Ultra-Low Temperature Considerations in Bioprocessing

One of the greatest scientific achievements of the 20th century was the development of hybridoma technology by César Milstein and his colleagues Georges Köhler and Niels K. Jerne in 1975. Hybridoma cells are generated through fusion between cancerous cell lines (immortal myeloma) and cells that are immune to a certain antigen (short-lived antibody-producing B cells) so that antibodies of the same type, monoclonal antibodies (mAbs), are expressed. This technique earned them the 1985 Nobel Prize for Physiology and Medicine. It also revolutionized the way we treat and prevent diseases and cancers and opened the door to the explosion of the biotechnology industry as we know it today.

The biotechnology industry is as vast as it is complex. Sectors range from environmental and agricultural to medical and pharmaceutical. Yet regardless of the individual sector, the overarching goal of biotech is to benefit society through the creation of pharmaceutical, diagnostic, agricultural, environmental, and other products that utilize living cells and cellular materials. These products necessitate the development of novel bioprocessing techniques as well as sophisticated equipment that can support each stage in the bioprocessing flow from product formulation, hydration, and cell culture (upstream) to harvest and finally separation and purification, finish, bulk storage, and filling (downstream).

DOWNSTREAM BIOPROCESSING EQUIPMENT FOR BULK DRUG PRODUCT STORAGE

The nature of ultra-low temperature refrigeration platforms has evolved since the mid-20th century when brute compressor force and volatile refrigerants were arranged in two-compressor systems designed to achieve the lowest temperatures possible through mechanical refrigeration. These systems were based on dual circuits in “cascade,” whereby one circuit cooled another and, in turn, cooled the process. Later, new “mixed-refrigerant autocascade” systems were developed using a single compressor system for bioprocessing, usually applied to vacuum cold traps.

While successful in process refrigeration, these systems were less effective in storage refrigeration. Lubricating oil was not always returned to the compressor sump resulting in “oil logging” and subsequent freezer failure. Such failures plagued the ultra-low temperature freezer industry for decades until new systems emerged with digital inverter compressors, natural refrigerants and sophisticated controls

embedded with predictive algorithms for continuous system feedback. Still, conventional platforms were acceptable for many process applications, yet more sensitive to applications for long-term storage.

Biopharmaceutical freezers are an essential piece of downstream bioprocessing equipment for the long-term bulk storage of biotech products. Yet not all freezers are created equal. This is true for both performance and energy consumption considerations, as well as selecting the most appropriate freezer for a specific application. For example, in bioprocessing, the vast majority of cell and gene therapy products require cryogenic storage temperatures (<-150°C) for long-term storage. However, when it comes to biopharmaceuticals, including mRNA vaccines, antibiotics, and more, ultra-low temperatures of -80°C or warmer are often suitable. These ultra-low temperatures can be achieved through one of three types of freezers: the conventional ultra-low temperature (ULT), the blast freezer, or the controlled rate, forced air convection ULT.

Of these three types of freezers: the conventional upright ultra-low temperature (ULT), controlled rate or blast freezer, or a chest style ULT freezer, each has an ideal use case and several drawbacks that make each solution difficult to use in many applications.

Most processes leverage standard upright freezers because of their ease of accessing materials, quiet operation, and small floor space footprint. However, many processes strain the capabilities of upright ULTs, leading to failures of equipment, loss of stored material, or a large volume of alarms that have to be investigated and documented.

COMPARING ULT TYPES FOR FREEZING AND STORING OF BULK PHARMA STORAGE

The difference between process refrigeration and sustained storage refrigeration in the biopharma environment determines the type of ultra-low temperature cooling platform best suited to the application. Process refrigeration, for example, can be applied to dedicated bulk freezing, solvent extraction and lyophilization systems where pharmaceuticals are brought through manufacturing sequences to near completion. These systems do not usually operate continuously. Instead, they are started and stopped as a function of manufacturing throughput and returned to a steady state, often at ambient temperature, between batches. Here, refrigeration compressor lubricating oil can be returned to the compressor pump, and the issue of oil logging endemic to past ultra-low temperature freezer models can be avoided.

To assess freezer performance in the bulk freezing of biological drug substances, however, it is also necessary to understand the heat removal required for a total load. In addition to variable freezing times from batch to batch in conventional ultra-low temperature freezers, the pulldown time for stored samples is significantly longer. Extra time is required because the refrigeration system is sized to account for the heat penetrating into the cabinet through insulation with a little extra cooling capacity for an occasional door opening. While the advances of auto cascade systems have remedied the issue of oil logging, the use of conventional ultra-low temperature freezers for bulk freezing causes these ULTs to be in a constant state of pulldown, leading to increased freezing time, higher energy expenditures, and additional strain on the freezer mechanics that cause premature failures. Furthermore, when conventional ultra-low temperature freezers are used for the initial storage of biopharmaceuticals, even for small volumes, the excess heat introduced to the system creates unnecessary stress on the biological components of products, jeopardizing biopharmaceutical viability.

Many bioprocessing facilities, therefore, rely on blast freezers for the rapid cooling of samples during the preparation process before being stored in an ultra-low temperature freezer. Blast freezers are able to provide the large cooling capacity that conventional ULTs lack. However, they are typically limited to freezing only, unlike controlled rate chambers which can rapidly freeze like a blast freezer, but also follow a specific controlled freezing or thawing profile for fragile molecules or biologic materials.

Decisions on how to store frozen biologic materials come down to a few factors- 1. how often will the freezer be accessed daily, 2. how temperature sensitive the stored material is, and 3. the available footprint for the freezer. In most cases, the use of a chest-style freezer makes sense to provide reliable cold storage and ensure sample safety when accessing the chamber to add or remove materials. However, floor space is at a premium as more and more demand for cold storage is made to support the biologic drug pipeline. This drives customers to either build larger spaces to accommodate the density-poor chest freezers or leverage upright freezers that have better footprints but require limited access to the chamber to protect the samples inside.

In most cases, the use of a chest-style freezer is effective in providing reliable cold storage and insuring sample safety when accessing the chamber to add or remove materials. However, floor space is at a premium as more and more demand for cold storage is needed to support the biologic drug pipeline. This may drive customers to build larger spaces to accommodate the density-poor chest freezers or leverage upright freezers that have more desirable footprints but require limited access to the chamber to protect the samples inside.

Upright Freezers that contain forced air convection technology can rapidly reduce the time to recover from a door opening, limiting temperature excursions, and have the high storage density/square feet that the industry demands. These freezers contain expansion valves to precisely control the refrigerant needs versus the fixed capillary tubes utilized by standard ULTs and chest freezers. The forced air system quickly exchanges the air in the chamber rather than waiting for natural convection to displace the warm air in the chamber (ULT and chest). This forced air convection comes at the expense of the small amount of energy used for a circulation fan. These units match the energy efficiencies of the top ULT freezers on the market today while significantly outperforming them in uniformity and temperature recovery from door openings.

Lastly, since forced air convection freezers utilize a self-contained refrigeration system, the chambers can easily be customized to accommodate operational process intensification efforts to reduce handling and steps.

TECHNOLOGY COMPARISON: FORCED AIR CONVECTION VS. COLD WALL

Customer Value	Forced Air Convection	Standard ULT
Designed for storage of materials already at set point temperature	✔	✔
Freeze material then store in same freezer	✔	–
Temperature uniformity of +/- 3°C *Empty chamber*	✔	✘
Designed for freezing & storage of bottles, bags and bulk material	✔	✘
Adaptable to manufacturing reconfigurations and volume changes	✔	–
Integrated predictive and preventative maintenance	✔	–
Material handling or storage flexibility using bottle carts, Metro shelving system- carts or pallet rack	✔	✘

ENSURING REPEATABLE FREEZING AND THAWING OF BIOPHARMACEUTICALS

Controlled rate chambers are the latest technology for bulk drug substance freeze/thaw. They are specifically engineered for consistent freezing of drug products from batch to batch and load to load. In a controlled rate freezer, forced air convection is used to remove heat from large volumes of materials. This ensures consistent freezing of all samples during bulk load freezing. Unlike in blast freezers, controlled rate chambers have configurable freeze rates that can be adapted to a wide range of biopharmaceutical manufacturer temperature specifications and storage volume changes.

Unlike standard ULTs, controlled rate chamber freezers are designed specifically for the repeatable freezing and storage of bulk material bags, bottles, and vials. Rapid, controlled bulk freezing of biopharmaceutical drug products creates uniform, consistent freezing of drug products. Ensuring consistent product quality attributes (PQA) during the freezing and thawing of biopharmaceuticals is essential to ensure dependable product quality across all samples.

Take Control. FARRAR's 4000 Series Controlled Rate Chamber provides uniform, repeatable, controlled rate freezing and thawing of your biopharmaceutical products.

WHAT WE DISCOVER, WE MUST SAVE.

The mechanical biological attributes of long-term ultra-low temperature storage call into play the physics and chemistry of refrigeration platforms. These are measured by the progressive orchestration of third-party components such as compressors, lubricating oils, refrigerants, electronics, cabinets, gaskets and other components that comprise an ultra-low temperature storage freezer. Thus, the structure of a single cell drives the equation for ultra-low temperature storage, whereby cell preservation is critical to all phases of a drug discovery effort. Here, where sheet metal and refrigerants meet the nuances of biological life forms, the symbiosis is complete. And because ultra-low temperatures retard or completely stop the metabolism of a living cell, restricting enzyme transfer and thereby shutting down the cell factory, the reliability of refrigeration systems deployed in the biopharma industry is critical. The objective, of course, is to preserve and maintain a biological specimen so that it can remain stable in the frozen state indefinitely and viable once thawed for use.

The performance of ultra-low temperature freezers, whether used in processing or storage, requires absolute control over pulldown, dwell, sustained archival, warm-up, and allied systems for documentation and monitoring. In short, conventional ULTs are not the ideal solution for freezing bulk biopharmaceutical drug products because they are designed to store products that are already frozen. Alternative ultra-low freezing chambers exist that are purpose-built for freezing and short and long-term commercial bulk storage of biopharma products, including mRNA vaccines and therapies, drug substances, biological samples, lot samples, clinical trial kits, and final drug products. They provide faster freezing rates that ensure continued mechanical reliability and are the only equipment on the market that can provide controlled rate thawing that helps ensure predictable PQAs.

Dependability Delivered. FARRAR's forced air convection Ultra-Low Chamber series (ULC-190, ULC-259, and ULC-311) delivers dependable batch freezing for drug substances, final drug products, biologic samples, lot samples and clinical trial kits at flexible temperature set points (20°C to -80°C).

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[1] Niels K. Jerne, Georges J.F. Köhler, César Milstein. “The Nobel Prize in Physiology or Medicine 1984,” The Nobel Prize. https://www.nobelprize.org/prizes/medicine/1984/summary/.

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[1] Johnson, Jeff. “New Approaches for Drug Substance Freezing and Storage.” New Approaches For Drug Substance Freezing And Storage. Bioprocess Online, January 12, 2023. https://www.bioprocessonline.com/doc/new-approaches-for-drug-substance-freezing-and-storage-0001.