middle of the freeze-dried cake.

In Figure 13, the obtained dry sample cakes are shown. The cake obtained by drying above T_g collapsed during freeze-drying, as can be observed in the shrinkage and the characteristic foamy appearance on top of the cake. SEM images confirm this. It has been shown that when samples collapse in the freeze drier, the obtained microstructures show more open and porous structures due to the viscous flow of the material while in the amorphous state. The sample freeze-dried below the T_g, on the other hand, maintained a nice even cake. The SEM images also show a well-ordered microstructure of sheets with small pores.

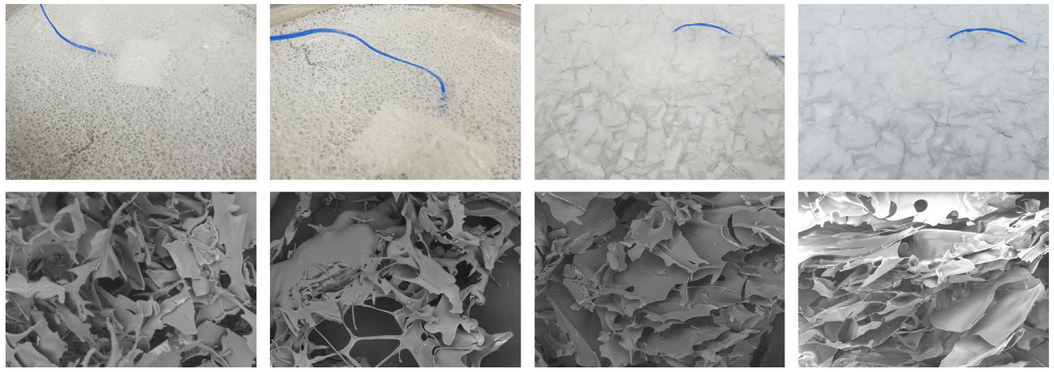


Figure 13: Photographs and SEM images of samples after freeze drying above the T_g (upper four images) and below the T_g (bottom four images).

7.4 Conclusion

Primary drying above the glass transition temperature can lead to the collapse of the sample and undesirable side effects. Care should be taken to optimize the freeze-drying process to remain below the T_g. This is especially important for pharmaceutical formulations where many actives and excipients possess a T_g.

8. Sample surface – The bigger, the better – shelf drying

8.1 Overview

In freeze-drying, the container surface and fill depth affect lyophilization. The product freeze-dries from the top down, building a layer of dry product on top of the remaining frozen product. The more the layer builds up, the more difficult it becomes for the vapor to escape. It should not be assumed that the fill volume or the vial size can be changed without effect. A larger vial will spread the same volume of product on a larger surface area and a lower depth, increasing the freeze-drying speed; a narrower container will cause the product to dry more slowly.

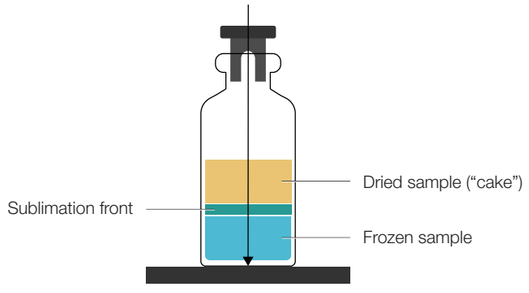


Figure 14: Sublimation front moving through the product from top to bottom.

8.2 Tall and narrow or wide and flat? Vial geometry and freeze-drying

In freeze-drying, a sublimation front moves from the top of the sample, i.e., air/solid interface, downwards towards the bottom of the vial, leaving a porous powder in its wake. The speed at which the sublimation front moves depends on the depths of the sample.

During freeze-drying, the amount of dried sample progressively grows larger on top of the frozen sample. However, a larger dried sample cake increasingly hinders the vapor from escaping from below. The thicker and larger the cake, the more impediments it presents. This can lead to a slowing down of the freeze-drying process and a decrease in the effect of sublimation cooling, which is the temperature drop a sample experiences in a freeze-drying chamber due to energy being used as heat for sublimation. That, in turn, can negatively affect the sample temperature and, in the worst-case scenario, can lead to premature melting of the sample before it is dried. Hence, increasing the surface area and decreasing the sample depths will lead to better results.

8.3 Influence of surface area on the freeze-drying rate

To investigate the influence of surface area on sublimation rate, 12 g of an aqueous poly (vinyl) alcohol (5 wt%, Mw = 15000 g/mol) solution was freeze-dried in three different sample vials of varying diameters (d = 24 mm, 37 mm, and 52 mm) reaching fill heights of 76 mm, 150 mm, and 330 mm respectively. Five vials of each vial type were freeze-dried together to calculate average sublimation. All samples were frozen at -40 °C and freeze-dried for 7 hours.

Figure 15 shows the sample temperature profiles for three different sample vials of varying diameters using the same freeze-drying parameters. The decrease of the sublimation cooling and subsequent increase in sample temperature can clearly be observed. Hence, the temperature profile for a vial with d = 24 mm (green) does not drop beneath -10 °C during the whole freeze-drying process, and the temperature profiles for d = 37 mm (blue) and d = 52 mm (red) show starting temperatures of -23 °C and -30 °C respectively. The plotted data shows that a higher surface area led to faster sublimation. This is most readily noticeable when comparing the slope of the three graphs. For the vials with the smallest diameter, there is almost no increase in 7 hours. In contrast, the slope of the temperature profile of the vial with the largest diameter steadily increased for 5 hours, after which it dramatically increased, signaling the approaching completion of the freeze-drying process.

The temperature profiles show that a higher surface area leads to faster sublimation. To illustrate this point better, the percentage of ice sublimated after 7 hours was plotted against the diameters of the vials (Figure 16). Using a wide vial (d=52 mm) leads to almost complete sublimation, i.e., 95% of the ice in the sample was gone after 7 hours. Decreasing the width to 37 mm resulted in a drop in sublimation to 77%, and when vials with diameters of 24 mm were used, only 51% of ice was sublimated after 7 hours.

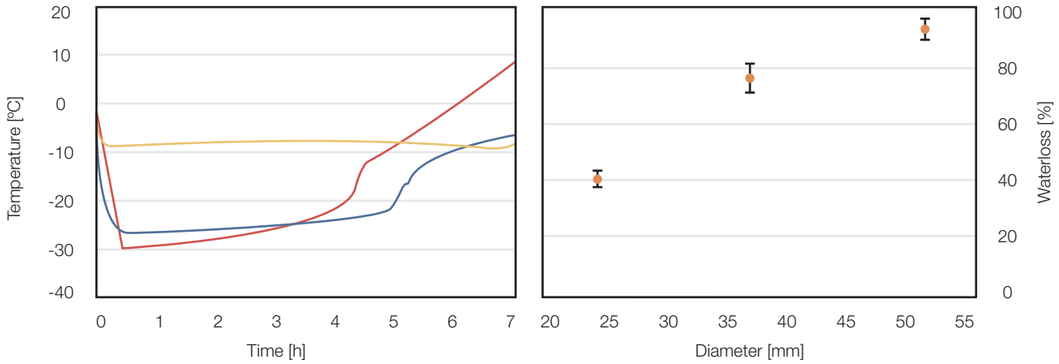


Figure 15: Sample temperature profiles using three different sizes of vials during a freeze-drying process (green, d = 24 mm – blue, d = 37 mm – red, d = 52 mm).

Figure 16: Percentage of water loss against vial diameter.

8.4 Conclusion

A common misconception regarding freeze-drying is that the sample container geometry and fill depths do not influence the freeze-drying process or the finished product. However, even small changes in vial diameter significantly influence the surface area and, as such, the sublimation rate. A larger surface area can cut the sublimation time almost in half and help maintain a low sample temperature, which is an important factor in avoiding sample collapse due to melting or sublimating above the glass transition temperature. Hence, carefully selecting the appropriate type of sample containers can accelerate the process, making the freeze-drying process more energy and time-efficient without negatively affecting the product quality.

9. Sample surface – The bigger, the better – manifold drying

9.1 Overview

In a freeze-drying process, sublimation occurs at the sample's surface; drying begins at the top of the product, and a layer forms where drying takes place. During the drying process, this well-defined sublimation front travels from top to bottom of the product. Drying is efficient at the beginning of the process but becomes more challenging as the sublimation front moves downwards. The sublimated water molecules must then pass the dried product (the cake") before leaving the matrix.

The drying rate at which a certain product is freeze-dried depends on various factors, with pressure and temperature being the key elements. However, the product itself also plays an important role, particularly the product volume to be freeze-dried and the kind of product arrangement. Generally, the greater the surface area relative to the volume, the faster the product dries. A larger surface results in more water molecules leaving the matrix.

9.2 How can drying efficiency be improved with manifold freeze-drying?

In manifold applications, if liquids are to be dried in flasks in a layer thicker than 1 to 2 cm, shell freezing may be beneficial. The product is frozen under rotation in a cooling bath. The rotation spreads the product on the wall of the flask, creating thin product layers (Figure 17). This freezing method increases the available sublimation area, reducing the freeze-drying time.

For example, freezing 200 mL of water in a 1L flask would result in a bulk of 4.5 cm high. By rotating the flask during the freezing process, the frozen product is spread on the inner wall of the flask to produce an even, homogeneous layer less than 1 cm thick (Figure 18).



Figure 17: Shell freezing using a Rotavapor® R-300 and the Dewar accessory.



Figure 18: Shell frozen flask with a homogeneous layer of water on the inner wall.

Experimental data (Figure 19) show that under similar manifold freeze-drying conditions, sublimation rate is more than doubled when the sample is frozen using shell freezing (23.9 gwater/flask/h; orange squares) than when frozen in bulk (9.9 gwater/flask/h; blue dots).

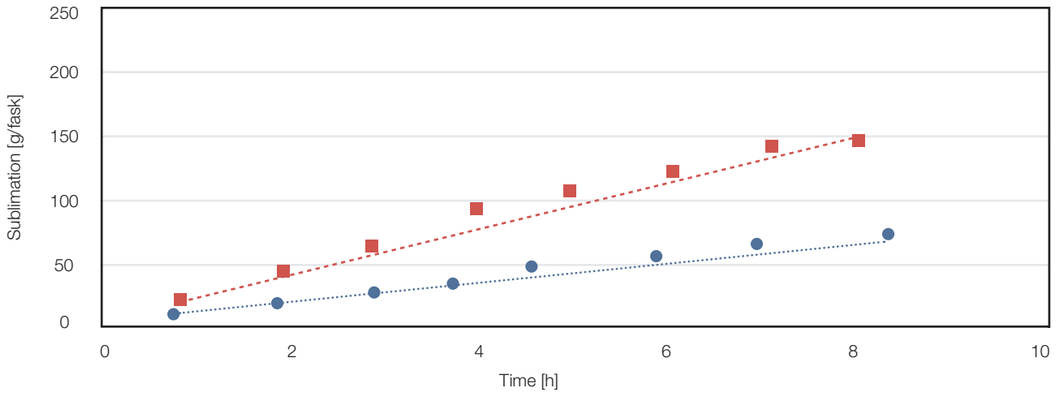


Figure 19: Sublimation in gwater/flask with (orange square) and without (blue dots) shell freezing.

9.3 Conclusion

The drying rate of a product generally depends on the surface area relative to the volume of product. The larger the surface area, the faster the product dries since more water molecules can leave the matrix. To increase product surface when using manifold freeze-drying, it is recommended to use shell freezing so that the product is spread on the inner wall of the flask while freezing. This recommendation is confirmed by experimental data showing a sublimation rate that is two times faster when shell freezing is used instead of bulk freezing.

10. One cycle fits all – the myth of the standard freeze-drying cycle

10.1 Overview

When starting a new freeze-drying process, it is common to use a cycle borrowed from other applications or products. Sometimes, this works. However, the cycle might turn out to be inefficient and/or lead to a high degree of product failure. Small changes such as container size, formulation changes, filling volume, or number of vials can significantly affect the process. Ideally, each cycle needs to be developed and optimized for a specific product, batch parameters, and freeze dryer.

10.2 What can be optimized in a freeze-drying process?

In general, a freeze-drying process can be divided into two cycles. The first cycle is called primary drying. Therein, most drying happens in the form of ice sublimated at low temperatures and pressures. Since sublimation is an endothermic process, the primary drying cycle can be accelerated when heat is applied via heatable shelves. However, if too much heat is applied too fast, heat-sensitive samples like sugars and proteins might deteriorate, or the sample might collapse because it is dried above the glass transition temperature. Cycle optimization needs to be adapted to the sample. The second cycle, called secondary drying, removes trapped water at high temperatures and even lower pressures. In this cycle, the product is dry enough not to run the risk of sample collapse. Heat-sensitive samples should still not be overheated to avoid degradation.

10.3 One cycle – four samples

To investigate the freeze-drying behavior of different samples at different concentrations, solutions of polyvinyl alcohol (PVA) (5 wt% and 1 wt%) and trehalose (1 wt% and 5 wt%) were freeze-dried under the same conditions. To that end, 30 mg (150 mg) of PVA or trehalose was added to 3 mL water in a 5 mL vial and frozen for 24 h at -40 °C. The samples were freeze-dried at 0.5 mbar for 10 hours until the sublimation was finished. The endpoint of the sublimation was determined as the point where shelf temperature and sample temperature meet. Figure 20 shows the temperature profiles.

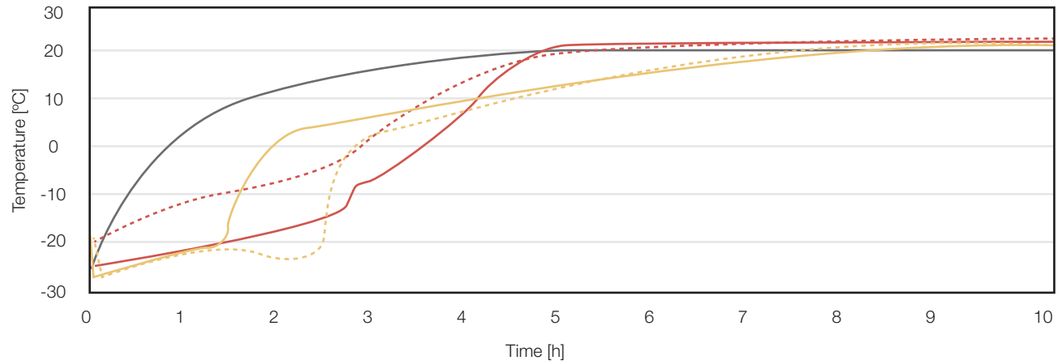


Figure 20: Temperature profile of a freeze-drying process using two different samples (polyvinyl alcohol in red and trehalose in yellow) at two concentrations (1% full line–5% dash line). The set shelf temperature is represented by full black line.

As seen in Figure 20, all samples show very different sublimation behaviors. The two most obvious differences are that while the sublimation of PVA is relatively steady and fast over the entire duration of the process, trehalose takes longer to sublimate. Another observation is that differently concentrated solutions behave differently depending on the ingredients. Solutions with a lower trehalose concentration sublimate faster than samples with a higher concentration of trehalose (Figure 20 yellow lines). In contrast, solutions of PVA show the exact opposite behavior, i.e., sublimation speed increased with concentration.

This simple experiment demonstrates that a freeze-drying cycle depends on the chosen sample. Hence, it is crucial to reevaluate any existing freeze-drying protocol when working with new or unknown samples.

10.4 Conclusion

Different product formulations will freeze-dry differently. Excipients have different thermal characteristics, and altering the formulation can affect the freeze-drying cycle. Modifying concentrations will change the product's processing characteristics and consequently change the drying time and parameters. Small changes in formulation, batch parameters, or equipment will impact the process requirements. Reusing existing freeze-drying cycles for a reformulated product is not recommended.

Proper optimization of a freeze-drying process will save time and energy while improving the quality of the obtained sample. Every optimization process must start with evaluating the sample properties, such as concentration and formulation. Although finding the perfect cycle for each sample takes time, the rewards are well worth the effort.

11. Secondary drying – What? Why? When?

11.1 Overview

In freeze-drying, samples are dried by sublimation, i.e., water or organic solvents are removed via a direct phase transition from solid to gas. Some unfrozen water cannot be removed by sublimation and remains in the pores of the freeze-dried material. This can be circumvented by adding a second phase to the freeze-drying process called secondary drying.

11.2 How does secondary drying work?

In the primary drying phase of a freeze-drying process, roughly 90% of water is sublimated at low temperatures and pressures. The last 10% of water remains unfrozen in the porous structure left after sublimation.
A secondary drying step is introduced to remove this residual water, where the pressure is reduced, and the shelf and sample temperatures are increased. This process evaporates the remaining water from the sample's structure, allowing the final moisture content to drop to as low as 1%.

11.3 Freeze drying with and without secondary drying

To investigate the influence of secondary drying on the final moisture content after the freeze-drying process, 200 mL solutions of 5 wt% polyvinyl alcohol (PVA) and 10 wt% trehalose were frozen at -40 °C and freeze-dried with and without a secondary drying phase, using the following parameters:

PVA and Trehalose were freeze-dried with and without a secondary drying phase. The temperature profiles and parameters, including and excluding a secondary drying step, are shown in Table 2, Figure 21, Table 3, and Figure 22, respectively. In both temperature profiles, it was observed that the primary drying phase was completed after approximately 16 hours, i.e., the sample temperature and shelf temperature were the same. To investigate if a secondary drying phase reduces the final moisture content, a moisture balance (Moisture Analyzer HR73 by Mettler Toledo) was used to measure the percentage of moisture. Table 4 shows that up to 1% more water could be removed in the freeze-drying process with a secondary drying step.

Table 2: Parameters of a freeze-drying process including secondary drying.

Phase	Primary Drying	Primary Drying	Secondary Drying	Secondary Drying
Duration [hh:mm]	01:40	20:00	01:40	03:00
Shelf Temp. [°C]	10.0	10.0	30.0	30.0
Shelf Temp. gradient [°C/min]	0.40	0.00	0.20	0.00
Pressure [mbar]	0.100	0.100	0.050	0.050

Table 3: Parameters of a freeze-drying process excluding secondary drying.

Phase	Primary Drying	Primary Drying
Duration [hh:mm]	01:40	20:00
Shelf Temp. [°C]	10.0	10.0
Shelf Temp. gradient [°C/min]	0.40	0.00
Pressure [mbar]	0.100	0.100

Table 4: Moisture content after freeze-drying with (+) and without (-) secondary drying.

Secondary Drying	+	-	+	-
Moisture content [%]	2.53	3.48	1.84	2.40

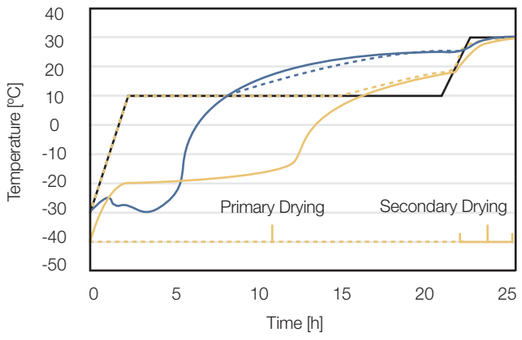


Figure 21: Temperature profile of the freeze-drying process of PVA (green lines) and trehalose, including secondary drying (blue lines). The primary drying and secondary drying phases are completed when the temperature of the shelf (dash line) meets the temperature of the product (full line). The set shelf temperature is shown as a full black line.

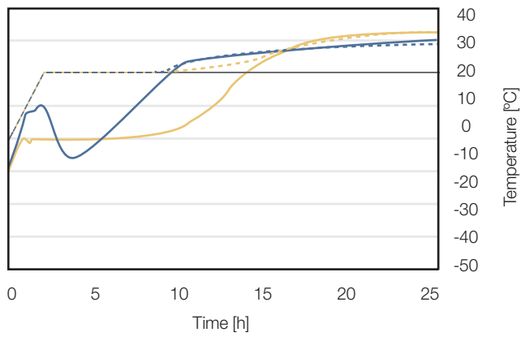


Figure 22: Temperature profile of the freeze-drying process of PVA (green line) and trehalose (blue line) excluding secondary drying (full line). Primary drying is complete when the product's temperature meets the shelf's temperature (dash line). The set shelf temperature is shown as a full black line.

11.4 Conclusion

It is not possible to obtain a product via freeze-drying without any moisture. However, by adding a secondary drying step after primary drying, trapped water can be removed, and samples with very low moisture content can be obtained compared to samples that have just been sublimated by primary drying. Secondary drying is a helpful and easy process optimization step when a very dry product is desired.

12. Influence of chamber pressure on the sublimation rate

12.1 Overview

The vacuum within the freeze-drying chamber is not intended to “suck out” the moisture but to create the necessary conditions for the ice to sublimate directly to vapor. If the chamber pressure is too high, the sublimation rate will slow down, and the product temperature will rise, leading to unwanted consequences such as sample melting. To counteract the effects of sublimation cooling, heat must also be added continually to the product. If the pressure chamber is too low, heat transfer will be decreased, leading to a slower process. Reducing the pressure below the vapor pressure of the condenser could even lead to sublimation of the ice from the condenser towards the vacuum pump.

12.2 What role does the chamber pressure play in sublimation?

A common misconception in freeze-drying is that lowering the chamber pressure will increase the sublimation rate. Sublimation, i.e., the direct phase transition from solid to gas, is an endothermic process. As such, heat is needed to facilitate the transition, and if no or little heat is supplied, the process can slow down or even stop altogether. Heat can be transferred to the sample via three main routes: conduction, convection, and radiation (Figure 23). In a freeze-drying chamber, heat transfer by convection is possible due to the remaining air molecules. At lower chamber pressures, fewer air molecules remain in the chamber, and the amount of heat transferred by convection decreases.

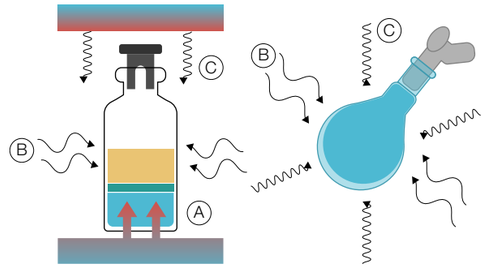


Figure 23: Heat transfer mechanisms in a shelf freeze-drying process (left) and manifold freeze-drying process (right). (A) Conduction (B) Convection (C) Radiation.

One of the main driving forces in freeze-drying is the pressure difference between the condenser and the sample in the drying chamber. Hence, the pressure in the chamber at the sample level must be higher for the vapor to move to the condenser and be solidified into ice. If the chamber pressure is too low, an equilibrium might ensue, significantly slowing the process.

12.3 How does pressure influence sublimation speed?

To show the influence of chamber pressure on the sublimation rate, the sublimation of 400 g ice was measured at two different chamber pressures, i.e., 1 and 0.2 mbar. In both cases, one sample was heated with a heatable shelf to 20 °C to add heat transfer by conduction, whereas the second sample was not heated and solely relied on convection. To follow the process, sample temperature data was recorded over 7.5 hours. A rise in the sample temperature signifies advanced sublimation. Plotting temperature against time gives a good approximation of the sublimation speed (Figure 24).

After 7.5 hours of freeze-drying, 28% and 32% of the ice were sublimated at 0.2 mbar and 1 mbar, respectively. The plotted data confirmed this by showing a constant sample temperature. However, when the sample shelves were heated, the sublimation rate dramatically increased at higher chamber pressures (Figure 24, red graphs). After 7.5 hours of sublimation, 61% of ice was sublimated when the chamber pressure was 0.2 mbar, whereas at 1 mbar, 75% of ice was sublimated in the same period.

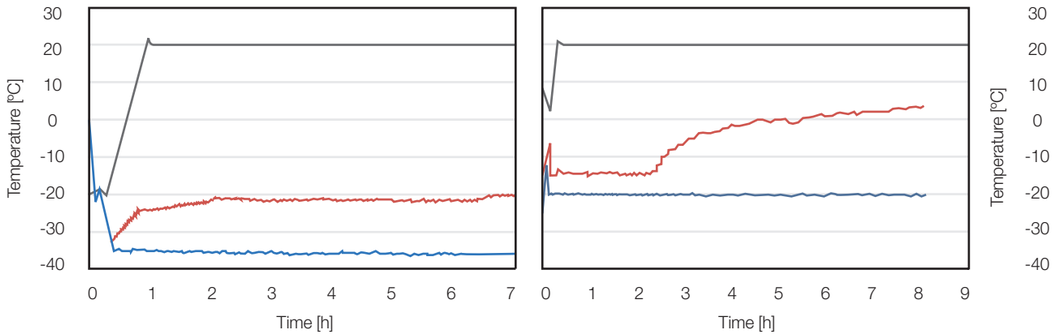


Figure 24: Heated shelf set temperature (grey) and sample temperatures of samples on heatable shelves (orange) and non-heatable shelves (blue) during a freeze-drying process samples at 0.2 mbar (top) and 1 mbar (bottom) chamber pressure.

12.4 Conclusion

Heat convection from remaining air molecules in a freeze dryer's chamber and a well-balanced pressure gradient between the chamber and condenser can significantly increase the sublimation rate in a freeze-drying process. For this reason, it is advisable to optimize the pressure settings when designing a freeze-drying method instead of applying very low pressure settings.

13. The benefits of heatable shelves

13.1 Overview

It is important to balance the heat input and output in freeze-drying. As the solvent escapes from the product, the product temperature drops through sublimation cooling. The rate of the drying slows down and needs to be counteracted by the addition of more heat to the product. This heat can be added via heatable shelves whose temperature can be raised or via the environment.

13.2 What are the heat transfer mechanisms?

For the phase change from solid to vapor to occur, energy, in the form of heat, needs to be added to the product. Heat is transferred by convection and radiation from the surrounding environment. For a shelf freeze dryer, heat can also be transferred by conduction from the shelf (Figure 23). The more energy transferred to the sample, the faster the process. A good balance between heat energy and sample critical temperature is needed to maintain product integrity.

The most common accessory to provide heat to the samples are heatable shelves. The temperature of the shelves is controlled and automatically adapted according to the programmed method.

13.3 How will heated shelves improve the freeze-drying process?

Heat energy must be added to the sample in a freeze-drying process. An increase in sample temperature of 1 °C has been shown to shorten the freeze-drying process by 13%, whereas an increase of 5 °C could shorten the process by 50%.

Experimental data show that for 400 g of frozen water on a plate, only 32% of the ice is sublimated in 7.5 hours with non-heated shelves. When the shelves are heated up to 20 °C, 61% of the ice is sublimated within the same time. The temperature profiles of the samples confirm these results during the freeze-drying cycle (Figure 25).

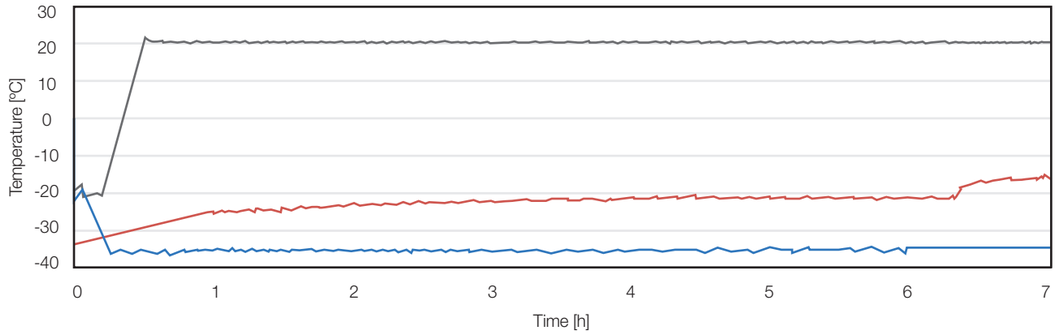


Figure 25: Heated shelf set temperature (grey) and sample temperature profiles during a freeze-drying process with non-heatable (blue) and heatable (orange) shelves.

13.4 Conclusion

During a freeze-drying process, 2800 Joules must be provided to convert 1 g of ice into vapor. This energy is brought as heat by the environment through radiation or convection or with heatable shelves by conduction. The more energy transferred to the sample, the faster the process.

It is recommended to heat the shelves to increase product temperature to influence the speed of the pro-cess. Attention must be paid to remaining below the sample's critical temperature. Heatable shelves al-low for precise control of the heat input, while thermocouples in the product simultaneously observe the product temperature and ensure the collapse temperature is not exceeded.

14. How to control negative shelf temperature

14.1 Overview

Since sublimation is an endothermic process, the primary drying cycle can be accelerated when heat is applied to the sample through the environment or via heatable shelves. However, if too much heat is applied too fast, sensitive samples like sugars and proteins might deteriorate or collapse because they are dried above their critical temperature. To avoid problems of this type, shelf temperature should sometimes be set to a negative temperature for primary drying. A negative temperature is difficult to set up if the instrument does not have cooling shelves.

Even though a freeze dryer does not have cooling shelves, this does not mean that negative shelf temperatures cannot be set. It is important to work with a full load of samples on the shelf so that the set temperature can be maintained throughout sublimation cooling.

14.2 Sublimation – energy requirements

During the freeze-drying process, the solvent is removed from the product through sublimation. Sublimation is an endothermic process, and 2800 joules are required to sublimate 1 g of ice. As more of the ice is sublimating, more energy is removed from the sample, and the more the sample cools down the shelf. This is called sublimation cooling. Usually, to counteract this sublimation cooling phenomenon, heat is added to the sample through heatable shelves so that the process can be completed faster.

14.3 How to set up a process?

When deciding how much heat will be applied to the sample, it is important to ensure that even though the shelf temperature might be higher than the sample's critical temperature, the sample remains below its critical temperature. When sample critical temperatures are below 0 °C, shelf temperature must also be set to a negative value. If the instrument does not include cooling shelves, the instrument will be able to regulate a negative temperature by finding a good balance between the heating process of the shelves and the sublimation cooling. It is, therefore, essential to leave enough time for the shelves to reach the set temperature, i.e., the right balance between heating and sublimation cooling. Since sublimation cooling is necessary to overcome the heat brought to the shelf by the heating system and by the environment, it is also mandatory to have enough sample sublimating to balance the process and maintain a negative shelf temperature. Therefore, it is important to work with a full load of samples on the shelf or to use vials filled with water to complete the loading.

Figure 26 shows the difference between drying profiles when a full sample load is freeze-dried or when only one vial is used for method development. It also shows the drying profile of an empty shelf. A solution of 5% glycine was used in this experiment, and the method was developed as shown by the grey dashed line in Figure 26. Seven hours were allotted for the shelves to reach -15 °C from a total of 27 hours of primary drying. The pressure was set at 0.1 mbar.

Results in Figure 26 show that, as expected, the temperature of an empty shelf (green dash line) without cooling power cannot be regulated at -15 °C degrees and that the temperature will increase due to energy input from the shelf heater and then the environment. When only one vial is placed on the shelf (blue profile), despite the sample being freeze-dried, the shelf temperature (blue dash line) cannot be main-tained at -15 °C through sublimation cooling since there is not enough sublimation to balance the heat from the shelf and the environment. The shelf temperature will thus follow the same trend as that of an empty shelf. When the shelf is fully loaded with sample (red profile), the shelf temperature (red dash line) is maintained at -15 °C while sublimation occurs. Once the sample starts to become dry, shelf (red dash line) and sample (red full line) temperatures both increase until they intersect, which means that the sample is dry.

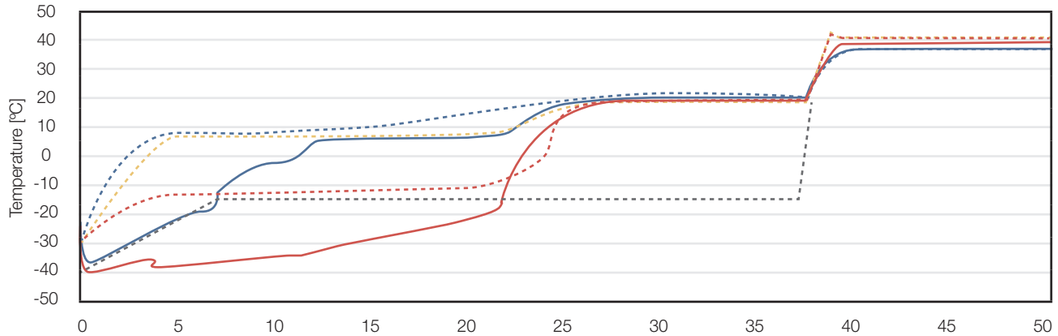


Figure 26: Temperature profiles of the freeze-drying process with shelf temperature set (grey dash line) at -15 °C. The red line shows the shelf (dash line) and the sample (full line) profiles when the shelf is full, the blue line shows the shelf (dash line) and the sample (full line) temperature profile when only one vial is on the shelf, and the green dash line shows the temperature profile of an empty shelf.

14.4 Outlook and conclusion

As shown in Figure 26, it is important to work with a shelf full of samples and to allow enough time for the set temperature to be reached so that the instrument can regulate a shelf temperature of -15 °C. When small amounts of samples are available, and only a few vials can be filled for method development, it is recommended to place the sample in the center of the shelf and to complete the loading with dummy vials containing water. This will help to bring enough sublimation cooling to maintain the shelf temperature at the desired value. The thermocouple should always be placed in the sample.

15. The influence of the environment on the process

15.1 Overview

The sublimation process requires the uptake of heat. This energy can be supplied via different mechanisms, i.e., conduction, convection, and radiation. Putting a freeze dryer in a sunny spot should be advantageous because more energy by radiation is available. However, too much sunlight can lead to sublimation that occurs too quickly, which can cause problems such as sample collapse or even the melting of the sample due to backpressure below the drying front.

15.2 Freeze drying with and without direct sunlight

To investigate the influence of direct sunlight on a freeze-drying process, solutions of polyvinyl alcohol (PVA) (5 wt% and 1 wt%) as well as Trehalose (5 wt%) were freeze-dried under the same conditions. To that end, 30 mg (150 mg) of PVA or Trehalose was added to 3 mL water in a 5 mL vial and frozen for 24 h at -40 °C. The sample was freeze-dried at 0.1 mbar for 3 hours in direct sunlight (Figure 27) and compared to samples of the same constitution dried at 0.5 mbar (which has been proven to increase the sublimation speed) in a room without direct sunlight. Figure 28 and Figure 29 show the temperature data obtained during the process without and with direct sunlight.

When the freeze dryer was in direct sunlight (Figure 29), the sample temperatures increased considerably faster than in a working environment (Figure 28).

Samples reached temperatures above 0 °C in the first one and a half hours and seemed dry after two and a half hours. Once the process was stopped, however, samples collapsed and redissolved due to the core of the sample not being properly dry (Figure 30). Direct sunlight not only influenced sample quality but heat and radiation going through the acrylic chamber also influenced the freeze dryer itself. The shelves' temperature increased above the set point, and the condenser temperature increased slightly, making the set pressure difficult to maintain.



Figure 27: Freeze Dryer in direct sunlight.

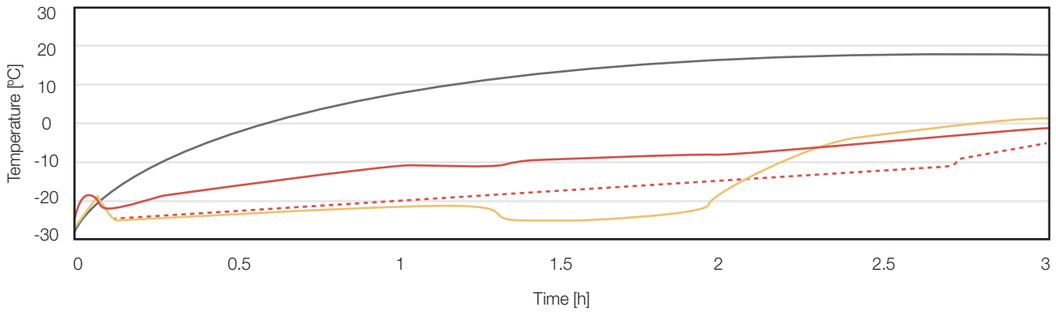


Figure 28: Temperature profiles of a freeze-drying process without direct sunlight. The black line shows the shelf temperatures, the red line shows the drying profiles of 1% PVA (dash) and 5% PVA (full), and the yellow line shows the drying profile of 5% trehalose.

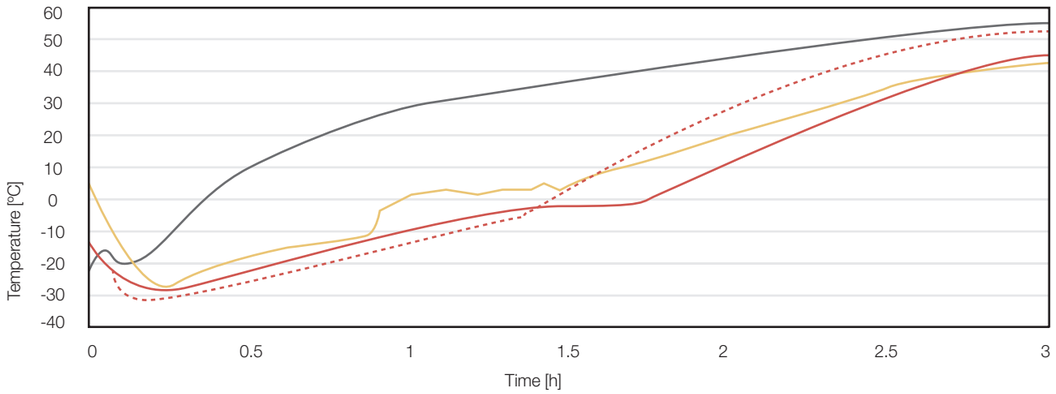


Figure 29: Temperature profiles of a freeze-drying process with direct sunlight. The black line shows the shelf temperatures, the red lines show the drying profiles of 1% PVA (dash) and 5% PVA (full), and the yellow line shows the drying profile of 5% trehalose.



Figure 30: Left: how the samples should have looked after freeze-drying; Right: Damaged samples after freeze-drying in direct sunlight.

15.3 Conclusion

Conclusion The placement of a freeze dryer plays an important role in the smooth running of the process and in extending the lifetime of the machine. A location that provides too much direct sunlight will not only hinder the machine in its function, i.e., target pressure cannot be reached, or the condenser is too warm, but it will also lead to heating up of the samples and, with that, catastrophic sample damages.

16. How to overcome the edge vial effect

16.1 Overview

Any vial that is not surrounded by six other vials is referred to as an edge vial. Edge vials receive additional heat transfer during primary drying due to radiation from the acrylic chamber. This higher energy input results in higher product temperatures and shorter primary drying time for the edge vials than the rest of the batch.

16.2 What is the edge vial effect?

In a shelf freeze dryer, even though most of the energy is transferred to the sample via conduction, the effects of radiation and convection also need to be considered for product quality and process control.

Radiation coming through the acrylic chamber will cause the product on the shelf's perimeter to dry faster than the product in the center. This phenomenon is well-known in freeze-drying and is called the edge vial effect.
A metallic ring (Ferrule #11065816) placed around the sample as a shield can help to reduce this phenomenon (Figure 31).

16.3 How to overcome the edge vial effect?

Experimental data (Figure 32) shows that when no shield was used, the samples on the perimeter of the self were dried in 19 hours, while those in the center needed 23 hours. Using a ferrule to shield the outer layer of the sample increased the drying time of the perimeter samples by 2 hours, meaning that 21 hours were required to dry the outside samples, whereas 23 hours were needed for those on the inside. Results also show that the first row of samples shields the rows in the middle and could be used as a shield instead of the ferrule.

16.4 Conclusion

Radiation coming through the acrylic chamber causes the product on the perimeter of the shelf to dry faster than the sample in the center. To achieve better product uniformity and process control, it is recommended to shield the outer layer of vials. Results show that using the ferrule alone might not be enough to achieve perfect uniformity. Shielding could also be improved by filling the outside row with vials containing water.



Figure 31: Placing a ferrule on the shelf around the samples will help protect them from radiation.

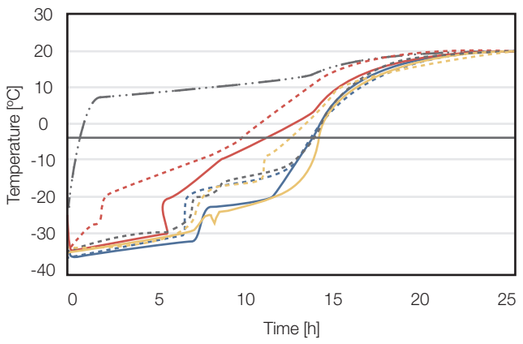


Figure 32: Freeze-drying processes without (dashed line) and with (full lines) ferrule as a sample shield. The red lines show the drying profile of the outside row on the shelf, and the grey long dash-dotted line indicates shelf temperature.

17. Shelf spacing, Moisture content & Condenser overload

17.1 Shelf spacing

Freeze dryers commonly have multiple shelves in the drying chamber to provide space for numerous vials filled with product. Therefore, adding more shelves in the instrument could optimize the process so that more product is freeze-dried at once.

However, it is important to ensure that there is enough space between shelves so that the product (together with the shelf) can be easily loaded into the drying chamber and that the room above the drying vessels (vials, plates, …) is adequate for the vapors to escape the containers unhindered. Insufficient space above the sample will most likely result in localized drying conditions due to the vapor leaving the container at a high rate and not being able to escape properly.

17.2 Moisture content

Frequency-modulated spectroscopy analysis of the final moisture levels across a freeze dryer shelf (Lighthouse Instruments).

Historically, the strategy of “the drier, the better” was frequently followed. This approach was appropriate for molecules with a direct degradation pathway triggered by water, but the approach was not well suited to the drying of biopharmaceuticals. Biological products such as cells, vaccines, or proteins typically require higher moisture content than chemicals. Studies have shown that proteins need small amounts of water to help maintain higher order structure, even in the lyophilized state. Other products, such as specific blood plasma formulations, need minimal water to achieve efficient dry-heat viral inactivation. Many products can be damaged by over-drying, and stability studies should be carried out to assess the ideal moisture content of a formulation. It is necessary to design a freeze-drying cycle that keeps all product vials within a specific moisture range, with minimum and maximum specifications.

Moisture analysis is commonly performed by Karl Fischer titration or thermogravimetric analysis. These methods are time-consuming and will destroy the sample. However, lyophilization chamber moisture mapping can be done using headspace analysis by frequency-modulated spectroscopy (Figure 33) (Lighthouse Instruments).
This method offers a quick and non-destructive analysis to measure the samples' moisture content accurately and monitor the quality of entire batches.

0.8	0.7	0.5	0.5	0.7	0.5	0.5	0.9	0.7	0.8	0.7	0.7	0.9	0.7	0.8	0.8	0.6	0.8	0.5	0.7	0.7	1.1	0.7
0.5	0.5	0.6	0.5	0.7	0.5	0.5	0.8	0.7	1.2	0.3	1.4	0.8	1.1	0.8	1.1	1.0	1.0	0.7	0.7	0.6	0.6	0.6
0.4	0.5	0.9	0.9	0.7	1.2	0.9	1.2	1.3	1.3	1.9	1.5	1.3	1.5	1.5	1.8	1.2	1.4	1.1	1.2	0.7	0.7	0.7
0.6	0.7	0.6	0.9	0.6	0.9	1.0	1.2	1.2	1.4	1.3	1.7	1.6	2.2	1.5	1.7	1.7	1.6	1.8	1.0	0.9	0.8	0.6
0.6	0.5	1.2	0.9	0.8	1.1	1.3	1.2	1.6	1.6	1.3	1.6	2.1	2.0	2.1	2.0	2.1	1.5	1.4	1.3	0.8	0.8	0.6
0.4	0.6	0.6	0.6	0.7	1.1	1.2	2.3	1.6	1.7	1.4	1.7	1.9	2.5	1.4	2.0	2.2	3.2	1.4	1.2	1.2	0.6	1.0
0.9	0.4	1.1	0.8	0.7	0.8	1.0	1.3	1.4	1.5	1.9	1.6	2.6	1.8	2.1	1.7	2.1	1.5	1.6	0.9	0.9	0.5	0.7
0.5	0.5	0.5	0.9	1.0	0.6	0.9	0.9	1.1	1.1	2.6	1.5	1.6	1.6	1.6	1.4	1.6	1.6	1.3	1.0	0.7	0.8	0.7
0.9	0.8	0.6	0.4	0.6	0.6	0.9	0.7	1.2	1.2	1.1	1.0	1.2	1.4	1.5	1.1	1.3	0.8	1.2	0.7	0.9	0.6	0.7
0.6	0.7	0.7	0.6	0.8	0.5	0.8	1.1	0.7	0.9	0.9	0.6	1.0	0.7	0.9	0.7	1.2	0.6	0.9	0.9	1.2	0.9	0.9
0.5		0.5		1.2		0.9		0.5		0.6		0.9		0.8		0.6		1.1		0.8		0.6

Figure 33: Frequency modulated spectroscopy analysis of the final moisture levels across a freeze dryer shelf (Lighthouse Instruments).

17.3 Condenser overload

When looking into condenser specifications, two parameters are important: the total capacity of the condenser (how many kg of ice/liter of water can be contained on the coil) and the condensing capacity or freeze-drying rate (how many kg/liters per 24 hours can be trapped on the coil).

These parameters are important because the total amount of solvent in the batch and the rate at which solvent sublimates can overload the condenser.

If the condenser cannot trap more vapor, the vapor will bypass the condenser and exit through the vacuum pump. Two situations could lead to this phenomenon: there might be more solvent to sublimate than the total capacity of the condenser, or the drying is faster than the condenser's drying rate. These figures are often determined using several standardized parameters and may not always apply to real-life situations.

Vapor will not escape the sample at an even rate throughout the cycle and drying is often faster at the start of primary drying when there is less resistance to the migration of the vapor. It is possible, that even when the performances of a condenser match, with process times averaging between 24 to 48 hours, some specific samples will generate too much vapor too fast for the condenser to trap, which will cause the condenser to overload.

Process temperature, container type, and batch size are all factors that can influence the rate at which vapor is generated. Applying too much energy at the start of primary drying can cause too much vapor production, which could lead to an increase in pressure and a rise in condenser temperature since the condenser will try to cope with condensing too much vapor. This must be kept in mind when designing a freeze-drying cycle.

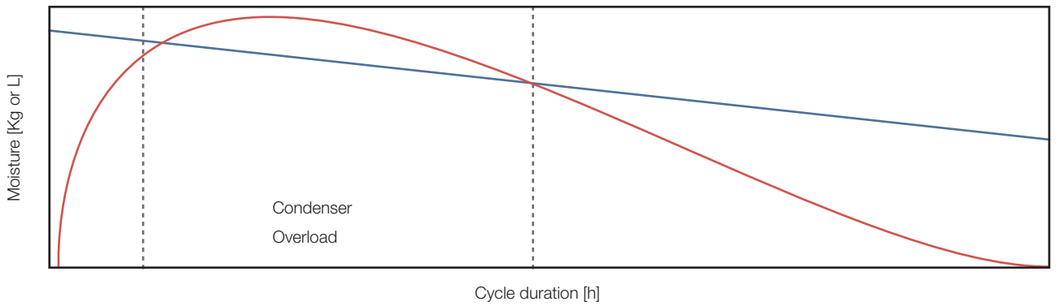


Figure 34: Condenser trapping capacity (blue) and vapor load (red).

18. Endpoint determination – temperature test

18.1 What is endpoint determination?

Out of the three steps of a freeze-drying process, primary drying is by far the longest, so optimizing it would be quite valuable. It could be advantageous to find a way to decrease the amount of time required during this step, as well as to make sure that the process is not terminated too early. Starting secondary drying before all the ice is removed from the product would certainly lead to product defects, such as collapse or eutectic melt.
The time required for primary drying is influenced by several parameters such as sample concentration, sample size, or sample container. The primary drying time also varies from run to run. For this reason, an automated process to detect the endpoint of primary drying would be economically advantageous.

Several analytical methods are available to assess the end of primary drying. The most basic and frequent method is to measure product temperature using thermocouples and to compare the measured temperature with the set temperature of the shelf the product sits on. The product temperature will be colder than the shelf temperature while sublimation occurs since heat from the shelf is needed for the phase change. When the sublimation of the ice is complete, the product temperature will increase and approach that of the shelf. Primary drying is complete when product temperature equals shelf temperature (difference to be determined, often below 1 °C). It is important to note that the vial containing the thermocouple does not represent the entire batch. Due to the wire conducting more heat, the sample will typically dry faster than the rest of the batch.
In bulk drying, the area around the thermocouple will similarly dry more quickly than other areas of the tray. Some additional drying time should thus be added after the thermocouple temperature increases towards the shelf temperature to ensure that the ice in the entire batch of product has been completely removed. How much time depends on sample characteristics and usually varies between several minutes to a few hours.
Thermocouples should always be placed in the center at the very bottom of the container (Figure 35). This is because the product dries from the top down. If drying in vials, it is also advised to place the vial containing the thermocouple in the middle of the shelf to avoid edge vial effects that cause the product in this vial to dry more quickly than the rest of the batch.

Additional endpoint determination methods that can determine the endpoint of primary drying on an entire batch are available. One such method is the comparative pressure measurement, which entails a comparison of the pressure readings between a Pirani gauge and a capacitance manometer. This method will be explained in the next chapter.

With the Lyovapor, endpoint determination of the primary drying using temperature measurement can be activated directly when programming the method. The temperature difference is measured throughout the process and once the difference is below a previously defined set point (normally around 1 °C), the system will automatically switch to the next phase.

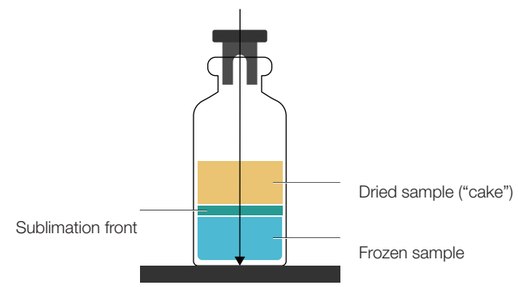


Figure 35: Because the sample dries from the top down, the thermocouple should be placed at the very bottom and center of the vial.

18.2 Freeze drying with and without endpoint determination

To illustrate the time-saving capacity of using endpoint determination, two experiments were run using solutions of 10 wt% Trehalose. A method was written (Table 5) using the Lyovapor™ software with a primary drying phase of 72 hours. The method was run with and without active endpoint determination. For the active endpoint determination, the software was programmed to start checking one hour after the start of primary drying if the requirement of delta T ≤ 1 °C was met. The test was counted as successful if the difference in temperature of 1 °C or less between the shelf and the sample was maintained for 20 min or more. Once these conditions were met, the software was programmed to switch automatically into secondary drying.

The temperature profiles of both processes, i.e., with and without endpoint determination (green and red graphs, respectively), can be found in Figure 36. The red graph shows the shelf temperature (dashed line) and the sample temperature (full line) of a 10 wt% Trehalose solution throughout the freeze-drying process without using the endpoint determination. After 20 hours, both the sample and shelf have reached the same temperature, and the primary drying phase is finished. However, since no active endpoint determination is programmed, the machine keeps running for the remaining 57 hours of the method before switching over to secondary drying. The green graph shows the same process with active endpoint determination and automatic phase switching (dashed line = shelf temperature, solid line = sample temperature). Compared to the first process, the Lyovapor™ automatically switches to secondary drying after 22 hours once the sample and the shelf are at a similar temperature (temperature difference of less than 1 °C for more than 20 min).

18.3 Conclusion

Using endpoint determination via temperature, a freeze-drying process can be optimized automatically to run for the shortest possible time. This can increase productivity, as well as save time and money. Due to the nature of the endpoint test implemented in the Lyovapor™ Software, it not only helps to reduce time, but it also helps to prevent premature switching of phases if sample compositions vary slightly.

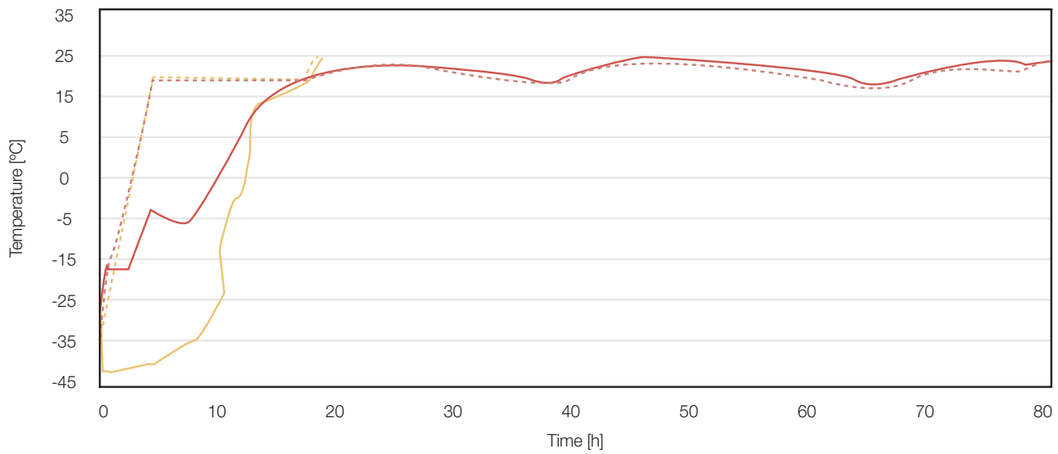


Figure 36: Temperature profile of a freeze-drying process, including primary and secondary drying. The green line shows the process with endpoint determination, whereas the red line shows the process without. The dashed lines represent the shelf temperature.

Table 5: Parameters of a freeze-drying process with and without endpoint determination.

Phase	Primary Drying	Primary Drying	Secondary Drying	Secondary Drying
Duration [hh:mm]	05:00	72:00	01:00	01:00
Shelf Temp. [°C]	20.0	20.0	25.0	25.0
Shelf Temp. gradient [°C/min]	0.17	0.00	0.08	0.00
Pressure [mbar]	0.100	0.100	0.050	0.050

19. Endpoint determination – pressure test

19.1 What is comparative pressure measurement (pressure difference)?

Comparative pressure measurement is a non-invasive endpoint detection method used to determine the end of the primary or secondary drying phase in freeze drying. The technique uses two different pressure gauges: a Pirani sensor and a capacitance manometer.

19.2 What do we need?

The Pirani sensor works on the principle that a gas's thermal conductivity varies with pressure. The gauge measures the pressure using a thin wire suspended in a gas and heated with an electric current. At high pressure, the wire loses heat energy to the gas due to the high collision rate of the surrounding gas molecules with the wire (Figure 37).

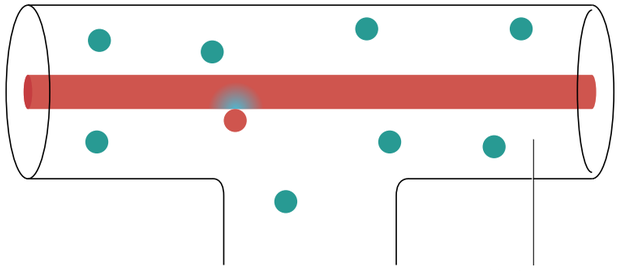


Figure 37: Pirani gauge – The core element is a heated wire. Gas molecules lose energy through collision and lower the temperature of the wire.

When the gas pressure is reduced (vacuum), the amount of gas molecules decreases, along with the conductivity of the surrounding media. The wire will then lose heat more slowly. Since this process relies on the thermal conductivity of the gas molecules and gas composition, a Pirani sensor will only display the correct pressure under its calibrated conditions, which are usually set at pure nitrogen or air environments.

In addition to the Pirani sensor, a capacitance manometer measures pressure independently of the gas composition. With this type of gauge, the difference in the capacitance signal is produced by physical changes within the manometer and not by changes in gas properties. Thus, pressure measurement by capacitance manometer is independent of the gas composition.

19.3 How does it work?

During the freeze-drying process, the gas in the drying chamber is almost entirely composed of water vapor due to the sublimation of ice. The more the sample dries, the more the gas composition changes and the water vapor is replaced by nitrogen or air until the chamber gas contains only pure nitrogen or air at the end of the drying process. Since water vapor's thermal conductivity is higher than nitrogen's by a factor of approximately 1.6, the Pirani gauge has a measurement offset of approximately 60% in a pure water vapor environment. The Pirani gauge and the capacitance manometer can only measure a similar pressure once the sample is dry, and the composition of the chamber gas mainly consists of pure nitrogen or air (Figure 38). It is important to note that pressure fluctuations prevent the difference between the two measurement tools from ever being zero. A suitable endpoint criterion should be higher than the difference caused by the fluctuations but low enough to ensure that the difference between the two displayed pressures is minimal over a given period before switching to the next freeze-drying step.

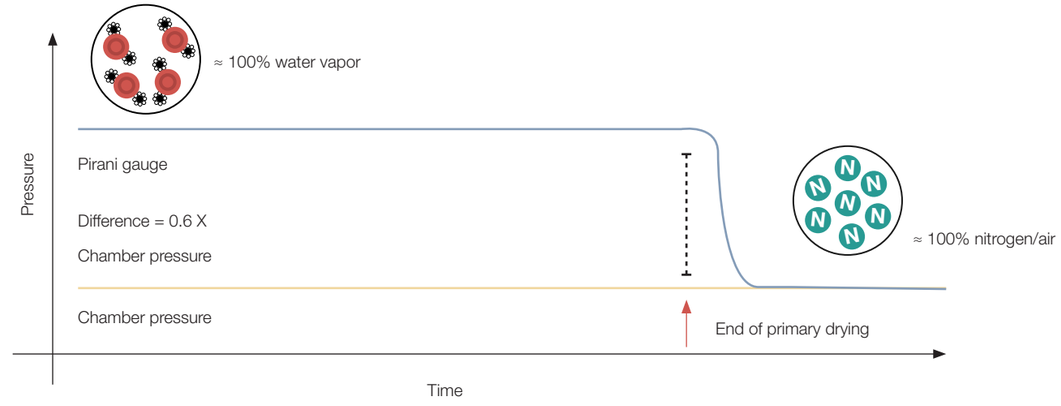


Figure 38: Comparative pressure measurement – during primary drying, the Pirani sensor shows a different measurement than the capacitance manometer. When the endpoint is reached, the gas is composed of pure air/nitrogen, and the two manometers show the same pressure.

19.4 How to find a suitable endpoint criterion?

Due to the pressure fluctuations in the chamber, choosing a pressure difference that is too small between the two devices will likely result in no endpoint detection at all since the criterion will never be met. To find a suitable pressure difference, multiple test runs with a test formulation are recommended. This study used a glycine solution (5% w/V in deionized water) as a test formulation. The solution was frozen over 24 hours at – 40 °C and dried at a pressure of 0.300 mbar. Before each freeze-drying cycle, a vacuum test must be carried out to calibrate the Pirani gauge. This step is mandatory to ensure the Pirani gauge displays the correct pressure before and after the drying phases.

Test runs were carried out without endpoint detection to ensure that the chosen pressure difference is not too low. The pressure difference between the two manometers after the two pressure curves merged was analyzed since this is the point where the sample is assumed to be dry. The analysis showed a maximum pressure difference between the two devices of 0.02 mbar — this should be considered the lower limit for the endpoint criterion.

Multiple test runs were carried out with different pressure differences as an endpoint criterion to find a suitable endpoint criterion. A successful endpoint detection was achieved when the temperature of the shelf met that of the sample, and the pressure of the two manometers merged. During the test runs, several pressure differences (0.05 mbar, 0.03 mbar, and 0.025 mbar) were tested and maintained for at least 60 min before the endpoint criterion was attained.

Figure 39 shows that only in the cycle with a pressure difference of 0.025 mbar as an endpoint criterion were the pressure and temperature curves merging simultaneously before switching to secondary drying. A pressure difference of 0.025 mbar or less maintained for more than 60 min can be considered a suitable endpoint criterion at the set pressure of 0.30 mbar.

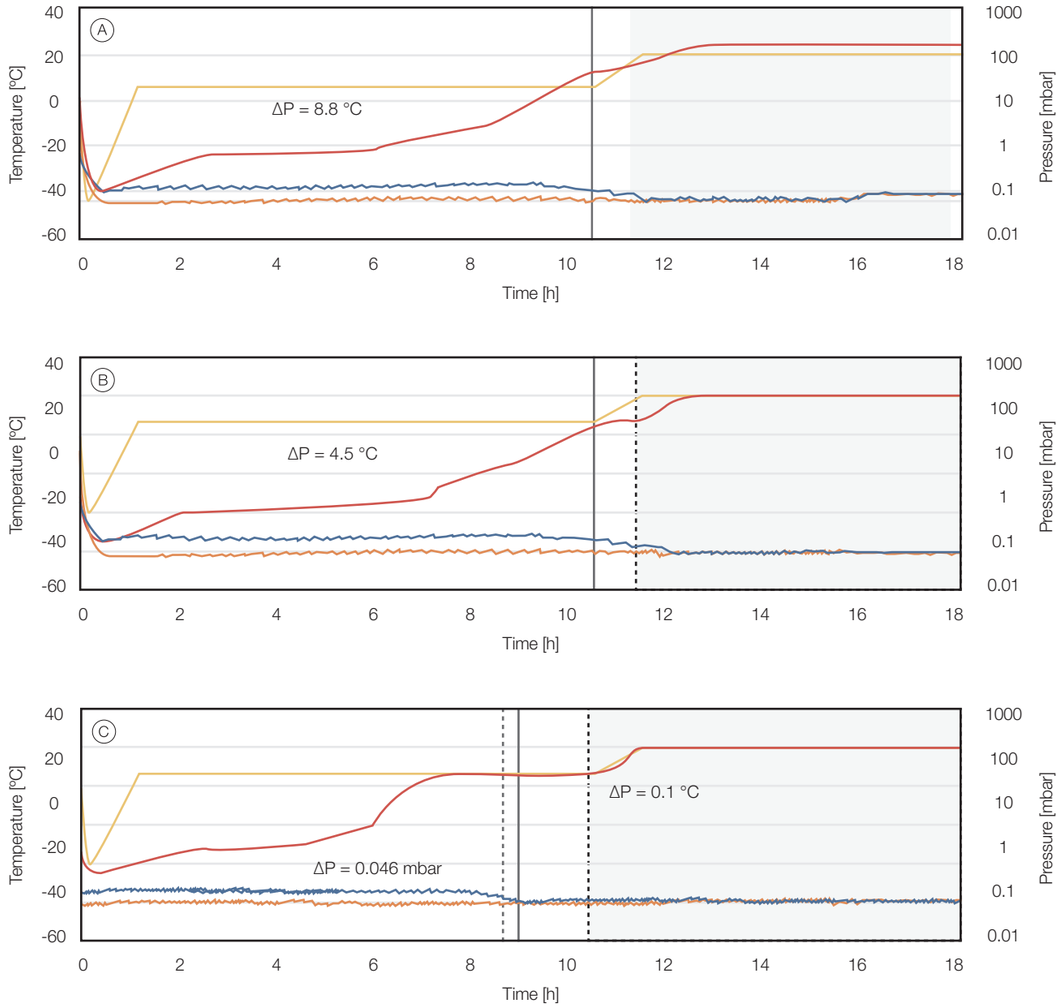


Figure 39: Freeze drying cycle of a glycine solution (50 mg/mL) with a pressure difference of 0.05 mbar (A), 0.03 mbar (B), and 0.025 mbar (C) as an endpoint criterion. For cycle C, a temperature difference of less than 0.5 °C was additionally used as an endpoint criterion. The shelf temperature (yellow), the sample temperature (red), the chamber pressure (green), and the Pirani gauge (blue) over primary and secondary drying are shown on the graph, together with the time at which the pressure (black) and the temperature endpoint criterion (black, dashed) are reached and the corresponding temperature and pressure difference at this point.

19.5 What about unconventional samples?

To verify this result, a freeze-drying run with strawberries was carried out. The strawberries were frozen for more than 24 hours at -40 °C and freeze-dried at a pressure of 0.300 mbar, a shelf temperature of 25 °C during primary drying, and 40 °C during secondary drying. The largest strawberry was also carrying a thermocouple so that the sample temperature could be compared to the shelf temperature.

Figure 40 A shows that the endpoint was reached after 37 hours and that the temperature and pressure curves merged simultaneously before the cycle changed to secondary drying. This demonstrates that the presented approach to evaluating an endpoint criterion is suitable for use with challenging samples such as strawberries.

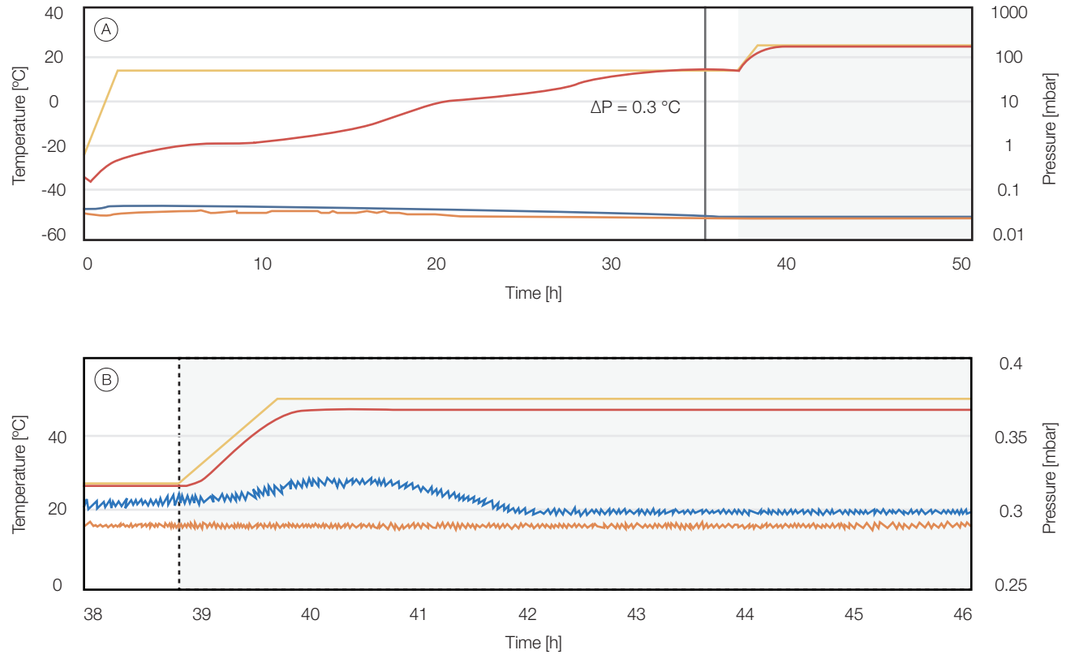


Figure 40: Freeze drying cycle of the strawberries, with a pressure difference of 0.025 mbar as an endpoint criterion. Figure (A) shows the entire freeze-drying cycle, whereas Figure (B) shows an extract of secondary drying. The shelf temperature (yellow), the sample temperature (red), the chamber pressure (green), and the Pirani gauge (blue) over primary (white shade) and secondary drying (grey shade) are shown in this graph together with the time at which the endpoint criterion (black) is reached and the corresponding temperature difference at this point.

19.6 Secondary drying

During the strawberries' secondary drying, the Pirani gauge showed a clear peak once the shelf temperature was increased (Figure 40 B) and evaporation began. This peak is usually missed when using temperature measurements for endpoint determination, which demonstrates another advantage of comparative pressure measurement.

19.7 Outlook and conclusion

It is important to point out that a suitable endpoint criterion is highly dependent on the chamber pressure during the freeze-drying cycle since the pressure difference during the drying phase is not absolute but will always be 60% of the chamber pressure. The process of finding a suitable endpoint criterion must be repeated if the chamber pressure of a freeze-drying method is changed. The endpoint criterion for the secondary drying stage should be adapted as well. Here, the maximum pressure difference usually does not reach 60 of the chamber pressure since only a small portion of water is left in the sample. Compared to primary drying, it might be beneficial to consider a smaller pressure difference, which needs to last for a longer period of time as an endpoint criterion. This approach, however, also needs to be evaluated with test runs.

20. The use of solvents in a laboratory freeze dryer

20.1 Overview

Freeze dryers were initially intended for use with water as a solvent. With new applications developing over the years, products that are not soluble in water are increasing in chemistry applications and freeze drying is being used more and more with solvents other than water. Organic-based solutions or inorganic acids and bases can commonly be used in research and development before lyophilization of the product. The behavior of these new solutions during freeze-drying needs to be considered prior to the process.

20.2 Hardware

Hardware Freeze dryers contain plastics (acrylic drying chamber), elastomer (seals and gaskets), and stainless steel (condenser), which can react adversely with inorganic and organic solvents. Before starting a cycle, it is important to check the system's state – seals, stainless steel, and acrylic chamber- and replace worn-out parts.

Most laboratory freeze dryers are designed with an acrylic drying chamber so that the product on the shelves is easy to observe. The presence of the organic solvent in the vapor stream can damage the acrylic chamber over time. It starts to look like some etched glass before the chamber fractures, leading to the inability to pull the vacuum. To minimize this problem and delay the need to replace parts, reducing contact between the organic solvent and the acrylic chamber is essential. A low-temperature condenser might prove to be helpful to collect the vapor. If the solvent's freezing temperature is above the condenser's temperature, most solvents are caught on the cold surface of the condenser. If the freezing point of the solvent is below the temperature of the condenser, the vapors either liquefy in the condenser or the stream rushes to the vacuum pump. In this case, the use of a dry pump is imperative. The collected solvents will be in contact seals in the drain valve during defrosting and cleaning. A regular replacement might be needed to avoid vacuum leakage. The material compatibility should be considered.

20.3 Process

Many organic solvents have a low freezing point, some of them well below that of the condenser surface temperature. To understand whether a solvent can be freeze-dried, four main aspects need to be verified using the vapor-pressure curve of the solvent (Table 6) (ice pressure curves for water are often not applicable):

- “How can the solvent be frozen?” – i.e., which method can be used to reach a temperature low enough so that the sample is fully frozen?
- “At which concentration can the solvent be frozen?” – i.e., does the solvent need to be diluted in order to be frozen?
- “Can the condenser collect the solvent?” – i.e., the temperature of the condenser is 15–20 °C lower than the solvent's freezing temperature.
- “Will the sample remain in a frozen phase during the process?” – i.e., can we maintain a low enough pressure so that the sample remains frozen?

Many aqueous samples can be handled easily during freeze-drying, and water will be collected entirely at the condenser during most experiments. The situation with organic solvents can, however, be quite different. Most of the time, organic solvents are tricky to freeze and quite often require dilution before freezing or the use of liquid nitrogen to reach a low enough temperature. Due to the very low vapor pressure of the solvents (low freezing points), temperatures reached in the condenser might not be low enough to trap the solvents completely. They will either liquefy in the condenser or leave the system through the pump as vapor. Choosing the right pump is crucial when solvent vapors are leaving the system, and a dry pump is recommended for all freeze-drying applications involving organic solvents. Even though the temperature of the condenser influences the amount and type of solvents being collected, many organic solvents have such a low freezing point that even a -105 °C condenser is unable to collect them. There is no major difference in capability between a -85 °C and a -105 °C condenser regarding vapor collection, as shown in Table 6. For solvents and acids with higher freezing temperatures, a -55 °C ice condenser might also be feasible, as long as the 15–20 °C difference can be maintained.

Due to the low freezing temperatures of the organic solvents, it might also be difficult to evacuate the system fast enough and to maintain a low enough pressure in the system to prevent the solvent from melting, even at ultimate vacuum. For diluted solutions, it is common to see solvents melting and evaporating while water remains frozen. The solvent vapors reach extremely large volumes in a very low-pressure environment. The limiting factor is the maximum volume that can be pumped out of the system. If this volume is too small, the pressure in the system will rise, which could lead to sample melting. In this case, organic solvents will evaporate from the sample first and will be removed at very high pressure; then, the water content will sublimate. Whether this is a problem depends on individual sample requirements.

It is also important to note that the concentration of the solvent has a considerable influence on whether a sample can be handled in a freeze dryer or not (see solvent list). Further dilution with water might help to dry the sample in a freeze dryer.

20.4 How to handle organic solvents in laboratory freeze dryers?

- When possible, eliminate as much of the solvent as possible before freeze-drying, using, for example, a rotary evaporator.
- Some small etching could create problems and require periodic replacement. Be prepared to replace components as needed.
- Good housekeeping is imperative; the freeze dryer must be carefully cleansed after each cycle.
- Do not allow condensate to sit in the condenser. Conduct the defrosting step of the instrument as soon as possible with the drain valve open. Wash out the condenser with water and ensure that the condenser is clean and dry.
- Use a dry pump when handling solvents other than water. Ensure that the pump's exhaust port sits in a fume hood to avoid any human exposure to the solvent.
- Use the defrost function to accelerate the melting of ice layers on the condenser. Choose an appropriate time setting to avoid vapor development in the lab environment.

Table 6: Triple points of commonly used solvents.

Solvent	T_{triple} [°C]	P_{triple} [mbar]
Water	0	6.1
Acetonitrile	-43.9	1.67
Acetone	-94.7	$2.33\ 10^{-2}$
Methanol	-97.7	$1.86\ 10^{-3}$
Ethanol	-123.15	$4.3\ 10^{-6}$

The following tables give a review of solvents frequently used in combination with water and show whether they can be removed by freeze-drying on the Lyovapor™ L-250 and Lyovapor™ L-300.

Due to the very low freezing temperature of the solvents listed in the tables below, very low pressure (lower than 0.05 mbar) is required to keep them in solid form. Since most of these pressures cannot be reached in the instruments, it is recommended to use an ultimate vacuum. The pressure can be set higher for DMSO since its freezing point is 18.5 °C.

Most solvents will not be trapped by the freeze dryer's condenser and will reach the vacuum pump. When solvents are involved, it is recommended to use a dry vacuum pump to avoid damage to the pump. BUCHI recommends Edwards scroll pumps for such use: Edwards nXds 6ic with the Lyovapor™ L-250 and Edwards nXds 10ic with the Lyovapor™ L-300.





	The sample can be freeze-dried properly. Sublimation occurs.
	The pressure in the drying chamber cannot be set to a low enough value to maintain the solvent in solid form. The solvent will melt while water will remain in ice form. The solvent will evaporate, and an increase in pressure can be observed until it is evaporated completely. The ice will then sublimate. Even though the solvent is not sublimating, evaporating it is good enough for many applications.
	NOT WORKING – NOT POSSIBLE.

Lyovapor L-250

Solvent	100%	60%	30%	10%	≤ 5%
Acetic acid					
Acetone					
Acetonitrile					
Dimethylsulfoxide					
Ethanol					
Isopropanol					
Methanol					
Trifluoro acetic acid					
Tert-Butanol					
Formic acid					
Dimethylformamide					
Dioxane					

Lyovapor L-300

Solvent	100%	50%	30%	10%	≤ 5%
Acetic acid					
Acetone					
Acetonitrile					
Dimethylsulfoxide					
Ethanol					
Isopropanol					
Methanol					
Tert-Butanol					
Trifluoro aceticacid					
Dioxane					

21. Conclusion – Lyophilization is a complex, intense process

Freeze-drying is not a quick process; cycles can take a few days and up to a week to complete.

Depending on the sample and the process, freezing can take a considerable amount of time (see page 19).

Primary drying is performed at very low pressure to maintain the sample's frozen structure, thereby inhibiting the drying rate (see page 31). Moreover, while drying, a layer of dry material builds up from top to bottom of the product and inhibits the flow of vapor, causing the drying process to slow down (see page 23).

During secondary drying, water is removed by desorption, not sublimation. At this point, the structure is relatively stable and unlikely to collapse, so higher temperatures can be involved. However, the process is still slow and may take hours to days to remove only 1–2% more moisture (see page 29).

Process duration is affected by the different characteristics and requirements of the samples.

Robust products such as fruits, vegetables, or ingredients can be freeze-dried quite aggressively without detriment. They have a good structure, and collapse is not an issue in foods since reconstitution is not a concern when being eaten. A freeze-drying process in these conditions will typically take 24–48 hours.

Pharmaceutical and biotechnology products will usually take more time to freeze-dry. Their critical temperature is quite low and requires the use of low pressures, reducing the speed of the process (see page 21). Reformulating can create a more suitable drying profile and enable a faster freeze-drying process (see page 27). Reevaluating the target dryness and assessing whether an increased final moisture can be tolerated could reduce secondary drying dramatically (see page 29). Importantly, the impact of any changes on the formulation, stability, and activity must be evaluated carefully.

During primary drying, the product temperature should not rise above the critical temperature of the sample. Otherwise, the frozen structure will start to fail, and defects, collectively termed collapse, will occur (see page 37). Freeze-drying should create a final product with an open, porous structure, allowing vapor to escape easily without affecting the remaining components. If the sample collapses, this structure is eliminated, and the vapor will not escape as easily. The drying will be less efficient, and a range of defects can be caused. The frozen structure is important for the freeze-drying process, and without it, it is not freeze-drying anymore but rather just drying.

Freeze-drying is not a straightforward process, requiring a good balance of several factors. It can be a difficult procedure to manage. The concepts explained throughout this booklet are the basis required for performing freeze-drying, and understanding them is crucial to achieving a successful process.

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