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Bioprocessing

Practical Technologies for Lyophilization

Understanding and Controlling Process Parameters Is Essential to Success

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Freeze-drying or lyophilization is a process that removes water from a substance. This dehydration process is performed under vacuum while the substance is in a frozen state. Lyophilization technology is used to freeze-dry products such as biologicals, bacterial cultures, analytical chemistry moieties, and therapeutic molecules (e.g., antibodies, vaccines, drugs, and heat-sensitive proteins). Lyophilizing such products, particularly liquid formulations, vastly increases their shelf-life and stability.

The lyophilization process comprises three essential steps: pretreatment, freezing, and drying.

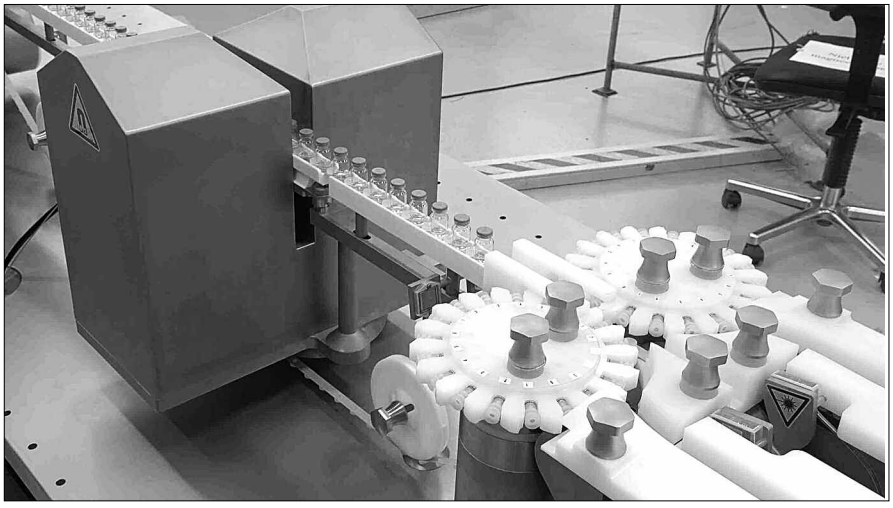
Pretreatment can be in the form of additives that stabilize the product, bulking agents that add mass to the product, or filtration to reduce volume and concentrate the product. The manner in which the product is frozen in the freezing step in large part determines the quality of lyophilization.

Snap or fast freezing causes smaller pore size in the resultant product cake. This, however, decreases lyophilization efficiency as vapor has a more difficult path to exit the product. Slow freezing causes bigger pore size and results in better lypohilization efficiency. During drying, the primary drying step removes about 90% of the water. The remaining 10% bound water is removed during the secondary drying step.

SMART Temperature Monitoring

Understanding process parameters, particularly the temperature conditions, during the lyophilization process is essential for a successful cycle. "The key is to keep the product in a frozen state and below its freezing temperature throughout the drying phase. Thermodynamic profiling of the product is critical for the drying phase," comments Leslie Mather, product manager at **FTS systems** (www.ftssystems.com).

FTS systems provides freeze-dryer



Vials passing through BOC Edwards' Non-Contact-Check-Weighing 7 (NCCW-7) system.

equipment to pharmaceutical and biotechnology companies for their formulations work and process development of parenteral products. "Our Smart Freeze-Dryer calculates product temperature and makes adjustments in the process to keep product frozen throughout the cycle. Smart freeze-drying technology uses the data from manometric pressure rise measurement in its proprietary algorithms to accurately calculate product temperature at the ice surface interface of the product.

"Smart Freeze Dryer equipment has an isolation valve between the product and condenser chamber. This valve closes for 25 seconds and the system measures pressure at the valve junction; 250 pressure readings are used to calculate product temperature. SMART then makes automatic adjustments in chamber pressure and shelf setpoint temperature to quickly bring the product temperature to a safety margin just below its collapse temperature. Since freeze-drying cycles can be long, ranging anywhere from six hours to one week, the Smart system can save money by eliminating the trial-and-error approach to cycle design," explains Mather.

Process Analytical Technology

PAT is based on the premise that quality assurance should be built-in or designed as part of the product. It emphasizes a process-driven approach vs. the more traditional data-validated approach. "The goal of PAT is to specify, monitor, and control processes to consistently ensure a pre-defined product quality," states Jos Corver, senior scientist at **BOC Edwards** (www.bocedwards.com).

PAT is emphasized by FDA for two reasons: the old-fashioned time-consuming data-oriented approach did not guarantee a flawless process or product quality and the release of new medical drugs was delayed by the time-consuming review of required data, which motivated the FDA to promote new technologies that increase the quality of monitoring data, leading to better process control.

"PAT can be quite powerful as understanding the science behind a given technology is its main focus. Knowing what you are doing, understanding important parameters, and applying science to control and improve process can highly improve product quality," says Corver.

NMR-based Noncontact Weighing Technology

Devising a well-controlled and predictable process requires accurate measurement of critical parameters, one of these being IPC (in-process control) of the filling system. Traditional balances rely on data derived from sampling vials taken out from the actual process and are subject to vagaries such as vibration, air-flow, and pressure fluctuations.

BOC Edwards has devised a noninvasive NMR-based, in-line, noncontact check-weighing (NCCW) technology. This system determines the weight of the contents of a vial with a single measurement, without taking the vial out of the transport system and without making physical contact with the vial. Since the response to an electromagnetic pulse is linearly proportional to the amount of product, a relatively simple calibration procedure is sufficient to apply this technology as a balance.

"It is critical that the amount of product in pharmaceutical drug vials is guaranteed within the label claim. Measurement of the contents of each individual vial is therefore an important aspect. Vials are typically overfilled to meet the stated claim. This NMR technique can be applied to liquid and solids. Having a system that gives feedback to filling stations at 100% rate allows for good process control. One can therefore produce at lower volume vials due to the narrower band fill and accuracy of the technology. Wastage due to overfilling can be avoided resulting in time and cost savings," says Dr. Corver.

Biopharma Technology (BTL; www.lyophilizationtechnology.com) markets a Lyostat 2 freeze-drying microscope and a Lyotherm 2 thermal analyzer. The Lyostat 2 Freeze Drying Microscope System enables identification of collapse and eutectic (freezing point) temperatures. In the Lyostat 2 system, an in-built stage chamber acts as a micro freeze-dryer. A small product sample is loaded onto a temperature-controlled block and subjected to freezing at a selected rate and observed while drying at a range of temperatures in order to determine its critical temperature(s). This process can be viewed in real-time either through the microscope or on a PC with image-capture software.

Microscope and Thermal Impedance

The Lyotherm 2 thermal analyzer enables one to perform differential thermal analysis (DTA) and electrical impedance measurement at the same time. This is an alternative low-cost technology compared to the Differential Scanning Calorimeter (DSC). The DTA allows determination of glass transition (Tg), eutectic (Teu) temperatures and crystallization events, while the data obtained from impedance analysis ($Z\sin\varphi$)—a method that is based on conventional resistance technology (as used in so-called eutectic monitors), but that has been demonstrated to be more sensitive to changes in frozen solutes—can be correlated to critical mobility-related events occurring in the frozen material.

The information gained from Lyostat 2 and Lyotherm 2 enables formulations to be refined according to behavior and

lyophilization parameters to be selected on a rational and product-specific basis.

Importance of Formulation

"Formulation composition is important when devising a product lyophilization process. For example, bulking agents may be added when one wants to freeze-dry small amounts of potent material, protective agents are added when one is dealing with heat-sensitive enzyme, antibody, or protein to prevent aggregation, and thermal stabilizers like dextran or PEG may be added to remedy a situation where a freeze-drying cycle is protracted due to the formulation having a low critical temperature," explains Kevin Ward, Ph.D, director of R&D at BTL.

Developing the right formulation depends on factors such as the final dosage form of product, the stage of clinical development, and the desired product concentration. For example, many antibody therapeutics require high product concentration to demonstrate efficacy after subcutaneous (SC) or intra-muscular (IM) administration. In such cases, freeze-drying may be useful not only for maintenance of product stability, but also to facilitate concentration of product via reconstitution in the small volumes (≤2mL) required for SC and IM injection.

KBI BioPharma (www.kbibiopharma.com) adopts a multi-step approach to formulation development. The company is a contract development organization for analytical, formulation, and process development for biotherapeutic proteins. The conformational and thermal properties of a product are first characterized using biophysical techniques such as differential scanning calorimetry, circular dichroism, FTIR, and fluorescence spectroscopy.

Statistical design of experiments is used during preformulation development to identify the optimal formulation conditions (buffer type, pH, ionic strength, excipients), which result in chemical and conformational stability of the product. The final concentrations of product, bulking agents, and cryoprotectants are optimized during freeze-drying cycle development.

"Technology transfer to the manufacturing facility is a critical element for a successful production run. Development is usually performed at a smaller scale using partial product loads, based on API availability and cost. Differences in scale as well as freeze-dryer design differences must be evaluated as part of tech transfer. Hence, involvement of the development house with production facility is imperative for successful process transfer," states Tim Kelly, Ph.D., vp of biopharmaceutical development at KBI BioPharma.

Stopper Selection

Stopper selection, particularly for drug vials, is an important component of the freeze-drying process. The stoppers should aid sublimation without much moisture absorption or migration into product composition.

"The functional requirements of the stopper need to be maintained in the context of product compatibility, process compatibility, moisture absorption, and moisture transmission," comments Fran DeGrazio, vp of marketing and strategic business development at **West Pharmaceutical Services** (WPS; www.westpharma.com). West Pharmaceutical Services markets several lyophilization stoppers with various treatment options.

"It is critical to choose the right elastomeric components to prevent stopper extractables from leaching into the drug product. WPS improves stopper performance by also adding coatings and films to these elastomeric components. Stoppers sticking to the lyophilization shelf can be prevented with the appropriate lubricious coating," explains DeGrazio.

FTS systems' LyoStar II R&D Freeze-Dryer for process development.

One of the company's offerings is LyoTec™ Stoppers. These stoppers are enhanced by Westar RS (ready to sterilize) or RU (ready to use) processing. The Westar stoppers receive a pharmaceutical-grade wash and are certified for pyrogen removal, bioburden, particles, and silicone.

"Westar RS stoppers are available for small volume production so one can use the same stopper right from lab testing through clinical trials into production runs. This takes a lot of variability out of the process," says DeGrazio.

The LyoTec stoppers are also available with B2 or FluroTec® coating. B2 coating is polymerized silicone coating that reduces friction on stoppers as they pass through the filling lines. This results in improved movement and line speeds. FluroTec film coating (made from ethylene-tetrafluoroethylene copolymer) is applied to the top and plug portion of stoppers. It improves compatability between the drug and closure, and provides a barrier against potential extracta-

Biopharma Technology's Lyotherm 2 provides an integrated differential thermal analyzer and an electrical impedance capability in one instrument. Lyotherm 2 has been designed to measure glass transition, eutectic melt, and melting temperatures relevant to freeze drying.

bles. The FluroTec coating eliminates the need for silicone oil lubrication thus preventing particulate contamination.

"A key point to note with respect to any stopper is that it should be already dry prior to placement on the product vial preceding lyophilization. Lyophilization cycles are designed with drug product characteristics and not stopper characteristics in mind," comments DeGrazio.

Material Science Approach

Baxter Biopharma Solutions (www.baxterbiopharmasolutions.com) offers services that support freeze-drying formulation and process development from the research and development stage all the way to commercial production runs for clinical and commercial products. Its freeze-drying facility in Bloomington, IN, can handle products of varying batch sizes and volumes using one or more of six commercial size and one clinical size freeze dryers.

From a technology perspective, Baxter adopts a materials science approach in developing a freeze-drying process, according to Steven Nail, Ph.D., senior research scientist at Baxter. "We perform a comprehensive thermal analysis, and freeze-dry microscopic analysis to optimize process parameters. For freeze-dried protein formulations, we have the capability to interface FTIR with the freeze-dry microscope to examine secondary structure of proteins in real-time during freeze-drying. Currently, Baxter is the only company that applies FTIR/freeze-dry microscopy technology in freeze-drying process development."

Existing Challenges in Lyophilization

While freeze drying is the preferred method to remove water from a product, it has its limitations. Biomolecules can be adversely impacted due to the stresses of the freezing and/or drying process. "The challenge is how do we optimize the freeze-drying process to make a pharmaceutically acceptable product in a cost-effective manner. Drying operations can be quite expensive. For example, the cost of a production freeze dryer can be greater than $3 million. Because of the high value of the product produced in a typical batch of freeze-dried injectable product, the need for redundancy in refrigeration equipment and vacuum-pumping equipment adds considerably to the cost," says Dr. Nail.

Another challenge is achieving the target moisture content in a specific product. The last of the solute-bound residual water in the product is removed during secondary drying. The secondary drying process is critical as not all of the water is in frozen form so product has to be dried carefully. Over-drying can result in a less stable product. "Baxter will soon be acquiring a process analytical technology capability, an in-process mass flow meter that allows precise monitoring of water removal. This meter will integrate mass flow rate throughout a freeze-dry cycle, giving the cumulative mass of water removed," explains Dr. Nail.

Design of the correct formulation remains a challenge. "Customers typically determine their formulations in an empirical manner and it can be exceedingly difficult to convince a customer to change to a better formulation. Inappropriately designed formulations and cycle continue to be a bane, an issue that FDA is well aware of," states Dr. Nail.

Product comparability between freeze drying and liquid formulations is another issue. "Clients sometimes prefer liquid formulations for the final marketed product, while freeze-drying product has advantages during clinical development due to its stability, ease of storage, and flexibility for dosing at multiple concentrations. The transition to a different final dosage form late in development or post-development may require demonstration of product comparability," says Dr. Kelly. GEN