Considerations When Freeze Drying Tissue

By: T.N. Thompson
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Although freeze drying was originally developed for biological products, as described in publications as early as 1935, there has been very little written on the challenges of freeze-drying tissues.

Freeze-drying equipment design and methodologies have been primarily based on processing liquid formulations in containers such as vials or trays. The concept of freezing the liquid material on the shelf and then using sublimation and desorption to reduce the moisture level to a low enough level to maintain long term stability is well understood.

Tissue freeze-drying presents unique challenges due to the broad variation in the content and form of material and the containers used to handle and store the product. To develop a consistent and optimized freeze-drying process for tissues, the relationship between the application, the equipment, and 3 steps of freeze drying needs to be fully considered.

Freezing

Freezing is the foundation of the freeze-drying process. A robust method of freezing is required to produce an efficient and effective freeze-drying process.

Tissues may be placed in bags, trays, petri dishes, tubes, or other containers for handling during freeze-drying. Containers that can be placed on the shelf of the freeze-dryer can be frozen in a controlled manner by reducing the shelf temperature at a controlled rate. Some products are frozen by immersion in LN2, which leads to small crystal structures, so it is recommended that an annealing step is considered after the materials are placed in the freeze-dryer.

In some cases, tissues may be frozen by placing the material in a -80C freezer and stored for periods of time until ready for processing. When freezing in a low temperature freezer, care should be taken to ensure that the tissues are consistently placed in the freezer in a manner to provide consistent freezing across the batch. Therefore, stacking materials on top of one another should be avoided and a space should be left between the samples to allow convective air flow. The temperature of the freezer at the time of placing the product inside should always be the same and a rack to prevent the bags or trays from touching each other will provide consistent conditions, resulting in the same freezing dynamics for all the material.

The final temperature of freezing needs to be below -40C and should be -60C to -80C due to the glass transition temperature of tissues. Borgognoni (2) suggests that the glass transition temperature of Bovine Pericardium is below -80C and the critical temperature is -11.6C, therefore annealing should be performed around -20C for 1 to 3 hours.

Important: If the tissue is not fully frozen prior to reducing the chamber pressure the unfrozen solvent in the product may bubble and disrupt the structure.

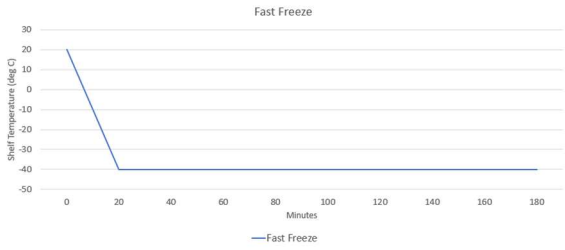
The method and rate of freezing produces very different ice crystal structures. For example, fast freezing (>10 C/min) results in a small ice crystal structure that can impede sublimation during the primary drying phase, while slow freezing (1 C/min or slower) results in a large ice crystal structure that is sublimation friendly. For some products, the initial rate of freezing needs to be rapid to maintain pH, eliminate product precipitation, or to prevent cellular damage from slow ice crystal growth. In these cases, an annealing step is added to enable the crystal size to increase which allows the sublimating vapor to escape the frozen structure more easily. As described by Borgognoni (2), slow freezing or slow freezing with annealing results in the lowest residual moisture content post-freeze-drying.

Studies on the effect of freezing rate and methods on tissues will result in a better understanding of how to further improve the freezing methodology.

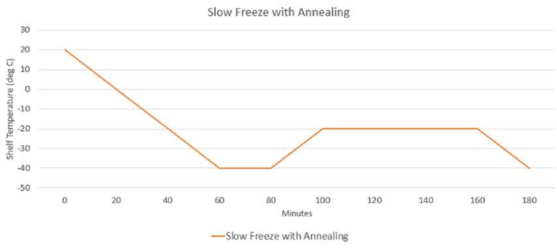
Summary:

- Freezing is the foundation of freeze-drying
- Freezing should be consistent and repeatable
    - The product should all be placed in the same manner in the freezer to ensure consistent freezing.
- Process control and validation is the paramount to a successful and consistent freeze-drying process
- Fast freezing leads to small ice crystals, which results in slower primary drying.
    - However, fast freezing may be needed for tissue to eliminate cellular damage.
- Freeze below the product glass transition temperature
- Add annealing to improve crystal structure and consistency

Freezing: Fast without Annealing



Freezing: Slow with Annealing



Primary Drying - Sublimation

Sublimation is the process of solid water changing to vapor, without forming a liquid, under low pressures. The process is endothermic, which means that a sublimating product absorbs heat from its surroundings, so heat must be added to drive the sublimation process. In freeze-drying, sublimation is used to remove the water from a frozen material. Heat is added to the product to change the frozen water into vapor and the vapor is collected on a cold condensing surface to enable continuous flow of water out of the product.

During primary drying the pressure in the freeze-dryer product chamber is reduced to well below 4600 mT, the triple point of water, to enable sublimation. For most freeze-drying applications, the chamber vacuum levels used are between 50 mT and 400 mT. Selecting the chamber pressure is a balance between efficient heat transfer and maintaining a low product temperature. In general, a lower chamber pressure will result in a slower sublimation rate due to the reduced heat transfer but will also maintain the product at a lower temperature. An optimum chamber pressure needs to be determined to enable good heat transfer to the product while still maintaining a product temperature below its critical temperature, which is the temperature at which the efficacy of the material is compromised.

Sublimation starts at the surface of the frozen structure and progresses inward as ice is removed and a dried layer remains. During the initial portion of the primary drying step there is no dry product and the surface of the material has no resistance to sublimation, so the process can be driven aggressively. However, as the dry layer thickness increases, the sublimating vapor has difficulty finding an exit path from the product, causing an increased pressure at the sublimation interface and a higher product temperature. It is important to keep the shelf temperature and pressure at levels that protect the product.

In vial freeze-drying there is good contact between the container and the temperature-controlled shelf. The temperature of the shelf is raised to add energy to drive the sublimation rate. The vial to shelf contact enables good process control.

The containers used for processing tissues vary widely. Many containers may have poor contact or no contact with the shelf. Trays and dishes may be placed on the shelf and will have good contact for process control. Tubes and bags however may not have contact with the shelf and the primary mode of heat transfer changes from conduction to convection and radiation. This can lead to product consistency issues if not fully understood.

When processing tissue in bags, it is not possible to have all the bags/material contact the shelf. For consistency, a method of racking the bags needs to be used. The rack method should keep the bags separated and out of contact with the shelf, since they can't all contact the shelf. A rack or other method of keeping the bags from contacting each other, while providing an orientation that enables the tissue to contact the bag for best heat transfer is recommended. By providing a uniform organization method the product consistency will be maximized.

Determining the proper shelf temperature and chamber pressure is critical to an optimized process. To do so, the tissues critical temperature must be known. The process should maintain the product temperature as close as possible to a safe temperature that is a few degrees below the critical temperature for the duration of primary drying for an efficient and robust cycle.

A common misunderstanding of the shelf temperature profile is that a low shelf temperature is used at the start of the cycle and then the shelf temperature is increased in a step fashion during the cycle. The step-up profile is very inefficient and potentially harmful to the product. Since the product sublimation rate is highest at the beginning of the primary drying cycle the shelf temperature can be increased to its highest temperature at the beginning of the cycle. A shelf temperature that maintains the product temperature below is critical point at the end of the cycle can be used for the entire cycle. At the end of the primary drying cycle, when the sublimation process has ended, the temperature of the product will be at its highest. It is recommended that a single shelf temperature be selected that can be used for the entire cycle for simplicity. Borgognoni (2) describes a simple primary drying cycle of a shelf temperature at -5C and a chamber pressure of 160 mT.

Primary drying ends when sublimation has completed, which can be determined by differential pressure measurement between a capacitance manometer and a Pirani vacuum sensor. The Pirani measurement is affected by the presence of water vapor. At the end of sublimation, the water vapor present in the product chamber has reached its lowest level and therefore the Pirani reading will be very close to the reading of the capacitance manometer, with the same differential that is present in a dry and empty chamber. At the end of primary drying the moisture content of the material is between 3% and 5%. To reach lower moisture levels a secondary drying step can be added.

- Primary drying notes
    - Sublimation is an endothermic process, the product temperature reduces during sublimation
    - Sublimation rate is highest at the beginning of primary drying since there is no resistance to the vapor leaving the product.
    - Increasing the shelf temperature in steps during primary drying is not a proper cycle, see above. Therefore, the shelf temperature can be raised to its optimum level at the beginning of the cycle.

Secondary Drying

Secondary drying for pharmaceuticals and other liquid-based products removes the bound water in the material which further reduces the moisture content to as low as 0.5%. This process uses higher shelf temperatures to help drive the bound water into the vapor state via desorption.

The target residual moisture for tissues is 5% or lower, which may be achieved in primary drying. When the application cannot reach these levels there may be a need for secondary drying step. The literature describes a secondary drying step at a 25C shelf temperature and at the same chamber pressure (IE: 160mT). The duration of secondary drying is typically about 30% to 50% of the length of primary drying.

The proper time can be determined by testing the residual moisture content at different times during the secondary drying phase.

Other notable information

Post-Processing – Cross Linking

When processing Collagen-based materials cross-linking may be used to improve the mechanical stability of the product. Cross-linking can be performed using a high shelf temperature, such as 105C.

Bulk material

When processing slurries in a tray there will be a much higher vapor load generated during primary drying due to the larger surface are of product and better contact with the shelf. For these applications it is critical to ensure that the freeze-drying equipment can handle the vapor load during the cycle. Verify that the condenser temperature and product chamber pressure level can be maintained for the entire cycle.

The thickness of the slurry in the tray can have a major impact on the primary drying cycle time. The thicker the material the longer the primary drying cycle. This is geometric in nature. For example, 1” of material thickness vs 0.5” of material thickness may take 4X as long to dry.

Bags

The vapor pressure inside the bag will be higher than the chamber pressure. The higher the vapor pressure the higher the product temperature.

You need to determine and control the product temperature during primary drying.

Equipment

Freeze-dryer Condenser design

Equipment designed with a ‘cold wall’ style condenser, one without exposed coils, measures the condenser temperature at the condenser inlet and not at the condensing surface. Therefore, the condenser wall could increase above -40C without any indication to the operator. Condenser temperatures above -40C will have a negative impact on the freeze-drying process.

Vacuum Control

Older freeze-dryers used Pirani vacuum gauges for process control. We have learned that controlling the vacuum level using a Pirani leads to variation in the cycle. As the water vapor present in the freeze dryer reduces, the Pirani reads a lower pressure and the result is an increased pressure in the chamber. The absolute vacuum level can rise as much as 100 mT.

It is suggested that a capacitance manometer be used for process control and the Pirani be used as a reference for end of primary drying determination.

Note, changing a cycle from a Pirani to a capacitance manometer (CM) takes some thought. The absolute pressure needs to be understood. Suggest running each cycle with a CM to watch the absolute chamber pressure before transferring the cycle.

21CFR Part 11 compliance

The industry is ever evolving and the need to meet FDA guidelines may be needed. In the pharmaceutical processing market the FDA requires the control system to be able to comply with 21CFR Part 11. This means a secure database, electronic signatures, change logs, and user level control. There for a PC/PLC control system is recommended.

References

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3- Lyophilization and homogenization of biological samples improves reproducibility and reduces standard deviation in molecular biology techniques, Molnar, 2021, Springer Nature