The Freeze Drying Theory and Process Things to Consider

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Freeze Drying, or Lyophilization, has become an accepted method of processing sensitive products that require long term storage at temperatures above freezing.

In this white paper we describe the typical types of equipment, the basic freeze drying theory and process steps – from pretreatment, freezing, primary drying to secondary drying. We also go into depth with some of the parameters that requires attention or understanding. Process monitoring and validation is also described as well as suggestions and recommendations for your choice of equipment.

Finally, we have listed several things to consider e.g. Sensor Designs, Live Data Option, Calibration, Data Analysis, FDA Compliance etc.

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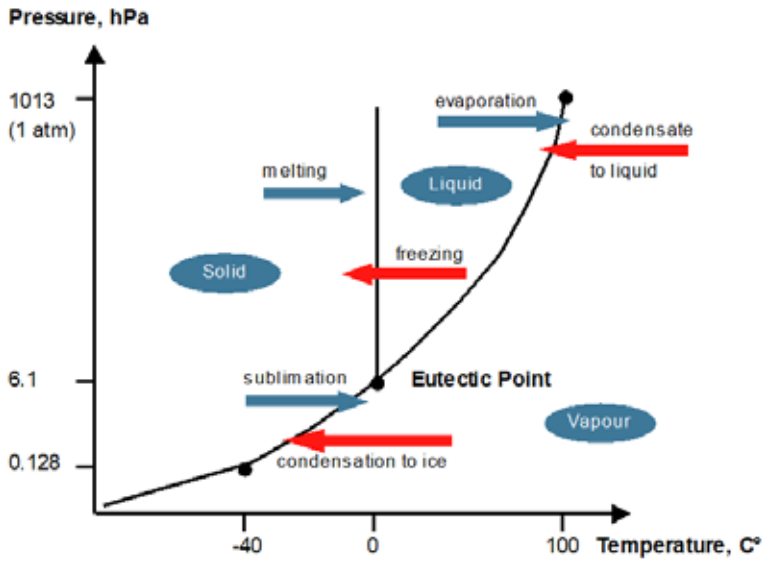
The Freeze Drying Process

Freeze Drying (Lyophilization) is a process whereby a product is dried under low temperature and vacuum. The water in the sample is first frozen to a solid and then removed directly by turning the ice into vapor. This is done under vacuum and without having to pass through the liquid phase. The unique advantage of Freeze Drying is that the samples are kept at low temperatures and remain frozen during the entire drying process, thereby preserving thermo labile components (proteins, flavors, colors), all while maintaining the original shape and size. The dried product can then be stored for long periods without the risk of changing composition (i.e. enzymatic, genetic) or being infected by microorganisms, which is all made possible due to the lack of water.

In other words, Freeze Drying is a dehydration technique. The aspect of the Freeze Drying process that makes it different from other dehydration techniques, is that dehydration takes place while the product is in a frozen state and under a vacuum. These conditions stabilize the product, minimizing the effects of oxidation and other degradation processes. Freeze Drying has become an accepted method of processing heat sensitive products that require long term storage at temperatures above freezing.

Freeze Drying is widely used in the pharmaceutical- as well as other industries and is one of the most expensive unit operations due to the high energy consumption. Conservative Freeze Drying cycles result in long processing times, which will increase the cost of production. Longer lyophilization cycles are not considered optimal, as they often turn out less robust and have an increased potential for equipment failure. Freeze Drying cycles should be optimized to minimize drying time without adversely affecting product quality.

The Freeze Drying process was developed as a commercial technique that enabled serum to be rendered chemically stable and viable without having to be refrigerated. The process was applied to penicillin, and became recognized as an important scientific technique for the preservation of biologicals. Freeze Drying is also used as a preservation or processing technique for a wide variety of products such as pharmaceuticals, diagnostic kits, restoration of water damaged documents, sludge from rivers prepared for hydrocarbon analysis, ceramics, viral or bacterial cultures, tissues prepared for analysis, production of synthetic skins and the restoration of historic/ reclaimed artifacts.



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Equipment Design

There are three basic components of any freeze dryer. These components are the product chamber, the condenser and the vacuum pump. Each component is vital for the functionality of the freeze dryer.

There are two basic types of product chambers, one for vials and one for trays (bulk). If a product is relatively sophisticated, chemically complicated, subject to resale or aseptic processing then a vial system would be favorable. This method allows operators to have ultimate control over the parameters that drive the Freeze Drying process.

When a product is processed in a vial dryer, the liquidized product is filled into vials and loaded onto the shelf trays of the freeze dryer. There, the product is pre-frozen to a temperature slightly below the freezing point of the product, also called the eutectic point.

During Primary Drying, the vacuum pump of a freeze dryer removes the non-condensable vapors. These vapors are created by leaks in the equipment and the constant release of non-condensable molecules from the product as the Freeze Drying progresses. The use of the vacuum pump establishes a free vapor path for migrating condensable molecules by removing the air from the chamber.

The Freeze Drying Process Steps

Regardless of the reason for utilizing the Freeze Drying process, there are 4 basic steps that require attention and/or understanding.

- Pretreatment
- Freezing
- Primary Drying (ice sublimation)
- Secondary Drying (moisture desorption)

Pretreatment

Pretreatment covers any method of “improving” the product prior to freezing. This may include concentrating the product, diluting the product, formulation revision such as the addition of components to increase stability and/or improve processing, decreasing a high vapor pressure solvent or increasing the surface area. In many instances, the decision to pretreat a product is based on the theoretical knowledge of Freeze Drying and its requirements, determined by the cycle time or product quality considerations.

Freezing

Freezing, also called Pre-freezing, is when the sample is frozen to a temperature below its “eutectic point” or safe freezing point. This is typically in the range of -40 to -60°C, whereas certain applications can go as low as -60 to -80°C. During pre-freezing, the freeze dryer works as a freezer in that no vacuum is applied. Pre-freezing could also be done separately from the dryer.

The freezing step is of paramount importance, as it determines the ice morphology and pore size distribution, which is essential for success later in the process. This seems rather elementary, but it is often the least understood and investigated step of the process.

The freezing of the product may result in either a sudden solidification of the liquid at a specific temperature (eutectic former) or a liquid which does not solidify, but rather just becomes more and more viscous (glass formers). The eutectic formers freezing temperature equates to the triple point of the product on the phase diagram. In this instance, the product is frozen in the classic sense and the temperature must be maintained below this level during the entire Primary

Drying step. To freeze a product properly, thermal analysis can be used in order to help better understand its properties.

Thermal analysis to detect the eutectic point can be done in several ways, but none of them are 100% effective.

- Time Versus Temperature Curve
- Differential Scanning Calorimetry
- Cryo Microscopy

Materials that have poor structural stability generally end up shrunken, puffed or may be glassy-looking and sticky. Such samples are said to have collapsed during Freeze Drying. Poor structural stability combined with longer drying times will also result in poor product quality.

Below are some collapse temperatures of typically freeze-dried products and solutions:

- Apple juice (-42°C)
- Citrate buffer (-40°C)
- Coffee extract (-20°C)
- Dextran (-9°C)
- Fructose (-48°C)
- Gelatin (-8°C)
- Glucose (-40°C)
- Inisitol (-27°C)
- Lactose (-32°C)
- Maltose (-32°C)
- Phosphate buffer (-80°C)
- Sorbitol (-45°C)

Once the freezing point (eutectic point) of the product is determined, the optimal rate of freeze must also be determined. The rate of freeze determines the crystalline size. It is important to remember that as the frozen liquid will eventually be subliming out of the product, the larger crystalline structure coming from a slow freeze rate, will produce a more porous and quickly dried product. Typically, this is advantageous for the optimization of Freeze Drying cycles but may not result in the best product in terms of rehydration (reconstitution).

On the other hand, a fast rate of freeze will result in a product that turns inactive at a faster rate and has a smaller crystalline structure, which in turn results in it being more granulated and therefore easier to reconstitute, even if it takes longer to freeze dry.

A rule of thumb for freezing products in vials, is that the product container should never be filled to more than half of its total volume.

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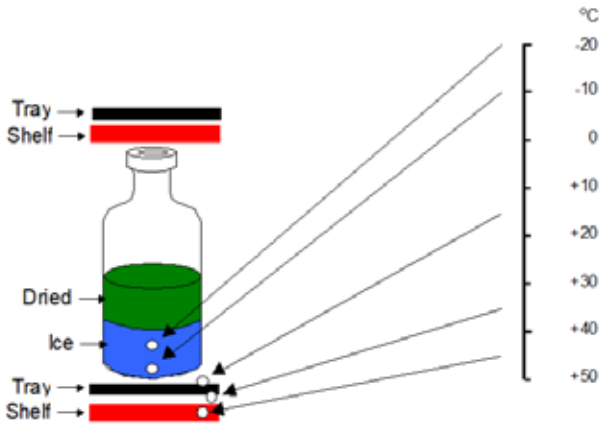
Indications that the cycle has completed the Primary Drying phase:

1. The product temperature is equal or very similar to the shelf temperature. This indicates that no heat transfer is occurring between these locations and that few vapor molecules (and their associated energy) are leaving the product.
2. The condenser temperature has returned to its original low temperature. This indicates that the condenser is no longer trapping high loads of vapor (and its associated energy) to result in a temperature rise.
3. The pressure in the system has returned to its original low value. Once again, this indicates that the movement of vapor molecules has decreased substantially.

Primary Drying

Primary Drying phase is where the ice sublimates (turns directly into vapor) under ultra-low pressure, typically down to 0.01 hPa (mBar) or lower, depending on the pre-freezing temperature of the sample. The driving force of sublimation is the pressure difference related to the corresponding temperature difference between the product ice surface and the condenser ice surface. Larger temperature differences mean larger pressure differences, which allow for a faster process. The vacuum speeds up the process by removing air molecules to allow sample vapor molecules to move easier from the sample, through the chamber and into the condenser. Typically, shelf temperatures during Primary Drying are ramped from –40 to +20°C during the process time, which can vary from a few hours to several days. The shelf temperatures indirectly influence the ice temperature of the sample by conducting heat (contact to the shelf) as well as the radiating of heat from the shelf above. Due to the low level of air molecules present in the chamber, highly limited amounts of heating stems from convection. Sample temperature(s) are monitored by tiny sensors inserted into the vials, which then follows the change of shelf temperature accordingly.



Schematic showing typical temperature relations between shelves and product

In order for a freeze dryer to be effective, the temperature of the condenser must be lower than the temperature of the product. This difference in temperature creates a pressure differential and the net migration of water vapor towards the condenser.

During the Primary Drying phase, it is essential to heat the product as much as possible (without passing the eutectic point) in order to increase the pressure differential between the product and the condenser. This also increases the temperature differential between the freeze-dried ice interface (condenser) and the product ice barrier. However, it is important to remember that the heat input constraints are often caused by the product's own thermal characteristics. If a product has a eutectic temperature of -10°C, then the product may be taken to a temperature of approximately -15°C. If the condenser is -50°C this will result in a much larger pressure differential (speed of process) than if the product temperature was left at -30°C or even -40°C. Since pressure differentials at very low temperatures are minimal, lowering the condenser temperature will have limited effect on the speed of process.

When using a vial chamber system, the operator can control the energy input to the product via temperature and pressure control. These controls allow the operator to optimize the Freeze Drying cycle. Typically, in Freeze Drying cycles, the product temperature will follow behind the shelf temperature, thus increasing the cycle duration. To overcome this issue, the pressure can be increased to raise the number of molecules available for heat transfer from the heat source (the shelves) to the product. Additionally, using vials with flat bottoms offer the optimal contact, which decreases the amount of heat transfer barriers between the shelves and the product.

Regardless of the Freeze Drying method employed, it is essential to remember that Primary Drying is a delicate balance between the energy input to the product and the pressure differential created between the product and the condenser by the temperature differential.

Also, Primary Drying is typically the part of the process that takes the longest and is therefore subject to optimization. This is typically done by adjusting the temperature and pressure in order to bring the product as close as possible to its collapse conditions, but without crossing the line.

Secondary Drying

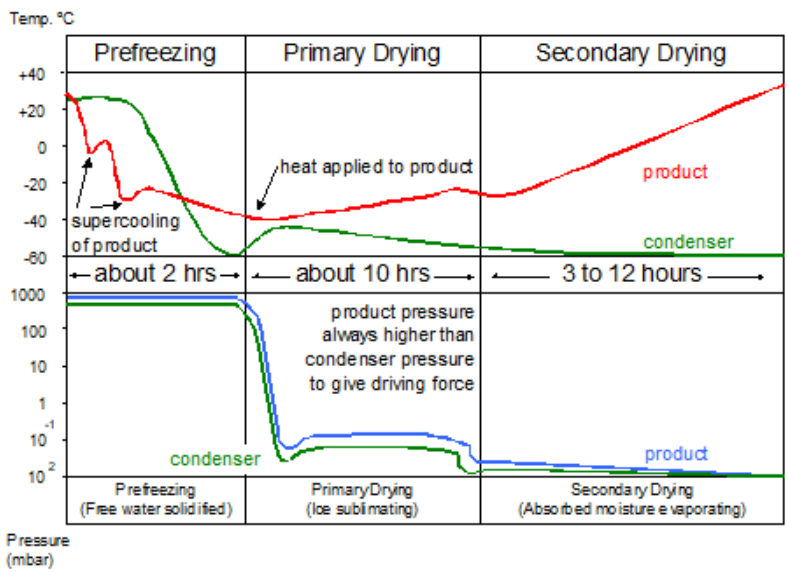
When the product reaches a temperature above its eutectic point, the Secondary Drying Process will typically have begun. During this step, the vacuum pump creates the low-pressure condition necessary for the removal of solvents, which often results in a product appearing dry. The solvent being removed during this desorption step is referred to as ”bound”. The amount of bound, or residual water, in the product is dependent on the amount of time the product remains in the Secondary Drying phase.

The removal is controlled and optimized by increasing the shelf temperature to its allowed maximum. This is, however, usually never raised above +42°C, as biological samples contain proteins that would denaturize as a result of this. Vacuum is at this point very high (low pressure) as no- or very few vapor molecules are present. This part of the Freeze Drying cycle typically represents less than half of the total cycle but is highly essential for the final moisture content of the sample. For pharmaceutical samples in vials, the required moisture level is often close to- or below 1-3% in order to secure the maximum shelf life. This can only be obtained through the use of Freeze Drying chambers equipped with stoppering arrangements for sealing the vials with e.g. rubber stoppers that close under vacuum, or by equalizing the chamber vacuum with an inert gas, such as nitrogen.

Typically, cell cultures, pharmaceuticals and diagnostic kits are subject to this low level of residual water content. Methods such as the Karl Fisher Titration Test or weighing the sample pre and post process may be used as a check of the residual moisture content. From a reconstitution point of view, it is not advantageous to aim for a lower residual moisture level than required, as it will get increasingly longer and more difficult.

Once the product is determined to be at the end of its cycle it must be removed from the freeze dryer. If bulk chambers are used, the system is brought to atmospheric conditions by “bleeding” air or nitrogen into the chamber before unloading the trays. Product processed in this manner will absorb the water vapor that it comes in contact with. Consequently, this product should be processed or stored as quickly as possible.

When drying the vials, they are usually closed off under vacuum (or back pressurized with N2 to atmospheric conditions), meaning that they do not require as quick treatment and only need appropriate storage.



Example of a typical Freeze Drying profile

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Scaling Up

The Freeze Drying process poses challenges in the scale-up from laboratory (Pilot Plan) to manufacturing. Full scalability between the Pilot Plan unit and the production lyophilization unit allows for the development or optimization of Freeze Drying cycle parameters, which can result in significant cost savings.

The Pilot Plan and production units' performances must be very similar in terms of the minimum temperature for the shelf and condenser, shelf and condenser cooling time, ultimate vacuum, vacuum pump down capacity and shelf temperature uniformity. It is important to determine the ratio of condenser surface area to shelf surface area, as well as the ratio of shelf surface area to the size of the gate opening between the chamber and condenser. These should be very similar for both types and will secure that the gate-valve can handle the ice vapor during Primary Drying, despite the different drying capacities.

Process Monitoring & Validation

To ensure a high quality and optimal production costs, the process is closely monitored using sample sensors. During each run, samples on each shelf are equipped with temperature sensors that log the data. The interesting spots are the ones where hot conditions can result in samples melting. At least one sample per shelf is usually equipped with a temperature sensor, but there could be as many as 5 per shelf, placed in each corner and center. Live data is typically only required during process optimization, whereas batch documentation and validations do not require live data. Online data can be obtained by using wired thermocouple systems or wireless data loggers, which have the advantage of easy positioning and no change of chamber integrity (no leaks). It is important to mention that freeze dryer loads often represent an extremely high value, which is why a vacuum leak, or any other failure, is intolerable.

The recommended best practice for measuring the product temperature within vials is based on the type, design and size of the sensor. First off, any sensor placed into a sample will introduce a bias in the monitored vials when compared to the unmonitored vials, as a sensor will/can act as a nucleation site for ice. Some studies have found that for specific products, this difference could be as high as 5°C higher in monitored vials. As freezing is slower, it will create larger ice crystals that potentially mean faster sublimation, which will not reflect the exact condition of unmonitored vials.

To reduce this difference, the sensor should be as thin as possible, as this will facilitate as little an impact on the sample as possible as well as help with the correct positioning of the sensor inside the vial. This position is located in the geometric center, almost touching the bottom of the vial. During the Primary Drying, this location will represent the last ice to disappear and therefore the potentially warmest part of the non-dried product. If the sensor is placed all the way at the bottom of the vial, the measured temperature will probably be influenced by the glass and therefore indirectly represent the shelf temperature instead. If the sensor is placed too high within the vial, it will represent more of the dry section of the sample. As the comparison between product temperature and shelf temperature is often used to determine the progress of Primary Drying, (when the product temperature equals the shelf temperature it signals the end of Primary Drying) it is highly important to measure.

People often use the term “thermocouple” to describe any temperature measuring device, but in reality, there are two distinct types available. Thermocouples operate by measuring voltage and RTD’s operate by measuring resistance. In either case, these measurements are converted to temperature. Both types have their pros and cons, but here are the most important aspects:

- Thermocouples measure temperature at a single point (welding spot) and can be delivered in any length, whereas RTD’s measure across the Pt element and have limitations in regard to cable length.
- Thermocouples have faster response times when operating with naked tips compared to RTD’s, which require additional protection around the Pt elements.
- Thermocouples require a feed through system, which is hard to keep absolutely vacuum sealed - wireless loggers are placed directly within the chamber(s).
- RTD’s are often used with wireless systems (data loggers) and have better accuracy, precision and stability (low drift over time).

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On a regular basis, once a year or when major components are serviced/exchanged, freeze dryers are re-validated or qualified to ensure proper functionality according to ISO 13408-3 by the following tests:

- Mapping of the shelf temperature without load is performed to verify the uniformity of temperature across the shelves (machine performance).
- Mapping of the shelf temperature with load to determine the impact on samples. The standard procedure typically involves a star pattern model with temperature probes that are inserted in the copper plate at all four corners as well as in the middle of each shelf.

Further tests that are involved in freeze dryer validations:

- SIP (Steam-In-Place) temperature mapping that ensures proper on-site sterilization of the unit. This works as if the freeze dryer was a sterilizer and the norms are identical (EN 285 & EN17665) for proving sterility.
- A vacuum pump test whereby the vacuum system is tested for ultimate vacuum – and pull-down time.

Choice of Equipment for Temperature Test

As the information implies, the ideal solution would be a wireless data logger system with a naked tip thermocouple gauge, size 30 (very thin) for optimal product monitoring.

This system would combine the best of both worlds – thermocouples with fast response times and small dimensions in conjunction with wireless data loggers – this combination would eliminate almost all challenges associated with product temperature measurements during Freeze Drying.

By using data loggers instead of cable systems, potential leak issues and time-consuming cable positioning are avoided. As Freeze Drying is a relatively long process, using several hours or days with temperatures down to -80°C, it is essential that the hard- and software being used is reliable and able to secure the integrity of data over long periods of time.

Choice of Equipment for Vacuum Test

Using a wireless data logger that can operate at extremely low temperatures and be equipped with a highly accurate vacuum sensor is required. Look for a vacuum sensor that is accurate enough to provide data for flux measurements throughout the freeze dryer – or simply determine the end of the primary and secondary cycles through pressure rise testing (PRT) in order to optimize production.

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Things to Consider...

Temperature Sensor Designs

It is immensely important to measure the core temperature of the product in its actual container (vial, etc.) with as little interference as possible. Besides using a wireless data logger with a thermocouple sensor, using special flexible PTFE sensors with fittings and fixtures to measure the temperature accurately is also viable. The PTFE cables should be thin (max. 1.8 mm in diameter) and offer the use of special vial stoppers designed for Lyophilizers - Lyo Stoppers – which helps to simulate the actual processing conditions. At the same time, the design ensures precise mounting of sensors at the hot spots inside the vials, which is typically found 1 to 2 mm from the bottom of the vial. Only flexible PTFE or thermocouple sensors offer an accurate simulation of the actual process. Inserting stainless-steel sensors into the small vials, does not provide accurate results due to the high amount of heat conduction.

Long Process Duration

The battery life of data loggers should be required to last long at low temperatures. Each battery should offer up to 2-3,000 working hours when equipped with a single senor and slightly less with double sensors. The battery should be designed to function properly at extremely low temperature conditions and be easy to replace in the field.

Logger memory is also important. Look for a memory capacity with a minimum of 60-120.000 data points and a non-volatile memory when used with a single sensor. This means that:

- 16-32 hours operation with 1 sec. sample rate.
- 80-160 hours with 5 sec. sample rate.
- 400-800 hours with 25 sec. sample rate.

This guarantees a trouble-free validation for the entire cycle.

Live Data Option

Freeze Dryer validations usually require 5 measuring points per shelf – all four corners as well as the center - while batch control could require up to 1 measuring point per shelf for the product temperature batch control.

When operating with a real-time data option, the RF transmitter (SKY module) is mounted on a logger. It is all handled simultaneously by the same receiver (Access Point) and data is “streamed” to the software using a proprietary transmission protocol. The data loggers keep a “safe copy” of the data until it has been sent to the software.

Having a transmission range of up to 100 meters in open space is optimal, provided that it can guarantee a transmission range of at least 15 meters within enclosed chambers, such as a freeze dryer. This means that the receiver can be placed 15 meters away from the data loggers. If the Lyophilizer is very large and exceeds the 15 meters length, more Access Points can be connected.

Data Analysis, Data Integrity and FDA Compliance

The operating software should preferably be fully FDA 21 CFR, Part 11 compliant. For optimal use, data should be collected and saved in the data logger memory and then, if run online, be transferred to the software. The software should preferably have the options of being installed locally on a PC (client) or on a server for mutual use.

The software package should include all the required documentation, which includes IQ/OQ, Audit Trails and Electronic Signatures.

Sensor Contact to Shelves

One of the main challenges during lyophilization validation, is limiting contamination of the shelves when placing sensors for shelf mapping while also maintaining a proper shelf contact. Typical methods would involve using thermal paste when adhering sensors to the shelves or using special tapes that can withstand ultra-low temperatures.

To avoid these practices, which can be difficult to perform deep within a large chamber, special contact pucks are available for use, made of special grade stainless steel. This system secures good shelf contact as the tip of the sensor can be inserted into the puck and be placed onto the shelve.

A special contact puck mounted within a Lyoshuttle with integrated vials would be the ideal and complete tool for this application.

Sensor Interchangeability

Validating freeze dryers require several measuring points, so in order to avoid allocating all data loggers to the same application, acquiring a data logger system that can be applied to a wide range of applications could prove beneficial. A system that allows complete interchangeability between temperature sensors, as well as sensors that cover other measuring parameters like vacuum, humidity, CO_2, conductivity or pressure, could allow for a more cost-efficient solution.

Calibration

The possibility to perform manual, semi-automatic and fully automatic calibrations with the same software, as well as being able to use any available bath/dry block, would be highly beneficial. A temperature standard/reference instrument should also have the ability to communicate with the software. The sensors used in conjunction with data loggers, usually consist of Pt1000 RTD elements, which are highly stable over longer periods of time. Due to this stability, pre and post calibrations are not always required.

Regular Monitoring of the Process Online

The wireless data logging system could also be used to regularly monitor the hot spot temperature of the vials during production runs in order to guarantee that the process is kept safe. The software should, therefore, allow for predefined alarm conditions that sends an alarm to the software as well as the operators e-mail address should the temperature exceed or decrease beyond the predefined limits. These types of safety alarms help ensure that necessary steps are being taken to avoid loss of batch.

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Conclusion

Ellab is a world leading manufacturer of complete thermal validation solutions for pharmaceutical, medical, food and other industries. We offer both wireless data loggers and thermocouple systems with high accuracy for reliable validation.

For freeze drying / lyophilization, we recommend TrackSense® Pro X/XL wireless data loggers that can operate under extreme conditions. The loggers offer the unique interchangeable sensor system facilitating high performance pressure, vacuum and temperature sensors, even with tiny thermocouples for product temperature measurement. As an option we suggest the SKY module for real-time data communication.

Adding the intuitive and FDA 21 CFR part 11 compliant ValSuite® software for data analysis and documentation plus a long list of accessories such as the LYO shuttle fixture and LYO 28 packing gland makes the overall solution unique for freeze drying.

Explore our options and contact us for more information.