biopharma group

Introduction to Freeze Drying

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Introduction to Freeze Drying

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Introduction

Written for those who wish to expand their existing knowledge or learn from scratch, this booklet introduces the fundamentals and benefits of freeze drying and is one of two free guides on freeze drying.

To download a copy of 'Misconceptions in Freeze Drying', please visit: www.biopharma.co.uk/misconceptions-in-freeze-drying/

Questions or suggestions?

Please feel free to direct any comments or questions to bps@biopharma.co.uk

Introduction to Freeze Drying

Freeze drying, or lyophilisation, is a process that removes the solvent from a product to a level where the it shows significantly increased stability. This process has applications in the preservation of many different types of materials, from small amounts of chemicals to large structures where both the material and the three-dimensional structural integrity can be maintained.

Typical applications include:

- **Fine chemicals, drugs, and laboratory reagents** – extending the shelf life and managing the labile or reactive properties of a molecule.
- **Therapeutic and industrial enzymes and biological agents**, such as proteins and DNA – preserving biological activity.
- **Preservation of cells** – used as an alternative to storing cells in liquid nitrogen or in freezers.
- **Tissues for research and medical use** such as blood, bone, and tendon – used for preservation and as a patient safety step.
- **Food stuffs** – enabling reduced transport costs, extending shelf life and maximising flavour and nutritional value.
- **Preservation and recovery of rare, sentimental, and valuable artefacts** such as waterlogged books, bridal bouquets, and archaeological finds.
- **A concentration step** – used to recover materials from dilute samples for further investigation.

Different applications and products have differing processing equipment requirements; condenser temperatures and capacities, through to the types and complexities of control systems will vary. Biopharma Group works with leading freeze dryer manufacturers to supply the optimal equipment for each individual customer's needs. Refer to the section on ‘Types of equipment’, page 14, for more information.

Freeze dried products, clockwise from left: instant coffee; vials of freeze dried product; bulk tray freeze dried fruits; injectables; nutriceuticals.

Benefits of freeze drying

Freeze drying offers a range of unique benefits:

- Freeze dried material can be stored at warmer temperatures, often up to ambient-room temperature, without suffering degradation. Without needing cryogenic or freezing facilities for cold-chain storage, the energy cost and risk of product loss from power failure is greatly reduced.
- Freeze drying can be performed in a more controlled environment than some other forms of drying, reducing the possibility of contamination.
- Drying can be extended until the specified moisture level is achieved, whether that be a residual moisture level of 5% or 0.1%.
- Because product is dried without using excessive heat, proteins and other products that would be thermally denatured can be successfully preserved without loss of activity.
- Freeze dried products have a large surface area which enables them to be reconstituted quickly. This is particularly important in the case of emergency vaccines and antibodies, which need to be administered as soon as possible. Instant soups, coffees and powdered milks are day-to-day examples demonstrating the benefits of quick reconstitution.
- Vials can be sealed under vacuum or inert gas, which helps to preserve products sensitive to oxygen. When drying in vials or ampoules the filling volume can be accurately maintained.
- As products are much lighter when they are dry, freeze drying also offers the benefit of reducing transportation costs.

Containers for freeze drying

Freeze drying can be carried out in a variety of different container types. In the pharmaceutical and biotech industries, vials are the most commonly used, whilst the food industry generally dries product loose in trays (known as ‘bulk’). For research purposes and within laboratories, flasks, ampoules, and microtiter plates are used most often. Newer innovations in the field of freeze drying include containers sealed with semi-permeable membranes used to prevent cross-contamination and loss of product, and blister packs.

Flasks

Freeze drying flasks are used with 'manifold' style adapters, such as the four-port side manifold kit with quick seal valves for the AdVantage Pro benchtop system (see pages 14 and 16).

The flasks are usually frozen in another piece of equipment, such as a shell bath freezer.

If the container is small enough it can also be frozen inside the unit condenser. Once the product is frozen, the flasks are attached to the manifold and a vacuum applied. Ambient heat from the atmosphere provides energy to the drying process.

Ampoules (see picture on page 7)

Ampoules are also typically attached to a freeze dryer manifold by means of an adaptor but can also be dried on a shelf.

Microtiter ('96 well') plates

Special microtiter plates are available for use in freeze dryers. They are cast in solid aluminium enabling the fastest and most uniform heat distribution to the product. These plates can be used in manifold freeze dryers when the manifold is a 'drum' style (see photo overleaf) or in shelf freeze dryers.

Bulk

Product dried in bulk include solid foodstuffs, APIs, individual items such as tissues or water-damaged artefacts, liquids, or slurries. The key issues when drying in bulk are:

- **Volume** – ensuring that the amount of liquid to be removed is not greater than the capacity of the condenser.
- **Fill depth** – the greater the depth of the product to be dried, the longer the drying process will take. During primary drying, pure water will freeze dry at a rate of roughly 1mm per hour. Use of thermocouple probes embedded into the product can help determine when it has been dried throughout. A large surface area accompanied by a low fill depth will deliver more efficient freeze drying.

Vials

Vials are available in a wide range of dimensions and varieties. The industry standard is tubular glass vials that comply with 'DIN' sizing.

The DIN standard details all vial dimensions, to ensure repeatability across batches. The tubular method of manufacture, rather than blown glass, ensures a smoother, more even wall thickness for consistency of drying and filling.

For all pharmaceutical products vials and other glass containers must be of 'Type 1 borosilicate' glass. Most vials are supplied clear, but amber glass is also available for products that are especially photosensitive.

Products freeze dried in vials are typically stoppered within the chamber, while under a partial vacuum or inert backfill. Stoppers are partially inserted before the vials are loaded and then manually pushed, or shelves collapsed on each other, to push the stoppers into the vials to seal them once the cycle is complete. Aluminium seals are often added afterwards to further protect the product by ensuring the stoppers remain firmly inserted. Both seals and stoppers are available to fit DIN-sized vials. See the section on Stoppering (page 10) for more information.

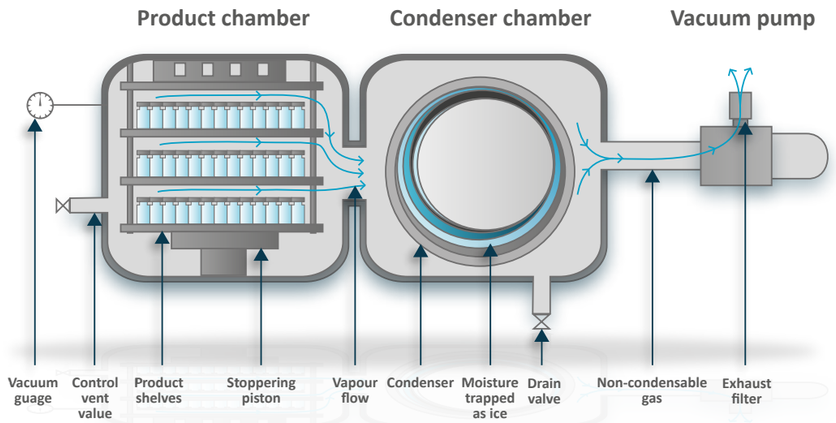
When calculating the capacity of your freeze dryer or writing a specification for a new freeze dryer, it is vital to remember shelf spacing. Sufficient gap must be allowed for the height of the vial plus the partially inserted stopper and a further allowance must be made for vapour flow. For production machines these calculations can be complex, but for smaller machines a rough guide is to allow 8-10mm for the partially inserted stopper and a further 10mm for clearance. For example, a DIN10R vial has a height of 45mm, so shelf clearance should be around 65mm.

Left to right: fruits drying in a bulk tray; 'drum' manifold; ampoules in an adapter.

Design of a freeze dryer

Freeze dryers come in many configurations. The following outlines the most essential and common components.

Product Chamber



The Product Chamber is the chamber in which product is placed for freeze drying.

In most cases the product chamber contains a number of shelves, temperature and vacuum sensors, as well as mechanisms which allow the shelves to move for stoppering purposes. The number of shelves that can be fitted is determined by the size of containers or products to be dried. The product chamber is designed to withstand the forces it is subjected to including vacuum, very low freezing temperatures, and positive pressure at elevated temperatures in the case of steam sterilisable systems (SIP). The product chamber may also contain devices and systems to allow automated cleaning (CIP) plus other process-dependent monitoring and control systems.

Most commonly, freeze dryers are able to freeze products loaded onto their temperature-controlled shelves, but it is not essential for freezing to take place within the same piece of equipment. For example, when dealing with products that are bulky or require special handling, it may be preferable to freeze the product before loading it into the freeze dryer. In such cases, the shelves can be pre-cooled to the required temperature to match the product temperature.

The product chamber should be easy to clean with smooth walls, curved corner surfaces and free draining.

Condenser

The process condenser may consist of plates, coils or other surfaces that are cooled to a very low temperature and on which the solvent vapour condenses and freezes. The condenser is usually located inside a separate chamber (an 'external condenser') but can also be situated inside the product chamber ('internal condenser'). External condensers can be below, behind, beside or above the product chamber without affecting trapping performance. Note that interconnecting pipework should be as short as possible with as large a diameter as possible, to allow unimpeded vapour flow from the drying product in the product chamber. Valves are commonly installed between the product and condenser chambers to allow them to be isolated.

Condensers are described in terms of capacity and operating temperature. Capacity is usually given in kilograms or litres but it is important to note that the stated condenser size may refer to total capacity or the deposition rate over a set period, depending on how the manufacturer has put together their specification. The required condenser temperature will be dependent on the thermal characteristics of the product to be dried. It will usually be several degrees lower than the minimum shelf temperature in order to maintain a temperature and vapour pressure gradient.

A correctly specified condenser should trap condensable solvent vapour during processing. Design considerations are similar to those described above for the product chamber.

Vacuum system

A vacuum is applied during the drying stages of freeze drying. Rotary vane vacuum pumps and, increasingly, dry pumps are most commonly used for freeze drying, although other types are used.

Freeze drying is initiated by achieving a vacuum lower than the vapour pressure of the frozen product. However, a higher vacuum (lower pressure) does not correlate with faster drying. Convection of heat from the remaining gas molecules into the product is an important source of energy in the sublimation process. If pressure falls too low, decreased convection can increase drying time. At the same time, sublimation of the solvent adds to the overall gas load and will act to decrease the vacuum. Therefore, throughout primary drying the vacuum level needs to be carefully controlled. In contrast, secondary drying is often facilitated by reducing chamber pressure to a minimum and increasing product temperature to encourage desorption.

A correctly specified condenser will trap all condensible vapours, but consideration should still be given to any non-condensible vapours and the possible effects on the vacuum pump.

Vacuum gauges need to be robust and suited to the working environment within the freeze dryer. For laboratory-type systems hot wire gauges such as thermocouples or pirani-type designs are sufficiently robust. For production systems that might be subject to SIP and CIP, the life of a hot wire gauge would be minimal and it is more common to use diaphragm-type capacitance manometer gauges.

Inert gas injection, most commonly nitrogen, can be used to aid control of chamber pressure if the product is sensitive to normal atmosphere.

Stoppering

Stoppering systems are needed when drying product in containers, most commonly vials; it is necessary to seal containers under controlled atmospheric conditions (e.g., controlled vacuum or inert gas) before removal from the dryer. Rubber stoppers are partially inserted after the vials are filled. At the end of freeze drying they are pushed fully home by pressing the shelves together either hydraulically, electrically or manually.

Control systems

Freeze dryer control systems range from simple push-bottom controllers to complex fully programmable SCADA systems, compliant with the latest regulatory requirements such as 21CFR11. A range of sensors provides information on the temperature and pressure of the equipment and the product, which in more sophisticated systems is provided in a reviewable format for post-process analysis. Control systems may also control other functions such as stoppering, sterilisation and alarms. There are many types of alarms, from maintenance ones reminding end users to change the vacuum pump oil, to warnings such as condenser overload, suggesting that the recipe parameters might be outside of the machine capabilities or there is an issue with the freeze dryer itself. It is important to familiarise oneself with the controls manual, to assure appropriate use and longevity of the equipment.

The freeze drying process

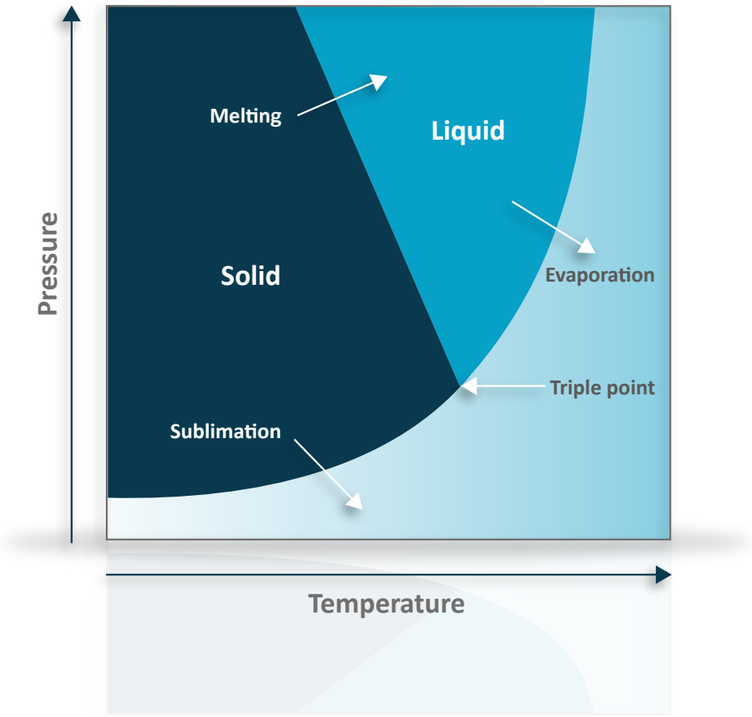
Freeze drying is a three step linear process: freezing, primary drying, and secondary drying. Each step is outlined below.

Freezing

The first stage in the freeze drying process is for the product to be frozen. The method of freezing is of vital importance as it will affect how the product will dry. The behaviour of a solution depends on the nature and concentration of the solutes present, which Liquid will be affected by the formation of the ice structure. Therefore, the optimal freezing parameters must be determined to protect the sample and produce a freeze dried product that will have acceptable characteristics.

In products where the solute will crystallise readily, product freezing will result in a complete mixture of ice and solute crystals. This behaviour is termed as eutectic freezing.

Right: a simple phase diagram, showing the relationship of pressure and temperature.



In other products the solute may persist, together with unfreezable water, as an amorphous, noneutectic mix. In practice, many solutions will cool to produce a partially crystalline, partially amorphous mix.

The lowest temperature in a system where the residual liquid phase and solid phase are in equilibrium is called the eutectic point. Above the eutectic point ice and solute concentrations persist, and below it a mix of ice and solute crystals are produced. For two part water/solute products the eutectic temperature is a discrete quantifiable temperature. For more complex multi-solute systems an eutectic zone may be observed, where the minimum eutectic temperature is lower than any of the individual eutectic temperatures within the product.

Key issues with freezing

The structure of the ice matrix dictates the flow of vapour out of the product and therefore the manner of drying. Ideally, to minimise impedance to the vapour flow, the ice crystals should be large, wide and contiguous, extending from the product base up to the surface. Small individual crystals prevent moisture from escaping, prolonging the drying time and increasing the risk of collapse. The speed and manner of freezing will influence the type of structure formed. Annealing, a technique of raising and then lowering the temperature of a frozen product, can also be used to encourage crystallisation or to provoke a more favourable ice structure.

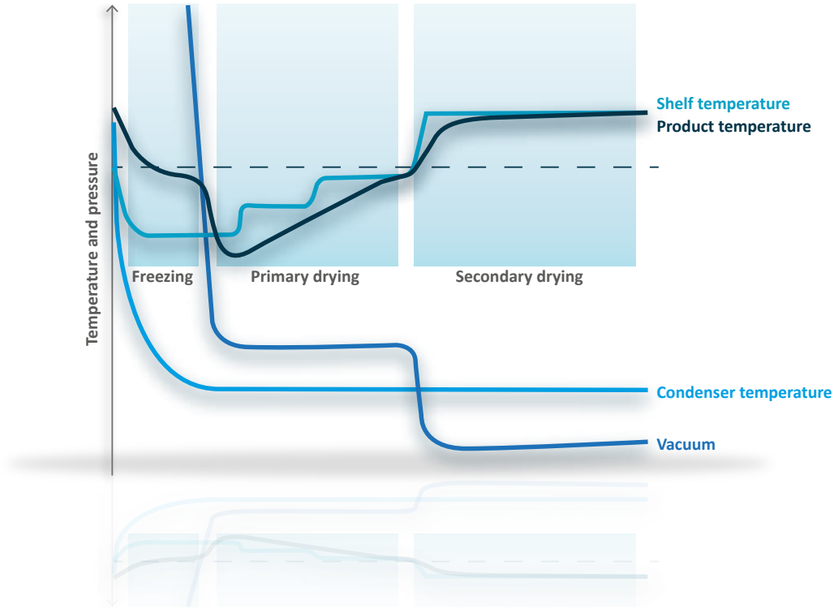
Many freeze dryers are able to freeze the product that is loaded into it, but it is not vital for freezing to take place within the same piece of equipment. For some products that are bulky or require special handling, it may be preferable to freeze the product before loading. It is also common for freeze-drying flasks to be frozen within separate equipment. Ice formation is not instantaneous or homogenous. Therefore, the essential aspect of freezing is to ensure that the product is cooled both low enough and long enough to facilitate the completion of the ice formation. The latest developments, such as ControLyo technology, are now offering control of ice nucleation at R&D level and at production-scale too.

Primary drying

Once frozen, product is dried first by a process known as sublimation. The product temperature is kept below its critical (glass or eutectic) temperature while a vacuum is pulled until the pressure/temperature balance is such that the ice sublimes directly into a vapour without melting.

This part of the process is most critical for the product to ensure that it does not melt or collapse. Sublimation leads to evaporative cooling which will lower the product temperature. Consequently, to maintain a constant temperature heat must be applied to compensate for sublimation cooling.

Failure to add sufficient heat will result in the product cooling and the process slowing. Conversely, excess heat input risks melt or collapse of the ice structure. The energy transfer during sublimation is therefore one of balance, where the mass of the product remains frozen and just enough heat is used to provide for the change of state.



Above: simplified freeze-drying cycle chart.

Collapse is often visually obvious with the product bubbling, shrinking, or forming a sticky residue, but it can also occur in products that appear to have produced a good cake. Collapse results in the loss of product stability and reduced activity. Collapsed product will not reconstitute as fast or as well as one that has successfully dried. It is relatively simple to maintain the heat input/vapour output relationship early in the freeze drying cycle. However, as drying takes place the drying front (or sublimation interface) progresses through the frozen product from the surface to the base. As the dry layer develops in thickness, the water vapour finds it increasingly difficult to migrate from the drying sample out and the rate of sublimation decreases. Typical product depth range in vials is 12-15mm.

Heat enters the product by several mechanisms: direct contact between the container base and shelf; conduction across the container and through the frozen mass to the drying front; by gaseous convection between the product and residual gas molecules in the chamber; and by radiation. Of these, convection has significantly more impact on product heat than the other mechanisms. As the pressure rises in the chamber, the effect of convection is greatly increased. Control of the pressure (or vacuum) in the chamber is therefore another way of influencing the overall speed of the process.

Freeze drying can be carried out at atmospheric pressure using blasts of dry air, but this process is difficult to control and is slow-paced.

Secondary drying

Caution should be exercised in defining 'primary' and 'secondary' drying too rigidly. Simplistically, primary drying is when ice is present in the product and secondary drying takes place in the absence of resident ice. Secondary drying is a desorption process where water which is chemically bound is removed. This occurs when the product is unlikely to melt and is therefore relatively stable.

The moisture level at the beginning of this stage may be around 5-10%. Depending on the final moisture level required (how dry it needs to be), secondary drying can take a long time as the process is quite slow.

In contrast to primary drying, which uses low shelf temperatures and a moderate vacuum, desorption drying is facilitated by raising shelf temperatures and reducing chamber pressure to a minimum.

Stoppering

A freeze dried product will have an exposed surface and the dried product is typically hygroscopic. Exposing the dried product to atmosphere will result in it reabsorbing moisture. Both air and water can damage a dried sample, resulting in degradation and poor stability. Consequently, for many products, especially pharmaceutical products, it is necessary to seal product into airtight containers as soon as possible. In the case of vials this means stoppering. Taking place within the freeze dryer chamber and usually under full or partial vacuum, rubber stoppers are automatically pushed into the vials to seal them. To prevent product foaming, it is also common to backfill with an inert gas just prior to stoppering. Nitrogen is commonly used, although it may not be completely inert to all bio products. In these cases, helium or argon may be used.

It is good practice to backfill to a slight negative pressure to ensure that the stopper is held within the vial. If the vial is backfilled to full atmospheric pressure, subsequent exposure of the vial to changes in temperature may cause the atmosphere within the vial to expand and the integrity of the seal may be compromised.

Product storage

A freeze dried product should exhibit minimal degradation and should tolerate atmospheric temperatures for distribution purposes. However, it would be dangerous to assume that freeze dried products are completely shelf-stable. The rate of decay will still be linked to storage conditions such as temperature as well as the original product formulation, product moisture content, and the sealing atmosphere. Exposure to light can also affect a product's shelf life, but as most products will generally be stored in boxes exposure is minimal in practice.

Occasionally reconstituted pharmaceutical or biotech products may be affected by ions or components leached from the vial or stopper. Vials and stoppers designed specifically to combat these problems are available.

Types of equipment

Freeze dryers can be roughly grouped together into three categories:

- **Bench-top systems** for preliminary research.
- **Pilot scale systems** with more sophisticated control and feedback systems.
- **Production systems**.

Benchtop freeze dryers

Benchtop systems, such as the AdVantage Pro series (shown overleaf) are regularly used in universities and research institutes because of their flexibility, small size, and cost-effectiveness. They feature temperature-controlled freeze drying shelves, the same as those found in larger systems, making them more comparable to pilot scale systems than to manifold systems, but in a compact frame ideal for laboratories pressed for space.

Right: 'AdVantage' benchtop shelf freeze dryer

The control system and optional software are more sophisticated, allowing the user to collect accurate product and process data. This type of system is perfect for teams undertaking formulation development and product characterisation work. For more information on our benchtop systems, see page 16.

Pilot scale systems

These larger, free-standing systems, such as the VirTis Genesis and Ultra (pictured below), can handle more product than bench-top systems. The shelf area typically ranges from around 0.5m2 to 2.0m2, with condenser capacities in the range of 25-50 litres. They are commonly used in universities and research institutes, for small-scale production, and in research departments of larger organisations. They are ideally suited to interim development work such as refining formulations to make them easier to freeze dry, optimising and scaling-up freeze drying cycles for production, and producing validatable batches for analysis and trials.

Left to right: 'LyoStar 4.0' freeze dryer; 'Sample Thief' device that can be fitted to the LyoStar; 'Genesis' freeze dryer; 'Benchmark' production system; 'Ultra' pilot scale system.

Such freeze dryers can be fitted with more sophisticated control systems to make them compliant with modern regulatory requirements and can also be modified to enable them to be used inside a cleanroom. Stoppering and backfilling are common. Options include sample thieves for in-cycle monitoring and analysis of products, and sophisticated software designed to streamline the cycle development process (such as the SMART freeze drying software available on the FTS LyoStar).

Production systems

Production systems can vary greatly in terms of shelf size, condenser capacity, control systems, design and construction, as required. The type of product being processed is also important as foods, artefacts, pharmaceuticals, and other chemicals will all have different processing and regulatory demands. The size and layout of the equipment components (chamber, condenser, vacuum system) is important especially when integrating a new machine into a compact space/predefined space/space with limited footprint or facilities restrictions. Other important equipment factors to consider are automated cleaning and sterilisation, loading/unloading systems, and regulatory requirements such as GAMP, 21CFR11 and cGMP.

Specifying a production system is extremely complex. Biopharma Group has extensive experience of helping customers specify the right system for their needs and draws on the expertise of four internationally recognised equipment manufacturers.

If you would like more information on production freeze drying systems and how we can help, please contact us directly on +44 (0)1962 841092.

Left to right: tray loader; production freeze dryer for the food industry.

Benchtop 'Advantage Pro' freeze dryer

Left to right: Advantage Pro freeze dryer; control system synoptic screen; processing in vials.

A wide range of features and extensive freeze drying capabilities are key attributes of the VirTis Advantage Pro Freeze Dryer, and it is small enough to fit on most laboratory benchtops!

Ideal for small-scale freeze drying applications, such as those occurring in pilot or research and development laboratories, the AdVantage Pro contains up to three fully temperature-controllable shelves usually only found in much larger models. It also has state-of-the-art control systems to ensure precise freeze-drying, particularly important for handling extremely valuable or sensitive biological materials. Multiple separate recipes, each with freezing and primary drying steps, can be stored for complete flexibility in cycle formulation. Popular system configurations include:

Vial processing configuration:

Advantage Pro EL with two shelves and stoppering. Stoppering enables vials to be sealed under vacuum or inert backfill. Two 35 x x25cm (10 x 14”) shelves, -82˚C condenser, 6 litre capacity.

Bulk processing configuration:

Advantage Pro EL with three shelves. This system features low condenser temperature for a wider range of solvents and is ideal for those processing in bulk trays. Three 35 x x25cm (10 x 14”) shelves, -82˚C condenser, 6 litre capacity.

Pilot 'Genesis' freeze dryer

Clockwise from left: Genesis freeze dryer; Merlin control system synoptic screen; LyoS freeze drying cycle dataset

A versatile Pilot Lyophilizer available for product development and small batch production

This highly configurable system has many options which can to be tailored to suit your application:

- Condenser options
  - 25 litre or 35 litre capacity
  - Condenser temperatures to -82°C
  - Hot gas defrost and smooth wall for quick turnaround
- Range of configurable control systems providing best fit for your application
  - Optional Pirani capacitance manometer and/or barometric end point determination
- Systems configured with between three and six stainless steel shelves, plus one radiant
  - Capacity offering 0.14 m2 to 0.85m2
  - Uniform shelf temperature control by recirculating fluid
  - Temperatures from -55°C to +65°C, ±1°C
  - Optional stoppering (up to five shelves only)

Shelf capacity quick comparison: up to 3135 x 2ml vials (14.75mm OD x 40mm high with partially inserted stopper) using a 5 shelf stoppering unit

Control: Merlin or LyoS™ (LyoS™-P option is 21 CFR Part 11 compliant)

LyoStar 4.0 R&D freeze dryer

Improve your efficiency with higher fidelity

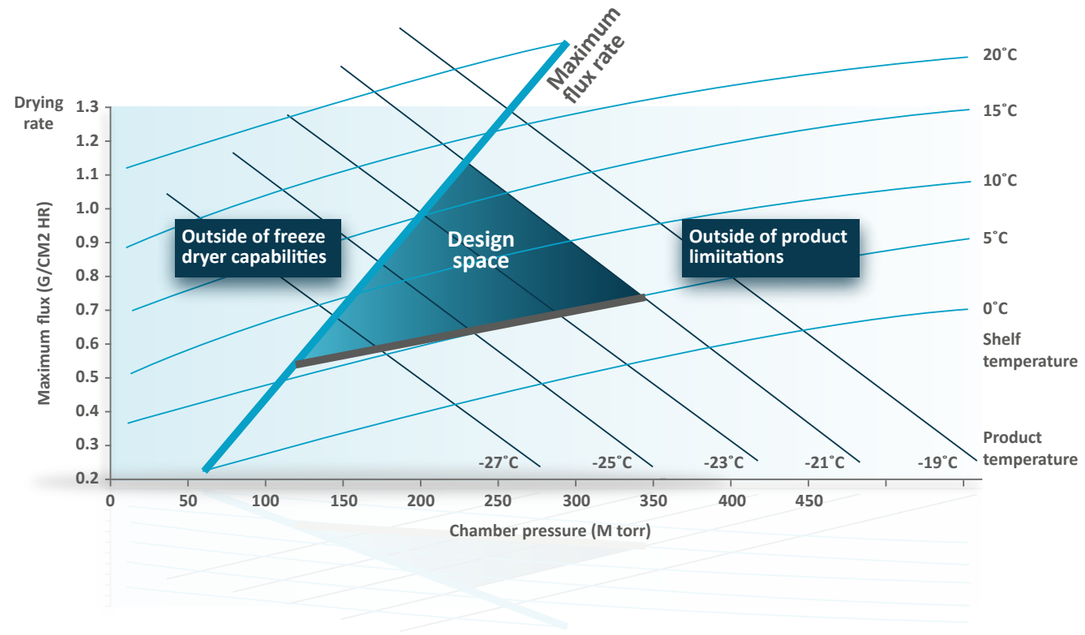
Designed and built to enhance the speed to market of biopharmaceutical products, LyoStar represents a significant advancement in freeze dryer engineering. The instrument serves as a core piece of machinery at R&D level, capable of replicating process/software controls in conjunction with fully scalable PAT technology – a necessity when facilitating a 'Quality-by-Design' (QBD) strategic approach to drug manufacture.

The level of data available through the LyoStar 4.0 suite of PAT technology allows the operator to thoroughly consider all aspects of the product post drying.

Process Analytical Technology (PAT) tools provide:

- In place to allow users the opportunity to overcome critical freeze drying challenges during development, scale-up and manufacturing of a biologic.
- SMART™-MTM Technology: Patented, SMART Technology delivers primary drying optimisation in one cycle by automatically adjusting shelf temperature. Using proprietary algorithms and high-speed measurements during primary drying, SMART delivers the additional, invaluable process data of dried layer resistance, ice thickness, product temperature at the ice surface interface and heat flow and mass transfer.
- ControLyo® Ice Nucleation on Demand Technology: SP exclusive ice nucleation technology that delivers nearly instantaneous ice nucleation as part of the LyoStar 4.0's automatic recipe. Rapid depressurisation from positive pressure above 20 psi using nitrogen or argon gas creates safe, uniform, nearly instant induction of ice crystal formation. Product resistance values decrease dramatically (when compared with data using traditional stochastic freezing).
- A 3D modelling package for computational fluid dynamics and process monitoring.
- Tempris Wireless Sensors advance your existing lyophilisation workflow with accurate product temperature measurements, placement, and cutting-edge process analytical technologies.
- LyoFlux, more commonly known as Tuneable Diode Laser Absorption Spectroscopy (TDLAS), a nearinfrared (NIR)-based PAT tool ensures the LyoStar® 4.0 delivers real-time, continuous monitoring.
- Endpoint Determination & Control Software: The LyoStar 4.0 is equipped as standard with both automated barometric endpoint testing (pressure rise testing) and Pirani/Capacitance Manometer convergence in the software. Both methods can be set up by the operator to simply monitor detect the absence of ice in the product and/or control the transition to secondary drying based on the results.

Cycle development times reduced by as much as 79%
Annual cycle development costs reduced by 60%



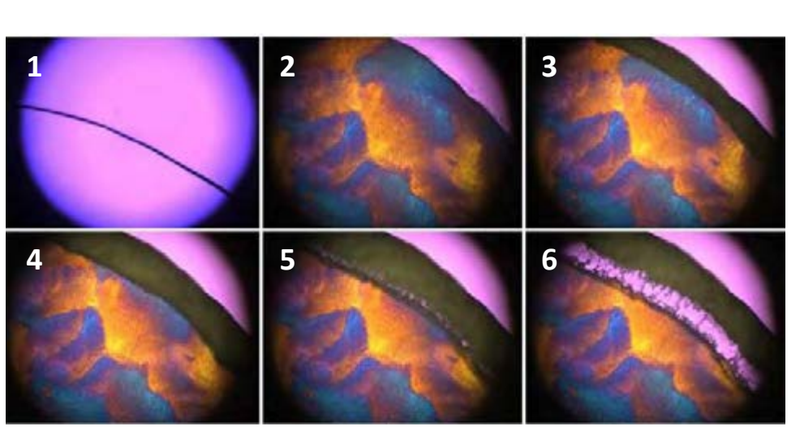
Above: illustration of design space

Scale, don't fail!

Configured with the latest innovations in freeze drying technology, the LyoStar® 4.0 is the most effective choice for formulation screening, cycle development, optimisation and scale-up. Delivering pinpoint process control and reliability to protect your most valuable products, the exclusive combination of Line of Sight™ technologies and robust software meets the needs of the most demanding lyophilisation workflows.

Lyostat5 Freeze Drying Microscope (FDM)

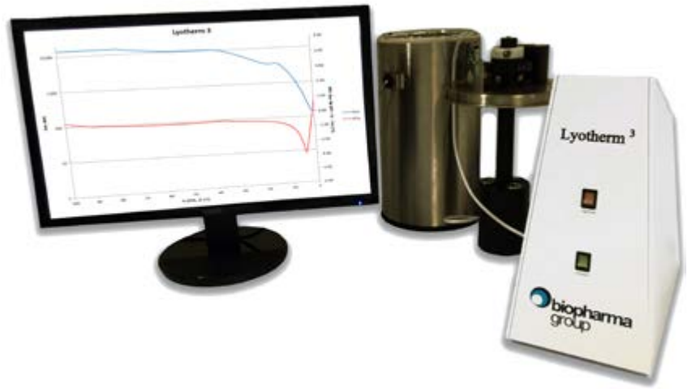
Essential Lyophilisation parameters at your fingertips Lyostat5 is our cutting-edge freeze drying microscope (FDM) system, enabling quick and easy identification of a formulation's collapse temperature. Product collapse is not always clearly visible and yet can lead to severely reduced activity, inefficient reconstitution and shorter shelf life. Identification of these critical temperatures can help redesign the cycle to be more robust, more efficient, and provide validation evidence of the success of the cycle.



Left to right: still images from a Lyostat analysis – the expanding black line indicates product drying with good structure, and images 5 and 6 show product collapse as the temperature is raised; the Lyostat5 instrument.

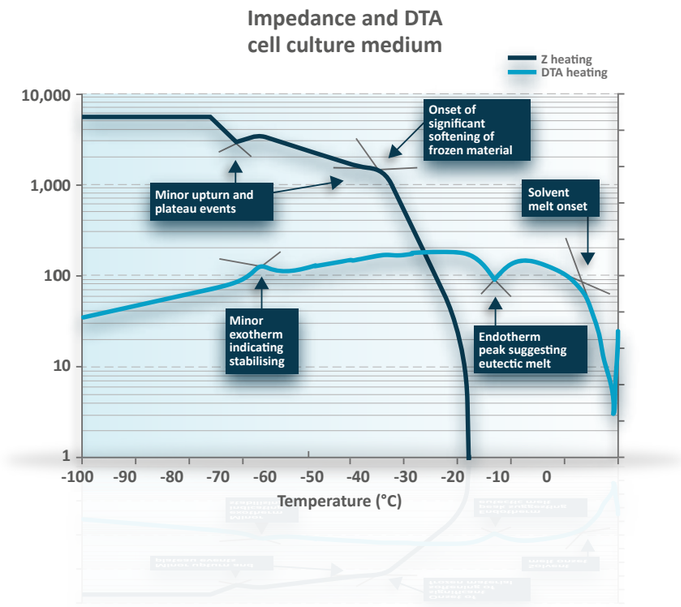
Lyotherm3 Frozen State Analyser

Simple but specific analysis for freeze drying



The Lyotherm3, developed by Biopharma Group in collaboration with lyophilisation specialist Professor Louis Rey, enables you to do two analyses at the same time, by providing you with a Differential Thermal Analyzer (DTA) and electrical impedance capability in a single combined unit.

Introduction to Freeze Drying Essential for any scientist currently involved with freeze drying development work, Lyotherm3 gives a more complete picture of thermal events occurring within your product than conventional thermal analysis. Impedance Analysis (ZSinc) is a fixed frequency dielectric analysis; it can provide an indication of the molecular mobility of a sample in its frozen state which could be attributed to changes within the formulation. The DTA measures the difference in temperature between a sample and a reference as heat is applied or removed from the system; this allows determination of the temperatures of significant endothermic or exothermic events e.g. crystallisation.



Above: explanation of the events discovered by Lyotherm analysis.

Glossary of terminology

Ablation

Entrapment and blow-out of product with the vapour flow is termed ablation. This is most common in formulations which have not produced a cohesive cake but remain loose and friable.

Absorption

A process in which one substance permeates another.

Adsorption

Adhesion of the molecules of gases, liquids, or dissolved substances to a solid surface, for example to the freeze dryer or container.

ALUS

Automatic Loading and Unloading Systems. Product, typically in vials, placed in a freeze dryer for freeze drying and removed after completion of the cycle entirely by machine, reducing the cost, contamination and safety implications of loading/unloading by hand.

Amorphous

A solid which is not crystalline. An amorphous solid may also be called a glass.

Ampoule

A small glass vessel in which liquids, mainly for injections, are hermetically sealed. Freeze drying can be performed in ampoules, usually attached to a manifold as the ampoules have a round bottom and therefore will not stand up on a shelf.

Annealing

A process involving controlled heating and cooling of a solution to encourage crystallisation.

Aqueous

Containing water; a solution in which the solvent is water.

Aseptic

Free from any living organisms.

Back-filling

After freeze drying the chamber can be filled with an inert gas, i.e. nitrogen, prior to stoppering. This prevents the product oxidising while in storage and also reduces the potential loss of product on opening the vial to atmosphere.

Backstreaming

When oil and diffusion pumps are used in low-pressure systems, vapours from the pump can sometimes migrate back into the chamber and into the product. Bulk

Product can be freeze dried in a variety of formats, including ampoules, vials and in bulk. For liquid product this generally entails being poured into stainless steel or plastic trays.

Bulking agent

A substance that is added to a formulation to increase solute density.

Cake

Freeze dried product in a vial is sometimes called a cake or plug.

Chamber

The product or drying chamber is the section of the freeze dryer in which the shelves are situated and the product is placed during freeze drying. The condenser chamber contains the process condenser and is a separate vessel, linked by a valve. In some freeze dryers the condenser is situated in the same chamber as the shelves.

CIP

Clean-In-Place. A method of cleaning the interior surfaces of equipment, such as pipework, chamber surfaces and fittings, without disassembly. See also SIP.

Cleanroom

A room designated for sterile research or production, in which the amount of airborne particulates, moisture, etc. is precisely controlled.

Collapse

The failure of a frozen product to maintain its structure, due to an unsuitable product temperature during sublimation. Collapse temperatures are often gradual in onset and can be difficult to measure.

Condenser

The part of the freeze dryer that traps the moisture that has been expelled from the product and holds it as ice. It also provides the driving force for the sublimation of the solvent.

Critical temperature(s)

The temperatures that reflect the points at which key changes occur in a solution, for example freezing point and collapse/eutectic temperature.

Cryoprotectant

A substance that is added to a formulation in order to protect the active ingredients during the freezing stages.

Crystalline

A structure that is crystalline has a uniform molecular structure.

Cycle

A freeze drying cycle describes operationally the entire freeze drying process. The control of parameters such as shelf temperature, condenser temperature, and chamber pressure (vacuum) is described in a stepwise manner. The aim is to precisely record the process of producing a satisfactorily dried product so that it can be repeated. Freeze drying cycles are programmed directly into a freeze dryer (or freeze dryer control system) and recorded electronically for recall whenever required.

Dehydration

The removal of water from a solution.

Defrosting

The process of removing the ice from the condenser by melting or mechanical means. Many freeze dryers contain hot air or water systems to speed up defrosting, especially in systems with large condensers which will hold a lot of ice.

Denature

To alter the structure of a molecule so that its biological activity is disrupted or entirely lost.

Drying

The removal of solvent from a solution.

Primary drying: the first drying stage of the freeze drying process, involving sublimation of mobile (rather than adsorbed) ice molecules.

Secondary drying: the second drying stage of the freeze drying process, which aims to remove (or desorb) the water molecules that were adsorbed.

Drying front

The freeze drying front describes the moving interface within a frozen product where the drying (sublimation) is occurring. Drying starts from the outer edge of the ice and progresses inwards, with the product in the bottom and centre of the container drying last. The freeze drying front can be viewed in real-time using a freeze drying microscope.

Eutectic point

The temperature at which a crystalline solid melts. Very few solids are purely crystalline, therefore, it is more relevant to talk about glass transition and collapse temperatures for most formulations.

Evaporation

The transformation of a liquid into a gas.

Excipient

A substance that is added to a formulation to provide benefits during the processing of the active ingredient, i.e., to increase critical temperatures or provide protection. Any component of a finished dosage form other than the active ingredients.

Flask

Freeze drying can be carried out in specially designed flasks. They are typically round- bottomed and fitted with a rubber lid which attaches to the manifold of a freeze dryer. They are most used in the research stage of drug development.

Free water

Water in a solution that is not chemically or physically bound.

Freeze drying

The process of drying a material by first freezing it and then encouraging the ice within it to sublimate, also known as lyophilisation.

Freezing

The solidification of a liquid, usually with the removal of heat. A product must be thoroughly frozen for freeze drying to take place effectively. The freezing point is the temperature at which a solution crystallises.

Glass

A solid which has a non-crystalline (amorphous) structure. The temperature at which the glass first exhibits a change in viscosity is termed as the glass transition temperature. GMP Good Manufacturing Practice. The FDA's guidelines on production for various industries, including Biotech and Pharmaceutical. cGMP stands for Current Good Manufacturing Practice and GAMP for Good Automated Manufacturing Practice.

Impedance

A function of capacitance, resistance and inductance that allows detection of changes in the molecular mobility of a product in its frozen state.

Lyophilisation

An alternative name for freeze drying.

Lyoprotectant

A substance that is added to a formulation to protect the active ingredients. Note that lyoprotectants protect during the drying stages whereas cryoprotectants protect during the freezing stages.

Manifold

On a freeze dryer the manifold is a pipe leading up from the condenser and typically branching into four, eight or twelve branches. Flasks or ampoules with pre-frozen material are attached to the manifold for drying. Manifolds can be 'drum', wide enough to accommodate small shelves or trays, or 'vertical', which can hold only flasks.

Matrix

The system of crystals in a frozen product is called the ice matrix or frozen matrix. The structure created and retained by the product after freeze drying is called the freeze dried matrix.

Meltback

A less common name for collapse and eutectic melting.

Melting

The change from a solid to a liquid. The Melting Point is the temperature at which this occurs. Note that the melting point and the freezing point are not necessarily the same (freezing is a random process).

Non-aqueous

A solution that does not contain water.

Nucleation

The process by which (ice) crystals form. When a product first starts to freeze, crystals initially form on particles in the formulation or cluster around existing crystals, and the ice matrix expands outwards from these nuclei.

OME

Oil Mist Eliminator. Oil mist escapes from the exhaust port of rotary vane pumps when in operation. An OME retains this oil in the exhausted gases.

Phase diagram

A graph which shows how the phase (solid, liquid, gas) of a substance is related to temperature and pressure.

Process development

Researching the relevant properties of a product and the processes by which it is obtained, and attempting to refine and streamline production to increase efficiency and improve effectiveness.

Product

A generic term referring to whatever is to be freeze dried. In pharmaceuticals or biotechnology, 'product' can refer to either the complete formulation or just the active ingredient.

Reconstitution

Adding a solvent to a freeze dried product to return it to its original wet or liquid condition.

Recrystallisation

The growth of large crystals at the expense of smaller crystals.

Refrigerant

The refrigeration system in a freeze dryer cools both the shelves and the condenser. The most common refrigeration systems in freeze drying are cooling by liquid nitrogen – an open system, where the refrigerant is used once and lost to the atmosphere; and mechanical refrigeration, utilising a compressor. Different refrigerants have different thermodynamic properties and may be better or worse suited to different applications. Common refrigerants used in freeze dryer compressors include R408b ('Suva 95'), and R404a ('Suva HP62').

Resistivity

The ability of a material to resist the flow of an electric current through itself. Changes in the structure of a substance as its temperature is raised or lowered will affect its resistivity. In freeze drying analysis resistance is usually measured in ohms and resistivity is derived by calculation.

Shelf life

The length of time a product can be stored before it degrades beyond usefulness.

Shell freezing

A method of pre-freezing product in flasks, prior to freeze drying. Flasks are placed in a chilled bath and rotated, forming a shell of frozen material around the inside of the flask in either a conical or cylindrical shape, depending on the type of equipment. Shell freezing provides a high surface area for sublimation, meaning that a larger volume of product can be processed than if it were simply poured into the bottom of the flask.

Shelves

In most freeze dryers the shelves function as heat exchangers, removing heat from the product during freezing and supplying heat back to the product during drying. They are chilled or heated by means of controlled fluid circulating through them. Stoppering systems, where fitted, require the shelf stack to be collapsible under hydraulic or some other power. Shelves can be manufactured up to 4 square metres.

SIP

Sterilise-In-Place. A method of sterilising the interior of equipment, such as pipework, chamber surfaces and fittings, without disassembly. SIP tends to use superheated steam, whereas CIP uses chemicals.

Skin

A layer found on the surface of some freeze dried products caused by a concentration of solute. This can be a result of a high concentration of solute excluded by the ice matrix during freezing. Often relatively impermeable, the formation of a skin impedes the drying of the product below it. Also known as a crust.

Sorption

The taking up of and retention of one substance by another, either absorption or adsorption.

Stopper

Stoppers are ventilated rubber lids for vials. A stopper is partially seated into the vial neck to allow water vapour to escape during drying, and fully inserted at the end of the process to fully seal the vial. 'Stoppering' is usually a function carried out by the freeze dryer while a vacuum or partial vacuum is still present, to help preserve the product.

Sublimation

The change from a solid into a gas without passing through the liquid phase. In freeze drying, the majority of sublimation takes place during the Primary Drying phase.

Supercooling

The process of cooling a liquid below its freezing point, without it becoming a solid. Without a nucleation point for a frozen structure to form around, the liquid phase can be maintained at a much lower temperature.

Thermal treatment

Another name for annealing.

Trays

Trays are used to hold product that is to be loaded onto freeze dryer shelves. Trays can be manufactured in a variety of materials including stainless steel, aluminium, plastic or glass. Trays are ideal for product that is to be freeze dried in bulk, either as a liquid or other format (for example drying of food products). For product in vials the trays can be made in two parts; an outer ring that keeps the vials in place, and a removable bottom. Once the vials are on the shelf the bottom half is removed, allowing the vials to sit directly onto the shelf and thereby allowing better heat conduction.

URS

User Requirement Specification. A document issued by the customer detailing their requirements.

Vacuum pump

All freeze dryers require at least one vacuum pump to function. The most commonly used vacuum pumps in freeze drying are rotary vane pumps or dry pumps.

Vapour pressure of ice

Temp	Vapour Pressure			Temp	Vapour Pressure		
(°C)	(milliTorr)	(milliBar)	(pascal)	(°C)	(milliTorr)	(milliBar)	(pascal)
0	4,584.000	6.1115	611.148	-46	48.000	0.0640	6.399
-2	3,883.000	5.1769	517.689	-48	37.700	0.0503	5.026
-4	3,281.000	4.3743	437.429	-50	29.500	0.0393	3.933
-6	2,765.000	3.6864	368.635	-52	23.000	0.0307	3.066
-8	2,325.000	3.0997	309.974	-54	17.900	0.0239	2.386
-10	1,949.000	2.5984	259.845	-56	13.800	0.0184	1.840
-12	1,630.000	2.1731	217.315	-58	10.600	0.0141	1.413
-14	1,359.000	1.8118	181.185	-60	8.100	0.0108	1.080
-16	1,130.000	1.5065	150.654	-62	6.160	0.0082	0.821
-18	936.800	1.2490	124.896	-64	4.660	0.0062	0.621
-20	774.400	1.0324	103.245	-66	3.510	0.0047	0.468
-22	638.200	0.8509	85.086	-68	2.630	0.0035	0.351
-24	524.300	0.6990	69.901	-70	1.960	0.0026	0.261
-26	429.400	0.5725	57.248	-72	1.450	0.0019	0.193
-28	350.500	0.4673	46.729	-74	1.060	0.0014	0.141
-30	285.100	0.3801	38.010	-76	0.780	0.0010	0.104
-32	231.200	0.3082	30.824	-78	0.570	0.0008	0.076
-34	186.800	0.2490	24.905	-80	0.410	0.0005	0.055
-36	150.300	0.2004	20.038	-82	0.290	0.0004	0.039
-38	120.600	0.1608	16.079	-84	0.210	0.0003	0.028
-40	96.300	0.1284	12.839	-86	0.150	0.0002	0.020
-42	76.700	0.1023	10.226	-88	0.100	0.0001	0.013
-44	60.800	0.0811	8.106	-90	0.072	0.0001	0.010

Vapour pressure over ice chart

Vial

Small, flat-bottomed glass bottle with a short neck. They are usually fitted with a stopper. Vials are perhaps the most convenient and certainly the most common container used for parenteral products. Vials are available in a wide range of sizes and dimensions.

WFI

Water For Injection. Water that has been purified to a high degree, for use particularly in drugs that are to be injected.

About Biopharma Group

Biopharma Group has dedicated divisions covering the UK, France, Ireland, and USA. We meet the needs of our customers' projects appropriate to the size and stage by augmenting their in-house expertise whether to buy equipment, a single cycle run/analysis or a full formulation development program.

Equipment Sales & Service

www.biopharma.co.uk

Established in 1989, this division is a leading supplier of equipment to the pharmaceutical, biotech process industries, specialising in freeze drying & associated technologies from freeze dryers, solvent evaporation systems & aseptic fill-finish processing lines to high pressure homogenisers, cryopreservation storage solutions, analytical & preparative HPLC systems, and airflow lab equipment such as microbiological safety cabinets, LAF cabinets and fume hoods. The key to our success, is many years of expertise in the processing industries plus in-depth knowledge of the equipment we supply. Additionally, we have an experienced technical service department enabling us to provide ongoing maintenance and support for the working life of your equipment.

CDMO Services

www.biopharmagroupcdmo.com

Biopharma Group has over 30 years of practical experience in freeze drying, spray drying & liquid formulations, with highly experienced teams dedicated to R&D, production and analytical lyo lab services. We have successfully completed over 5,000 projects, working on a wide range of products including small molecules and APIs, biopharmaceuticals, proteins, therapeutic delivery systems, diagnostics, whole organisms, cells and bacteria, vaccines, antibodies, blood components and food to name a few. Biopharma Group is your strategic CDMO choice with services able to be categorised in to three key areas:

- Contract R&D
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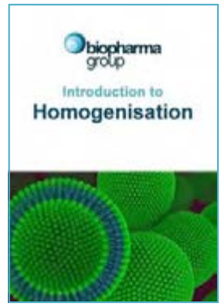
- Consultancy & lyo training courses.

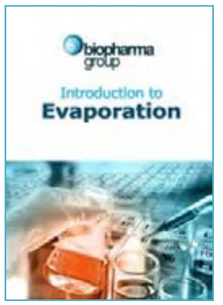
Biopharma Technologies France (BTF) offers a combined proposition of equipment sales of Biopharma Group with the addition of dissolution & absorption solution from Pion Scientific plus the CDMO services expertise giving our French-speaking clients a full end-to-end solution. www.biopharmatech.fr

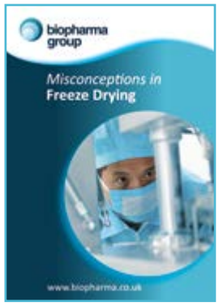
Other Booklets

These introductory booklets are designed to be a helpful reference for anyone new to the fields.

Click on the information guides below to download.







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Introduction to Freeze Drying

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