

## LYOPHILIZATION – THE PROCESS AND INDUSTRIAL USE

*This article presents a general overview of lyophilization and discusses the underlying principles of the process through the basics of: formulation, freezing, primary drying and secondary drying. In this article lyophilization is defined as a stabilizing process in which the substance is first frozen and then the quantity of the solvent is reduced first by sublimation (primary drying) and then by desorption (secondary drying) to values that will no longer support biological growth or chemical reactions. Special mention was made of the industrial use of the process and emphasis was placed on the lyophilization of pharmaceutical products and food industry products. Lyophilization equipment, as well as the formulation of materials that can be lyophilized, are described in sufficient detail to give information on the restrictions and advantages of lyophilization. Processing economics and comparison with conventional drying methods are presented. A historical overview of the process and future developments presented from the industrial viewpoint give an insight on the previous application of lyophilization and the prospects of its broad industrial use.*

### INTRODUCTION TO FREEZE DRYING TECHNOLOGY

#### Historical Overview

Historically, lyophilization can be traced back to prehistory when, for example, the Eskimo preserved fish by dehydration in the cold, dry Arctic winds.

The history of lyophilization as an industrial process is surprisingly recent. Although Altmann [1] used lyophilization for the preparation of histological sections as early as 1890, his technique went unnoticed for over 40 years. Shackell [2] independently rediscovered the technique in 1909 for the preservation of biologicals.

The industrial applications of lyophilization do not appear to have been appreciated until when there arose the need to process large quantities of heat-sensitive blood products and the recently discovered antibiotics. The efforts of Tival in 1927 and Elser in 1934 were rapidly followed by the important contributions of Flosdorf in the United States and Greaves in England who were largely responsible for making large scale applications of lyophilization possible. Stimulated particularly by a series of symposia in England and the United States and the renowned courses on lyophilization organized by Rey in France, the atmosphere in the 1950s and early 60s was one of optimism for the future of lyophilization, particularly in its application to foodstuffs [3].

Three serious practical disadvantages of freeze preservations:

1. the high cost of transporting these stocks of frozen product,
2. the associated high cost of transporting these stocks and
3. the potential for total loss of material due to failure of the freezing plant,

have encouraged the adoption of drying methods. Conventional drying techniques, using high process temperatures, while successful in dehydrating material often result in alterations of the organoleptic properties, a reduction in nutritional value or a decrease in biological activity in the case of an enzyme, hormone etc. Lyophilization is a well established alternative technique used to preserve labile materials often, but not exclusively, of biological origin [4–6].

With advances in vacuum and refrigeration technologies reliable commercial freeze-dryers were able to be developed [7]. Since that time the method has become fully integrated within the pharmaceutical industry, and is particularly useful for establishing an early lead when marketing a novel therapeutic agent.

#### Definitions

There are two terms used in industry which describe the same process but in a different way: Freeze drying and lyophilization, which means 'to make solvent-loving'.

Rey [8] first came up with the term lyophilization by taking into account the porous nature of the dried product and its "lyophil" characteristic to rapidly reabsorb the solvent and restore the substance. The term lyophilization has become more common because it is applicable to both aqueous and non-aqueous systems. It is interesting that lyophilization processes are often conducted in lyophilization equipment, although the descriptive term lyophilizer is becoming more prevalent. In this article, the process will be defined as lyophilization, and the equipment will still be referred to as a freeze-dryer.

Although the steps in the lyophilization process outlined by Rey [8] have been generally accepted, only recently has a definition been given to the term, lyophilization [9]. In its simplest form, lyophilization is defined as a stabilizing process in which the substance is first frozen and then the quantity of the solvent is reduced first by sublimation (primary drying) and then

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by desorption (secondary drying) to values that will no longer support biological growth or chemical reactions [10].

### The advantages of Lyophilization

Apart from the reduction in thermal inactivation of the product during freezing as noted above, other advantages of the method are:

- The avoidance of concentration effects – for example salting out of proteins. The distribution of components within the drying and dried product remain unchanged throughout the process.
- The water content of the dried product can be reduced to very low levels. In general the lower the water content the more stable the product, although overdrying may reduce product stability.
- Since the product is normally sealed under vacuum or inert gas, oxidative denaturation is greatly reduced.
- Loss of water equates to a loss of product weight and this may be important where transport costs are significant.
- Lyophilization operates in a more controlled environment than some other forms of drying, which potentially means less possibility for contamination.

Because freeze material has a porous structure, the product can be instantly reconstituted by the addition of a simple diluent [11].

### Materials That Can Be Lyophilized

The major types of material processed by lyophilization have been summarized by Adams [12]:

1. Non-biological where the process is used to dehydrate or concentrate reactive or heat labile chemicals
2. Non-living bioproducts – this comprises the major area of application and includes:
  - Enzymes, hormones, antibiotics, vitamins, blood products, antibodies, inactivated vaccines, etc. This sub-group includes pharmaceuticals which may be used diagnostically or therapeutically
  - Bone and other body tissues for surgical or medical use
  - Foodstuffs where organoleptic properties are important
  - Industrially useful bio products
3. Living organisms – where reconstituted cells after drying must be able to grow and multiply to produce new progeny. Examples include bacteria and fungi used as seed cultures or attenuated viral vaccines.
4. Miscellaneous – Flood damaged books, museum artifacts, etc.

Lyophilization is less suitable for materials which supercool to form glasses, for products which form impervious surface skins upon cooling, thereby inhibiting the evolution of subliming vapour, and is unsuitable for certain cell types (eukaryotes) which are able to retain viability, when frozen, only in the presence of special additives incompatible with the lyophilization.

## THE PROCESS

The following is a general overview of the lyophilization process. The intent of this section is merely to familiarize the reader with the basic steps in the process.

### Formulation

A formulation is defined as any system containing a solvent that, upon its removal, will enhance the stability of the substance.

Formulation preparation is often neglected when discussing lyophilization. It is important to remember that lyophilization is invariably the ultimate step in a preparation exercise and one should realise that lyophilization will never rectify damage caused during preparation but will rather exacerbate any such damage. Foodstuffs should be of good quality and may require special pretreatment. For example, muscle tissue should be sliced in such a way as to minimise the impedance to vapour flow offered by skins and membranes, while vegetables may require blanching to prevent oxidative browning. Solutions intended for pharmaceutical use generally consist of an active constituent and possibly other constituents such as protective additives that are added to stabilize the formulation in its liquid state for therapeutic reasons. Pharmaceutical formulations will require dispensing into suitable containers prior to freezing [13].

Containers must be regarded as an integral part of the processing equipment since their nature and design will influence heat transfer and impede vapour flow to a greater or lesser extent.

Foodstuffs, chemicals, bulk pharmaceuticals, etc may be dispensed into aluminium, teflon coated or stainless steel trays. While metals have superior thermal conductive properties compared to plastics, thin walled plastic trays are nevertheless becoming increasingly popular for processing bulk materials.

Pharmaceutical solutions are usually dispensed into glass vials or ampoules. Vials are small bottles fitted with a special ventilated stopper which permits the escape of water vapour during drying (Figure 2). At the end of the process the stoppers can be fully inserted into the vial using the stoppering device within the freeze-drier (See Section **Container-Closure System**) to produce a completely sealed container. While vials are particularly convenient for filling and lyophilization, the vial stopper hybrid has the potential to leak between the vial neck and stopper [14]. The all-glass ampoule theoretically overcomes the potential for leakage since the ampoule tip is heat fused at the end of drying. Practical difficulties associated with heat sealing ampoules within the freeze-drier require that the ampoules are sealed externally and complicated techniques must be adopted to prevent the ingress of damp air into the ampoule during sealing. Cammack and Adams [15] recommended that vials are used for products requiring only short term storage while

ampoules are more satisfactory where long term storage is essential. One should be alert to the possibility that poor heat-sealing can cause ampoule leakage during storage [16].

Ampoules and vials should be manufactured from good quality glass and ideally should be fabricated from glass tubing. The walls and bases should be oven and stress-free to enable a consistent product freezing and drying pattern to be obtained with minimum breakages due to thermal shock during prefreezing. Light sensitive products may require dispensing into amber or coloured vials prior to processing.

Rubber stoppers should be manufactured from ethically acceptable polymers and are usually lubricated with silicone oil as an aid to insertion. Because of the potential for silicone fluid to cause product denaturation, stoppers coated with alternative lubricants (such as teflon) have recently been introduced. The washing and pretreatment of stoppers should be carefully controlled prior to filling, since it is possible for stoppers to absorb significant quantities of water during washing and sterilization. Sufficient water may remain within the stopper even after lyophilization to cause product collapse and reduced stability upon storage. Product collapse is the loss of structure or the noticeable shrinkage of product observed in a vial during or following lyophilization. In extreme cases collapse will result in the formation of an amorphous, bubbled, glassy residue within the vial. Collapse may be confused with melting, although the two phenomena are quite distinct.

Recently, novel containers have appeared for specialist use. Sealed containers, holding both a plug of dried product and a quantity of diluent can now be processed enabling the clinician to reconstitute the freeze-dried material with simplicity. Such containers minimise the risk of product contamination or the mismatching of drug with diluent during reconstitution. Small beads and plastic wells holding type – specific sera, adhesive plasters impregnated with wound-healing freeze-dried hormones, nasal inhalers containing freeze-dried vaccines or anti-congestants and instantly dissolving tablets are among the range of novel containers and techniques being used for the processing and distributing freeze-dried products.

Lyophilization in glass containers will serve as the basis for an overview of the process, as the intention is to emphasis the use of the process in the pharmaceutical industry, where lyophilization in glass containers prevails [17].

### Freezing

The principal function of the freezing process is:

- to separate the solvent from the solutes.
- to minimise thermal degradation within the dispensed product.
- to immobilise the components in the solution thereby preventing their concentration during drying.

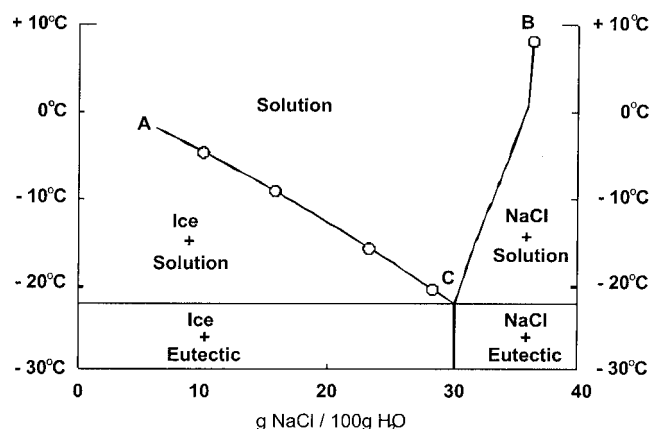


Figure 1. The eutectic phase diagram of sodium chloride solution [18]

- to prevent product foaming when vacuum is applied.

For an aqueous system, the water will form ice crystals and solutes will be confined to the interstitial region between the ice crystals. The temperature necessary to achieve complete freezing of the formulation will depend on the nature of the solvent and other constituents that comprise the formulation. Freezing may be performed in an external freezing unit or on the shelves of the freeze-dryer.

An example of the frozen ice-product matrix is given in Figure 2b. The freezing process is an important step in the lyophilization process.

Ideally prefreezing should result in the complete crystallisation of both the solvent (usually water) and the solutes within the solution. When discussing the formulation, we shall see that this idea is seldom achieved in practice. Figure 1 describes the eutectic phase diagram of an aqueous sodium chloride solution which could be translated into a description of events occurring during prefreezing. (The eutectic point is the temperature in a system at which a residual liquid phase is in equilibrium with the solid phases (Figure 1). In this case, cooling from room temperature to 0°C is followed by supercooling prior to the crystallisation of pure water as ice, thereby resulting in a concentration of the sodium chloride solution. Reducing the temperature still further will increase the concentration of the salt solution during the eutectic transition. A second supercooling inflexion is followed by the crystallisation of the remainder, of the water and the salt at the eutectic point, resulting in a mixture of ice and salt crystals.

### Rates of Freezing Prior to Sublimation

When considering the cooling rates obtained on the shelves of the freeze-dryer, one must appreciate that cooling proceeds from the base of the vial upwards through the depth of the liquid. In this case it is useful to regard freezing rates as depths of liquid frozen, in millimeters per minute. The optimal rate of cooling prior to sublimation is 1mm/min and such a freezing rate will

induce the correct ice crystal formation necessary to permit water vapour to escape freely from the frozen matrix. At freezing rates below 0.5 mm or much greater than 1.0 mm per minute, the structure of the ice matrix will be such that vapour flow will be impeded during sublimation and drying times significantly extended. It is not possible to achieve this optimal cooling rate when the depth of the fill in the vial greatly exceeds 10 mm and this depth should therefore be regarded as the maximum fill depth. Liquid foodstuffs, such as coffee, are often spray or droplet frozen prior to lyophilization.

#### Problems Associated With Incomplete Freezing

Incomplete freezing may result as a consequence of mechanical failure of the shelf or cabinet cooling system or a failure to allow an adequate prefreezing time to ensure complete product freezing. Incomplete freezing can also result from an inability to cool the product below an eutectic temperature. Suspensions of micro-organisms often contain several electrolytes and should be cooled below the lowest eutectic temperature to ensure complete freezing. It is imperative that a vacuum is not applied to an incompletely frozen product since foaming may occur resulting in an unacceptable product.

#### Primary Drying

Once the formulation has reached a completely frozen state, the pressure in the freeze-dryer is reduced,

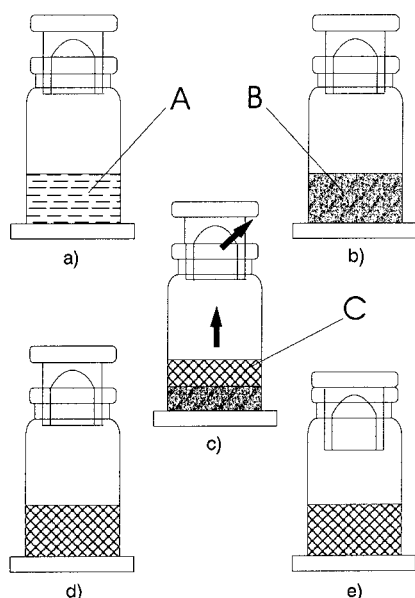


Figure 2. Lyophilization in glass containers [10]

Fig. 2a) shows a fill volume of a liquid formulation, denoted by the region defined as "A" in a glass container with a lyophilization closure positioned for the drying process; in Fig. 2b) The frozen ice-product matrix of the formulation is designated by the region "B"; Fig. 2c) illustrates the primary drying process and the interstitial cake portion is denoted as region "C"; the completion of secondary drying is shown by Fig. 2d), and the final product with the closure in its stoppered position is illustrated by Fig. 2e).

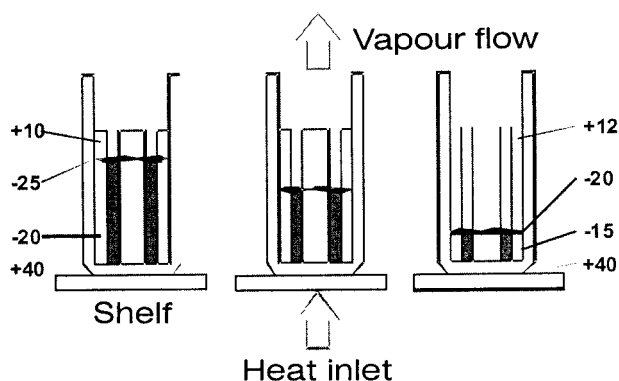


Figure 3. Sublimation

and heat is applied to the formulation to initiate sublimation of the ice crystals. The application of vacuum allows the free migration of water vapour from the frozen mass. The migration of water from the product may be regarded basically as a diffusion process and this will help to appreciate why lyophilization is a relatively slow process. The sublimating solvent vapors pass through the opening in the closure. As the sublimation of the ice crystals proceeds, the ice-gas interface recedes through the cake (Figure 2c). As there are latent heat losses during sublimation, heat must be applied to the product throughout primary drying. In the absence of heat, the product temperature would reach a level at which lyophilization would cease. It is important that heat is not applied until a vacuum of c.20 Pa has been attained or product melting may occur. In the case of pharmaceutical application of the process the vials are placed on shelves which facilitates heat exchange as shown in Figure 3.

Throughout most of the primary drying cycle the product will remain at a significantly lower temperature than the shelf because of heat loss with water vapour escape from the product and only toward the end of primary drying, when sublimation has virtually ceased, will the product temperature rapidly rise to that of the shelf (Figure 4). Completion of the primary drying process occurs when all of the ice crystals have been removed from the formulation and the volume occupied by the resulting cake is equivalent to that of the frozen

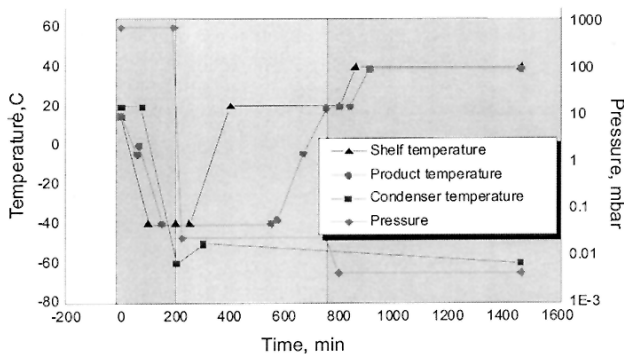


Figure 4. Idealised lyophilisation cycle

matrix. Only water adsorbed to the bulk powder or structurally integrated with the solute will remain. The product could be removed from the drier at this stage. In practice, since the water content is usually too high, 5–10% w/w for optimal stability, the drying cycle is extended.

### Secondary Drying

After the completion of primary drying (Figure 1.1d), there will still be some water adsorbed onto the surface of the cake. This moisture may constitute, depending on the temperature and the nature of the constituents comprising the cake, 5–10% w/w of the dried product. In many cases, such moisture values may be too high, and the final product may not have the desired stability. The desired stability is obtained by reducing the moisture content in the product by desorbing the moisture from the cake without reducing the volume of the interstitial cake. This prolonged drying stage is called secondary drying, water being removed by desorption. The remaining water removal is usually accomplished by increasing the temperature of the product and reducing the partial pressure of water vapor in the container. During secondary drying the water content is reduced to 2% or less and various methods to determine the moisture content are used [19].

Sensitive biological materials may be inactivated by overdrying [20]. In older accounts of lyophilization methodology, it was often recommended that primary and secondary drying should be completed using separate vapour traps within the freeze-dryer or even completed on separate machines. The greater efficiency of vapour trapping systems in modern plants no longer require special secondary drying devices.

### Container-Closure System

After lyophilization, the formulation must be protected from the environment. In most cases, the formulation in the container is sealed by a stoppering mechanism contained in the freeze-dryer, which depresses closure into the container (Figure 2e). The stoppering of the closure into the container temporarily protects the final product from the environment. Upon completion of the stoppering of the containers, the product can be safely removed from the freeze-dryer and the stopper crimp-sealed with a metal or colored cap to provide a permanent seal for the product.

## THE BASIC DESIGN OF FREEZE-DRYERS

The following is a brief, general description of the essential components and their functions in a freeze-dryer. The general layout of a freeze-dryer is given in Figure 5.

### The Lyophilization Chamber

The freeze-dryer chamber serves two main functions: to provide a safe environment for the product during the entire lyophilization process and to provide

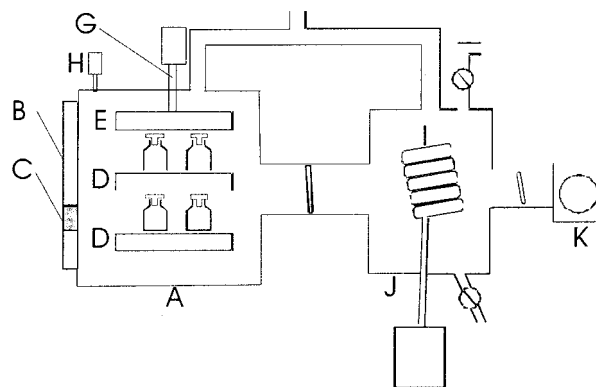


Figure 5. The general layout of a freeze-dryer

A is the drying chamber; B is the door to the drying chamber and C is a viewing port for a metal door. D represents the usable shelves of the dryer, while E is an unusable shelf. G represents the hydraulic ram system. H is the pressure gauge, I is the condenser surface housed in an insulated condenser chamber J, and K represents the vacuum pumping system.

the necessary temperatures and pressures to conduct each step of the lyophilization process.

The lyophilization chamber (A), as illustrated by Figure 5, is a metal vessel, generally constructed from stainless steel that can be accessed by a hinged door (B). The chamber door is either fabricated from metal such as stainless steel or from clear plastic. Figure 5 shows that the chamber is accessed by means of a metal door containing a glass viewing port (C). The door is fitted with an elastomer gasket (not shown) to form a vacuum seal with the drying chamber.

The drying chamber also contains a pressure gauge (H) and is equipped with an insulation covering over the entire chamber surface to prevent heat transfer to the shelves (D) and the trays (F) during the drying process.

### Shelves

A small research freeze-dryer may have only one shelf but all others will have several. The shelf design is made more complicated because of the several functions it must perform. The shelf acts as a heat exchanger, removing energy from the product during freezing, and supplying energy to the product during the primary and secondary drying segments of the freeze-drying cycle. Shelves must be flat and level to ensure there is proper contact with the product containers. The shelf construction must be heavy enough to ensure that a level surface is maintained and strong enough to support the forces of a stoppering system if fitted. All of the shelves are of a hollow construction that permits the serpentine flow of the heat-transfer fluid. The heat-transfer fluid can be chilled to freeze the product or heated to provide the necessary energy for the primary and secondary drying processes. A shelf stack may be fixed in to position where bulk products are handled or a hydraulic system (G) can move the shelves vertically to provide the necessary force to stopper the closures prior to opening the chamber door (B).

The shelves are connected to a silicone oil system through either fixed or flexible hoses.

The drying chamber presented in Figure 5 contains usable shelves (D) and an unusable shelf (F). The trays (F) containing the product, either bulk or in glass containers, are loaded onto the usable shelves. The unusable shelf (E) serves as a radiation shield for the top shelf.

### The Condenser Chamber

The main function of the condenser chamber is to house the condenser surfaces for the removal of water vapor from the gases that pass from the drying chamber. For the condenser plates to be effective, their operating temperatures must be a minimum of 20°C lower than the product temperature during the primary drying process. Unlike the shelves of the dryer, which are chilled by a heat-transfer fluid, the condenser surfaces are generally refrigerated by the direct expansion of a refrigerant.

Figure 5 illustrates an external condenser system in which the condenser surfaces (I) are housed in a separate insulated vacuum chamber (J). In some dryers, the condenser surfaces are housed in the drying chamber and are referred to as internal condensers.

### The Vacuum Pumping System

The vacuum pumping system, in conjunction with the condenser system, provides the necessary pressures for conducting the primary and secondary drying processes. Typically mechanical vacuum pumps used in freeze-dryers are oil lubricated, however, oil-free mechanical pumping systems are available. The vacuum pump (K) shown in Figure 5 compresses the non-condensable gases that pass through the condenser chamber (J) and discharges these gases directly into the atmosphere.

### Sensors

The temperature and pressure must be measured during the process [21]. The two parameters which are controlled are the fluid or shelf temperature and the pressure (or vacuum). Other temperatures which can be measured (during a lyophilization cycle) include product temperatures and the process condenser. The temperature measuring devices used are RTDs (PT 100) or thermocouples (normally Type T). Vacuum sensors include two main types – thermoelectric or Pirani gauges and capacitance manometers.

### Control System

Control may be entirely manual or usually fully automatic for the production machines. The control elements required are as mentioned above, shelf temperature and pressure plus time. A control programme will set up these values as required by the product or the process. The time may vary from a few hours to several days. Other data such as product

temperatures and process condenser temperatures can also be recorded and logged [22,23].

## APPLICATIONS

The following is a general discussion of the various applications of the lyophilization process in the various industries. The intent of the discussion is not to provide the reader with a comprehensive review, but with an overview of the scope of the lyophilization process.

### Healthcare Industry

The most extensive use of the lyophilization process is involved in the healthcare industry. This includes the lyophilization of pharmaceuticals such as chemical compounds, parenteral formulations, vaccines, and also in diagnostic products. Included in this category are biotechnology products that consist mainly of protein-based products [24,25]. A listing of pharmaceutical products given in Table 1 below is provided by courtesy of the Hemofarm Group and serves as an example of the variety of materials that are lyophilized for use in healthcare (Table 1).

Table 1. Materials that are lyophilized for use in healthcare

Product	Active Ingridient	Pharmacological Group
Lemod Solu <sup>®</sup>	Methylprednisolone	Corticosteroid
Hidrokortizon <sup>®</sup> (Hemol <sup>®</sup> )	Hydrocortisone	Corticosteroid
Azaran <sup>®</sup>	Ceftriaxone	Antibiotic
Fenobarbiton-Na <sup>®</sup>	Phenobarbitone	Antiepileptic
Nitroprusid-Na <sup>®</sup>	Nitroprusside	Vasodilator

### Veterinary

Perhaps the second most common use of lyophilization involves veterinary products. These products range from vaccines for individual household pets to large scale applications, such as the inoculation of herds of cattle and sheep or large flocks of poultry. These lyophilized products serve not only to protect the animals and the investment of the producer, but also to improve the quality of the product. These products also protect the consumer from diseases that may be transmitted by the consumption of animal products.

### Food

The most widely known freeze-dried food product is coffee. However, freeze-dried coffee is not, in the true sense, a lyophilized product. Its dark brown color stems not from the coffee solution but from the manner in which the product is prepared. The coffee is first extracted from the coffee bean with hot water and the water content is reduced to form a concentrated viscous extract. The extract is then carefully processed to form frozen granules. These coffee granules are then dried

under conditions that generate partial collapse or controlled meltback [26], which provide the granules with the dark brown color. If the coffee were truly lyophilized, the color would appear golden brown because of the reflection of light from the surfaces of the frozen coffee solids. In addition, lyophilized coffee would require a cup of lyophilized powder for each cup of coffee instead of the single teaspoon that is currently used. As a result of the application of the term freeze-dried to a form of instant coffee, lyophilization is no longer synonymous with lyophilization.

Other freeze food products are manufactured. These products may be used as additives, such as berries in breakfast cereals, or are complete products that eliminate the need for refrigeration, such as ice cream that can be eaten without reconstitution. The lyophilization of foods is also applicable for products where weight can be a factor, such as in backpacking. These freeze-dried meals, like freeze-dried beef, require reconstitution by the addition of water and enhancement of flavor by heating the product [27]. Except in special cases like freeze-dried coffee, freeze-dried foods cannot compete economically with frozen or canned food products.

#### Other Applications

Other applications of lyophilization include the lyophilization of floral products and taxidermy. There has only been limited success in the lyophilization of floral products; only the tile flower portion of the plant appears to respond to the drying process, and there is often a slight change in color. For example, red roses tend to take on a purple tint. The stems of the flowers tend to assume a brown tint, and the leaves become brittle and curled. In the drying of plants, the nature of the plant plays an important role. For example, cacti, in order to conserve water, are equipped with an outer layer that is nearly impervious to the transport of water vapor. A plant such as a cacti would prove difficult or nearly impossible to dry without first disturbing the outer layer to permit the transport of water vapor. While the preservation of flowers by lyophilization techniques could represent a major market, the growth of the market awaits the development of a better process. Pretreatment of the flower prior to lyophilization, when freshly cut or even during its growth, may be necessary in order to achieve a more natural product with a relatively long shelf life.

Lyophilization has been applied to the taxidermy of small animals. Many small mammals, such as squirrels and raccoons, have been successfully freeze-dried to provide a specimen having a lifelike appearance. In spite of the success of this technique, it is currently used on a very limited basis.

Lyophilization has been instrumental in the rescue of precious manuscripts, books, and documents that incurred water damage as a result of a fire or a flood. By quickly freezing all of the documents and then lyophilizing the documents in batches, many documents and valuable manuscripts have been restored to nearly original condition. While such services are not often

required, the fact that this technology has applications that can prove beneficial to later generations is indeed a rewarding legacy provided by this technology.

#### PROCESSING ECONOMICS

It is difficult to compare the economics of lyophilization accurately with conventional drying partly because of the problems of conducting trials using identical materials processed under similar conditions of scale and partly because the results of realistic trials would presumably be commercially sensitive.

#### Processing Time

Table 2 illustrates the comparative drying times for typical products spray, fluid bed or freeze-dried. The considerably increased processing time for lyophilization should be noted. Product capacity is further reduced since the depth of fill is insured to 1 cm for technical reasons.

Table 2. Temperatures and drying times for drying processes

Process		Typical Operating Temperatures	Drying Times
Spray Drying		80–100°C	< 10 min
Fluid Bed Drying		80°C	< 100 min
Freeze drying (1 cm product depth)	Milk	– 5°C	600 min
	Blood Serum	– 25°C	1200 min
	Cytomegalovirus Vaccine	– 40°C	3600 min

#### Equipment Capital Costs

Modern pharmaceutical freeze-driers are an amalgam of a refrigeration/vacuum/steam pressure plant and, consequently, equipment design and fabrication costs are high.

#### Operating Costs

In addition to the normal running costs involved with operating an industrial plant, the lyophilization process depends on the extraction or addition of energy to freeze, sublime and condense water vapour. This additional removal of latent heat increases the cycle of energy consumption considerably [28]. For a significant period of the cycle time during freezing, pharmaceutical freeze-driers are utilized only as a freezing cabinet thereby further reducing the economic efficiency of the plant. Incidental factors which increase production costs include the requirement to use high cost dosage containers and the additional costs of sealing the dried product.

#### Comparative Economics

From the limited data available, a cost comparison of processing foodstuffs would indicate a 5–8 fold

increase in drying costs between lyophilization and conventional drying [29]. However, the point should be made that when the entire process is compared, that is from raw material to finished product, this differential may be significantly reduced in favour of freeze drying and a finished product cost comparison of conventional drying: lyophilization of 1:1.33 may be feasible [30].

On purely economic grounds lyophilization is likely to compare unfavourably with alternative drying techniques and the process can only be justified on the basis of superior product quality.

### PHARMACEUTICAL USE OF THE LYOPHILIZATION PROCESS

The lyophilization process generally includes the following steps:

- Dissolving the drug and excipients in a suitable solvent, generally water for injection (WFI).
- Sterilizing the bulk solution by passing it through a 0.22 micron bacteria-retentive filter.
- Filling into individual sterile containers and partially stoppering the containers under aseptic conditions.
- Transporting the partially stoppered containers to the lyophilizer and loading into the chamber under aseptic conditions.
- Freezing the solution by placing the partially stoppered containers on cooled shelves in a freeze-drying chamber or pre-freezing in another chamber.
- Applying a vacuum to the chamber and heating the shelves in order to evaporate the water from the frozen state.
- Complete stoppering of the vials usually by hydraulic or screw rod stoppering mechanisms installed in the lyophilizers.

Figure 6 presents a typical flowchart of the pharmaceutical application of lyophilization used for manufacturing parenteral products in vials.

There are many new parenteral products, including anti-infectives, biotechnology derived products, and in-vitro diagnostics which are manufactured as lyophilized products. Additionally, inspections have disclosed potency, sterility and stability problems associated with the manufacture and control of lyophilized products. It is recognized that there is complex technology associated with the manufacture and control of a lyophilized pharmaceutical dosage form. Some of the important aspects of these operations include: the formulation of solutions; the filling of vials and validation of the filling operation; sterilization and engineering aspects of the lyophilizer; scale-up and validation of the lyophilization cycle; and testing of the end product.

#### Product Type/Formulation

Products are manufactured in the lyophilized form due to their instability when in solution. Many antibiotics, such as some of the semi-synthetic penicillins, cephalosporins, and also some of the salts of

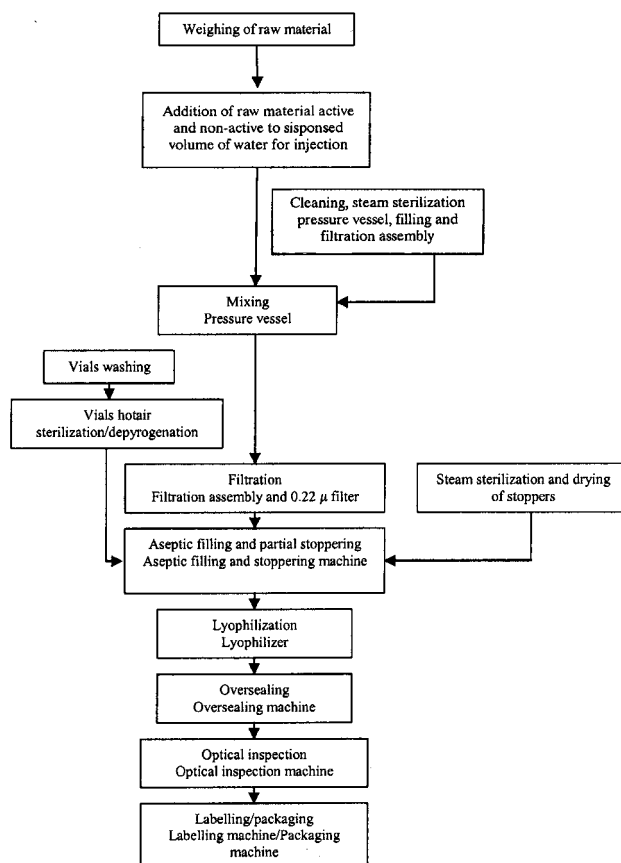


Figure 6. Typical flowchart of the pharmaceutical application of lyophilization – courtesy of the Hemofarm Group

erythromycin, doxycycline and chloramphenicol are made by the lyophilization process. Because they are antibiotics, a low bioburden of these formulations would be expected at the time of batching. However, some of the other dosage forms that are lyophilized, such as hydrocortisone sodium succinate, methylprednisolone sodium succinate and many of the biotechnology derived products, have no antibacterial effect when in solution.

For these types of products, the bioburden should be minimal and the bioburden should be determined prior to the sterilization of these bulk solutions prior to filling. Obviously, the batching or compounding of these bulk solutions should be controlled in order to prevent any potential increase in microbiological levels that may occur up to the time that the bulk solutions are filtered (sterilized). A concern with any microbiological level is the possible increase in endotoxins that may develop. Good practice for the compounding of lyophilized products would also include batching in a controlled environment and in sealed tanks, particularly if the solution is to be held for any length of time prior to sterilization.

#### Filling

The filling of vials that are to be lyophilized has some problems that are somewhat unique. The stopper

is placed on top of the vial and is ultimately seated in the freeze-dryer. As a result, the contents of the vial are subject to contamination until they are actually sealed.

Typically, vials to be lyophilized are partially stoppered by machine. However, some filling lines have been noted which utilize an operator to place each stopper on top of the vial by hand.

Once filled and partially stoppered, vials are transported to the lyophilizer by metal trays and loaded into the lyophilizer. The transfer and handling, such as loading of the lyophilizer, should take place under primary barriers, such as laminar flow hoods under which the vials were filled.

### Lyophilization Cycle and Controls

After sterilization of the lyophilizer and aseptic loading, the initial step is freezing the solution. In some cycles, the shelves are at the temperature needed for freezing, while for other cycles, the product is loaded and then the shelves are taken to the freezing temperature necessary for product freeze. In those cycles in which the shelves are precooled prior to loading, there is concern for any ice formation on shelves prior to loading. Ice on shelves prior to loading can cause partial or complete stoppering of the vials prior to lyophilization of the product. A recent field complaint of a product in solution and not lyophilized was attributed to preliminary stoppering of a few vials prior to exposure to the lyophilization cycle. Unfortunately, the firm's 100% vial inspection failed to identify the defective vial.

Typically, the product is frozen at a temperature well below the eutectic point.

It is desirable after freezing and during primary drying to hold the drying temperature (in the product) at least 4–5°C below the eutectic point. Obviously, the manufacturer should know the eutectic point and have the necessary instrumentation to assure the uniformity of product temperatures. The lyophilizer should also have the necessary instrumentation to control and record the key process parameters. These include: shelf temperature, product temperature, condenser temperature, chamber pressure and condenser pressure. The manufacturing directions should provide for time, temperature and pressure limits necessary for a lyophilization cycle for a product. The monitoring of product temperature is particularly important for those cycles for which there are atypical operating procedures, such as power failures or equipment breakdown.

Electromechanical control of a lyophilization cycle has utilized cam-type recorder-controllers. However, newer units provide microcomputer control of the freeze drying process. A very basic requirement for a computer controlled process is a flow chart or logic. Typically, operator involvement in a computer controlled lyophilization cycle primarily occurs at the beginning. It consists of loading the chamber, inserting temperature probes in the product vials and entering cycle

parameters such as shelf temperature for freezing, product freeze temperature, freezing soak time, primary drying shelf temperature and cabinet pressure, product temperature for the establishment of fill vacuum, secondary drying shelf temperature and secondary drying time.

For most cycles, stoppering occurs within the lyophilizer. Typically, the lyophilizer has some type of rod or rods (ram) which enter the immediate chamber at the time of stoppering. Once the rod enters the chamber, there is the potential for contamination of the chamber. However, since the vials are stoppered, there is no avenue for contamination of the vials in the chamber which are now stoppered. Generally, lyophilizers should be sterilized after each cycle because of the potential for contamination of the shelf support rods. Additionally, the physical act of removing vials and cleaning the chamber can increase levels of contamination.

In some of the larger units, the shelves are collapsed after sterilization to facilitate loading. Obviously, the portions of the ram entering the chamber to collapse the shelves enters from a non-sterile area. Attempts to minimize contamination have included wiping the ram with a sanitizing agent prior to loading. Control aspects have included testing the ram for microbiological contamination, testing it for residues of hydraulic fluid, and testing the fluid for its bacteriostatic effectiveness. One lyophilizer fabricator has proposed developing a flexible "skirt" to cover the ram.

### FUTURE DEVELOPMENTS

Lyophilized products are currently being manufactured by the batch. While freeze-dried coffee is perhaps the only product that is manufactured on a continuous basis, it is really not a lyophilized product. Lyophilized coffee has a light yellow color rather than a dark brown, in addition, the methods used to manufacture coffee would hardly be acceptable for heat-sensitive injectable products (i.e., the shelf temperature is greater than 100°C). The remainder of this section will first consider a batch system and, in particular, the use of automatic loading and unloading systems. Based on a typical lyophilization process, the feasibility of a continuous freeze-drying system will be shown only to be possible if and only if there can be a major reduction in process time. A prototype continuous dryer will be described and its production output compared to that of a typical batch production dryer.

#### The Batch System

Automatic loading equipment capable of loading and unloading a 90 m dryer in 2–3 min [30] has become increasingly popular in recent years. These systems are capable of loading either vials or vials in trays directly on the shelves of the dryer. One of the chief advantages of such loading devices is to significantly reduce the number of people in the clean room, and thereby approach the conditions of isolation technology during

the loading and unloading operation [31–33]. In this way, the probability of biological contamination of the product is greatly reduced.

### The Continuous System

In 1975 there was considerable interest in continuous freeze systems. At that time, the system used trays to move the product from one stage to another. Since the trays did not come in direct contact with the shelves, the heat mechanism to the trays was mainly through radiation. The thermal radiation was produced from horizontal heating plates that were arranged to create isothermal zones through which the product trays would pass in a stack configuration. Each stack consisted of 15 trays [34].

It is of interest to note that, in his keynote address before the annual meeting of the Parenteral Drug Association on 3 November 1994, Polster [35] expressed concerns regarding the use of emerging technologies. He predicted that those manufacturers (of pharmaceutical products) who resisted technological advances would suffer at the hands of their competitors. He envisioned that in the coming 21<sup>st</sup> century, manufacturing would be performed using complete barrier technology. In the case of lyophilization, he predicted that the process would be continuous and require less than 8 h [35].

Table 3. Process time of a typical pharmaceutical or biotechnology lyophilization process

Process phase	Time
Filling and loading 37,000 vials at 120 vials/min	6 h
Freezing (ambient to $-40^{\circ}\text{C}$ )	12 h
Primary drying process	48 h
Secondary drying process	24 h
Backfilling and stoppering	1 h
Total process time	91 h

Consider what would be involved to lyophilize a pharmaceutical or biotechnology product in 10 ml vials on a continuous basis [36], using the following lyophilization process:

The hypothetical continuous system would consist of three separate chambers. The first chamber would be maintained at  $-40^{\circ}\text{C}$  and at  $1.01295 \times 10^5$  Pa. The second chamber would be maintained at the shelf temperature and be pressure defined for the primary drying process. Secondary drying would be conducted in a third chamber maintained at the final shelf temperature and pressure. The system would accommodate three trays of vials (each containing 168 vials) abreast.

With a system capable of filling 3 trays at a time, it would require approximately 6 hr to complete the filling process and another 6 hr to complete the freezing

process. At a width capable of 3 trays across, the length of the freezing chamber would be 22 m. Assuming that each vacuum lock could accommodate only 3 trays abreast, then the length of the primary drying chamber would be 14 m, while the length of the secondary drying chamber would be 7. The estimated total length of such a system would be 44 m or about the height of a 7-story building.

Assuming that it would require 1 hr to load and unload a vacuum lock, the minimum time to transport all of the trays from the freezing chamber into the primary drying chamber would be 72 h. An additional 48 h would be necessary to complete primary drying and 24 hr to complete the secondary drying. The total process time would be 158 h, or 1.7 times the process performed in a batch freeze-dryer. The dimensions of the dryer could be reduced. For example, the length of the freezing chamber could be reduced by one-half; however, in so doing, the overall process time would be increased to 181 h.

If the above continuous dryer required sterilization, then the entire system would have to be fabricated as a pressure-coded vessel. This would certainly add to the cost of the dryer. The cost of such a freeze-dryer would certainly exceed the cost of present dryers having the same shelf capacity. When the additional processing time is factored in, the continuous dryer based on present lyophilization processes would hardly appear as an attractive alternative to present technology.

Only if the lyophilization process time could be reduced by a factor of 1/5th to 1/10th would a continuous freeze system be deemed feasible. Reducing the time of the lyophilization process would also lead to reduced dimensions for the dryer. Because of the increase in the drying rate, the cake would have to have a sufficient self-supporting structure during primary drying to withstand the force exerted by the flow of gas from the vial. Therefore, it is unlikely that a formulation having a solid content less than 2% would be a candidate for lyophilization in a continuous dryer.

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## IZVOD

### LIOFILIZACIJA – PROCES I INDUSTRIJSKA PRIMENA

(Pregledni rad)

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Ovaj rad daje osnovni pregled procesa liofilizacije i razmatranje principa procesa kroz osnove: formulacije, zamrzavanja, primarnog sušenja i sekundarnog sušenja. U ovom radu je liofilizacija definisana kao proces stabilizacije u kome se supstanca prvo zamrava i onda se sadržaj rastvarača redukuje sublimacijom (primarno sušenje), a zatim desorpcijom (sekundarno sušenje) do vrednosti koja više ne podržava hemijske reakcije i rast živih organizama. Načinjen je poseban osvrt na industrijsku primenu procesa a akcenat je stavljen na primenu liofilizacije u farmaceutskoj industriji i industriji hrane. Oprema koja se koristi za liofilizaciju kao i materijali koji se mogu liofilizirati su opisani dovoljno detaljno da pruže informacije o ograničenjima i prednostima liofilizacije. Predstavljeni su ekonomika procesa i poređenje sa konvencionalnim metodama sušenja. Istorijski pregled i trendovi u razvoju procesa, iz ugla industrije, daju uvid u to gde se liofilizacija nalazila i kuda je, danas, vodi njena široka industrijska primena.

Ključne reči: Liofilizacija • Liofilizator • Liofiliziranje • Primarno sušenje • Sekundarno sušenje • Komora • Polica • Kondenzator • Formulacija • Farmaceutska industrija • Industrija hrane •

Key words: Lyophilization • Freeze dryer • Freeze drying • Primary drying • Secondary drying • Chamber • Shelf • Condenser • Formulation • Pharmaceutical industry • Food industry •