ejpmr, 2024, 11(3), 461-468

SJIF Impact Factor 7.065

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Review Article

ISSN 2394-3211

EJPMR

A COMPREHENSIVE REVIEW ON LYOPHILIZATION (FREEZE-DRYING) TECHNIQUE

Selvi G.[1]*, Priya Darshini R.[2], Anushiya M.[2] and Deepakumari V.[2]

[1]Associate Professor, Department of Pharmaceutics, C L Baid Metha College of Pharmacy, Thoraipakkam, The Tamil Nadu, Dr. M.G.R. Medical University, Chennai 600097 Tamil Nadu, India.

[2]Department of Pharmaceutics, C L Baid Metha College of Pharmacy, Thoraipakkam, The Tamil Nadu, Dr. M.G.R. Medical University, Chennai 600097 Tamil Nadu, India.

***Corresponding Author: Selvi G.**

Associate Professor, Department of Pharmaceutics, C L Baid Metha College of Pharmacy, Thoraipakkam, The Tamil Nadu, Dr. M.G.R. Medical University, Chennai 600097 Tamil Nadu, India. **Email ID:** selviarunkumar@gmail.com

Article Received on 29/01/2024 **Article Revised on 19/02/2024** **Article Accepted on 09/03/2024**

ABSTRACT

In the realm of pharmaceutical formulation, lyophilization stands out as a crucial technique offering distinct advantages over conventional methods. Its ability to safely dry heat-sensitive vaccines, antibiotics, and protein-based products has rendered it indispensable in modern medicine. Particularly in the context of lyophilized powder formulations, the reconstituted product can be swiftly utilized post-lyophilization, facilitating ease of administration and storage. As the demand for injectable biopharmaceuticals continues to surge, lyophilized products have assumed heightened significance in the pharmaceutical landscape. The evolution and proliferation of lyophilization techniques underscore its escalating importance in 21st-century pharmaceutical endeavors. While historically perceived as an expensive process, the transformative impact of lyophilization on drug stability and efficacy cannot be overstated. In earlier times, it is primarily viewed as a means of drying; however, its role has since expanded significantly, with pharmaceutical formulations now heavily reliant on this method. This review places a spotlight on recent advancements within the field, shedding light on potential avenues for future exploration and innovation. By delving into the core principles, procedural nuances, formulation considerations, and overarching significance of lyophilization, this discourse seeks to provide a holistic perspective on this indispensable pharmaceutical process.

KEYWORDS: Freeze drying, Vacuum, Vapour Pressure, Dryer.

INTRODUCTION

The process of lyophilization or freeze drying involves freezing the sample and then removing the water through sublimation (Primary drying) and desorption (secondary drying). In freeze drying, moisture is removed from the product by sublimating it after freezing.[1] Biological and pharmaceutical products that are unstable in aqueous solutions can be stabilized by drying, a process called aqueous solution drying. 'Lyophilization' refers to the process of making products that "love dryness".[2] Traditionally, the term 'lyophilization' refers to a process used to produce products that appear to be more attractive to the dry state than to the frozen state. It is more accurate to use the term freeze-drying instead of lyophilization because the two terms are used interchangeably.[3] When aqueous solution stability is an issue, lyophilization is the most commonly used method for the manufacture of parenteral. Low moisture content (less than 1%) is necessary for materials that require sterilization and gentle preservation in order to maintain their stability.[4] Food and pharmaceutical industries have used freeze drying for many years for a variety of purposes. Among other things, this procedure can be used to stabilize living materials, including a variety of microbiological cultures, preserve whole animals for museum displays, restore books and other items damaged by water, as well as concentrate and recover reaction products.[5] Materials can be dried without being damaged by freeze-drying or lyophilization. Sublimation is an indirect method of converting solids into gases without undergoing a liquid state, it uses the sublimation phenomenon. The frozen product is vacuum-dried without thawing before packing. The parenteral industry has embraced freeze-drying. As recombinant DNA technology is becoming more prevalent freeze-drying process is considered apt for clinical and commercial production of proteins and peptides. As an alternative to freeze-drying, sterile crystallization or spray-drying and powder filling are also available for the production of sterile dry powder drug products. The freeze-drying process is the most common method used to manufacture drug products that are too unstable to be offered as solutions.[6]

lyophilization or freeze drying involves the removal of water from an ice-based product and placing it in the vacuum chamber, which allows the ice to directly change from solid to vapor without passing through a liquid phase.[7] It is necessary to sublimate ice at temperatures and pressures below the triple point during lyophilization. It is suitable for drying thermolabile compounds since it is performed at low temperatures and pressures. To produce lyophilized products with desired moisture content, sample must be prepared, frozen, then followed by primary and secondary drying.[8] A lyophilizer removes water from a solution by concentrating water vapor between the drying front and the condenser. When the temperature increases during the primary drying process, the vapor pressure of water increases. Therefore, it is crucial to maintain the primary drying temperature at its highest, but below the critical process temperature, to avoid losing the cake's structure. Amorphous substance collapses at this temperature, while crystalline substances melt at this temperature. The solution becomes maximally concentrated when ice crystals separate out during freezing. When the solute and ice are further cooled, phase separation occurs.[9]

The fundamental process steps

1) **Freezing:** A frozen product allows low temperature drying to take place.
2) **Vacuum:** After a product is frozen, the sublimation process takes place to allow the frozen solvents to be vaporized without passing through liquid.
3) **Heat:** It is necessary to apply heat to frozen products in order to accelerate the sublimation process.
4) **Condensation:** Solvent vaporizes in a vacuum chamber, is then converted back to solid form by a low-temperature condenser.

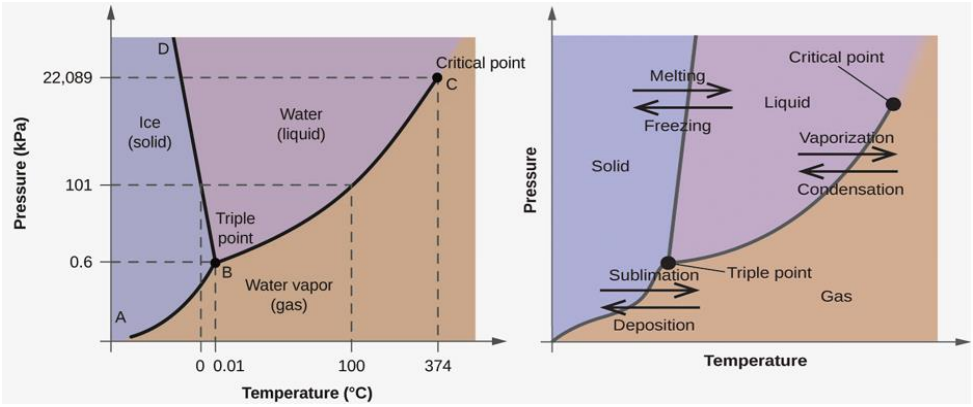

Figure 1: Lyophilization process.

Advantages of lyophilization

- Other drying and preserving techniques cannot compare with the benefits of Lyophilization Techniques.
- A heat-sensitive product can benefit from this drying technique.
- Products that are ready to reconstitute reduce weight and make transport easier, as well as maintaining the quality of foods/biochemical reagents and chemicals.
- A dry product may be more stable with the addition of this ingredient.
- In emergency medicine and hospital applications, the dried product can be reconstituted to facilitate safe use.
- An inert atmosphere (for example, nitrogen) can minimize the detrimental effect of lyophilized products that are susceptible to oxidation.
- Sublingual tablets can also be fast dissolving with this technique, in addition to parenteral products. Due to the fast-melting effect, tablets disintegrate quickly and have a great mouth feel.
- Other drying methods cannot achieve the same degree of sterility and freedom from foreign particles as this method.
- The properties don't change much because microbe growth and enzyme activity cannot take place when the temperature is low.
- Keeping the temperature at a normal level during transportation and storage.
- It takes a short time for reconstitution.
- The constituents of dried materials remain homogeneously dispersed.
- Liquid form of product is processed.
- There is a possibility of achieving and maintaining sterility of the product.

Disadvantages of lyophilization

- The process takes a long time.
- The product is more expensive because of its heavy manufacturing cost.
- Diluting agents must be sterile during reconstitution.

Traditional lyophilization technology

It takes careful balance between the product, the equipment, and the processing techniques in traditional lyophilization. A wide range of chemical components have been stabilized by lyophilization for nearly 30 years. These characteristics may make chemicals prone to degradation, require refrigeration, or require stabilization if they do not have a long shelf life. The

process of lyophilization effectively resolves these problems by suspending the activity of reagents when properly performed.[10] Chemical solutions that are stored at room temperature after lyophilization have a long shelf life. Solubility characteristics of the product provide rapid reconstitution due to the process' excellent solubility. In spite of their heat- and moisture-sensitivity, heat- and moisture-sensitive compounds remain viable. Aqueous preparations usually contain bacteria and enzymes that usually denature proteins during the process. Biological and chemical purity is thus maximized by lyophilization.[11]

Processing

Freeze-drying process

A freeze-drying process is primarily used to remove excess water from sensitive products, mainly biological products, in a way that prevents them from being damaged and enables them to be preserved in a permanent state and reconstituted by adding water. Drying takes place under atmospheric pressure by freezing the product. A primary drying phase describes the removal of water (ice) by sublimation; secondary drying describes the removal of water (desorption) by desorption. Drying under vacuum is called freeze drying.[12]

Pretreatment

All methods of pretreatment prior to freezing are included in pretreatment. Consequently, the product could be concentrated, corrected in its formulation (i.e., added components to improve stability and/or processability), or its surface area could be increased. In many cases, pretreating a product is a function of a product's cycle time or quality considerations, as well as theoretical knowledge of freeze-drying.[13] It can be said that pretreatments include freezing, concentrating in solution, formulating for product appearance preservation, stabilizing reactive products, increasing surface area, and reducing high vapor pressure solvents. Three parts are traditionally involved in lyophilization cycle design:

1) During freezing, some of the sample becomes pure crystalline ice while the remainder becomes a glassy state, where further crystallization is impossible.
2) Amorphous solutes are sublimated under vacuum at low temperatures to remove the ice formed during freezing, leaving a highly porous structure that is typically 30% water. Sublimation occurs at temperatures of –45 to –20°C during primary drying, at pressures between 10- 4 and 10-5 atmospheres.
3) The second drying step involves gradually raising the temperature of the sample while maintaining low pressure in order to desorb most of the remaining water from the glass.

Lyophilization equipment

Manifold freezedryers, rotary freezedryers, and tray style freeze dryers are the three types of freeze-dries. Every freeze dryer has two components: a vacuum pump to reduce the ambient gas pressure in the vessel and a condenser to remove the moisture by condensation on a surface cooled to between 40 and 80 degrees Celsius (112-112 degrees Fahrenheit). Depending on how the dried matter is interfaced with the condenser, manifold, rotary, and tray freeze-dryers differ.

Multiple containers with dried product are connected to a condenser using a short tube, usually circular. A large reservoir holds dried material in the tray and rotary freeze-dryers. Pellets, cubes, and other pourable materials are usually dried in rotary freeze-dryers. Rotating dryers dry substances more uniformly across the entire surface because the cylindrical reservoir rotates during drying. In a tray-style freeze-dryer, the reservoir is rectangular and there are shelves that can be used to carry trays, vials and other containers that contain products, such as pharmaceutical solutions and tissue extracts.

For liquid substances that will need to be dried quickly in small containers, multiple freeze-dryers are used in lab environments.[14] It is possible to dry the product to a moisture content of less than 5% with a manifold dryer. The removal of unbound water is the only method of drying without heat. In order to achieve lower moisture content, secondary drying requires an addition of a heater that will remove bound water.

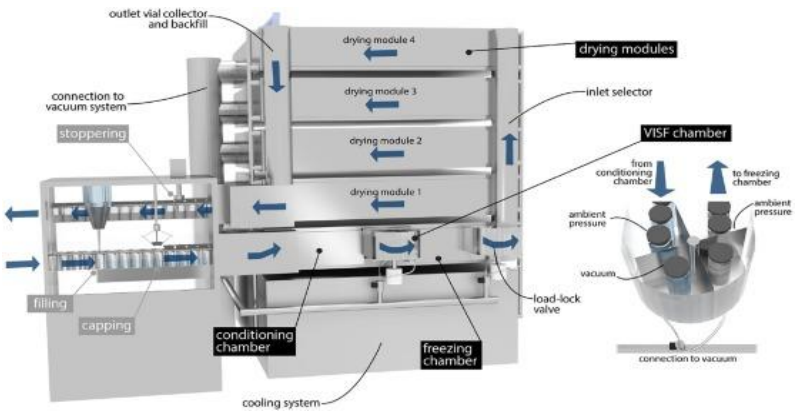

Figure 2: Lyophilization equipment.

It is often larger and more sophisticated to use a tray style freeze-dryer than a manifold dryer. Various materials can be dried with tray style freeze-driers. Long-term storage requires the driest product, which is produced in a tray freeze-dryer. This type of freezer freeze-dries products from both the unbound and bound water sides, so that the finished product is as dry as possible. A tray freeze-dryer can dry bulk goods as well as containers and vials containing products. By pressing a stopper into place, the freeze-dryer seals the vial before exposing it to the atmosphere when drying in vials.

A vaccine, for example, is stored in this way for a long time. To improve quality of the product and to produce the product faster with less labor, improved freeze-drying techniques are being developed to expand the range of products that can be freeze dried. As described above, a lyophilizer consists of a vacuum chamber containing shelves containing containers and their contents that can be cooled and heated. Associated controls are connected to the vacuum chamber, along with a vacuum pump, refrigerator, and associated controls.

Vacuum chambers are generally equipped with shelves that hold glass vials containing chemicals. Products are frozen inside shelves with cooling elements. As soon as the product is frozen, it is evacuated and heated with the vacuum pump. The shelf transfers heat to the vial, and then the product receives heat from the vial through thermal conduction.[15]

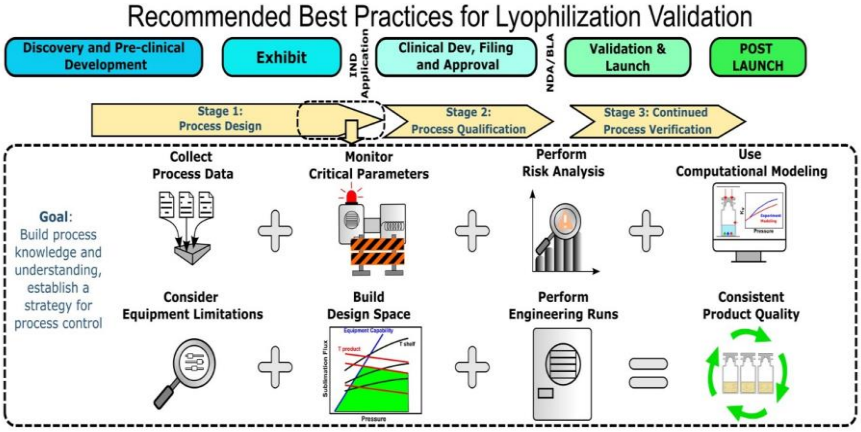

Figure: 3 Lyophilization validation process.

Excipients in lyophilized formulation[16]

The creation of a lyophilized (freeze-dried) pharmaceutical formulation is tailored to the specific needs of the active pharmaceutical ingredient (API) and the planned method of delivery. Such a formulation can include a variety of excipients, each serving one or multiple roles. These excipients are utilized for various purposes, including acting as buffers and pH adjusters, providing bulk, stabilizing the formulation, and adjusting tonicity.

Buffers

In the formulation of pharmaceuticals, maintaining a stable pH is essential, and buffers play a crucial role in achieving this. During the development of freeze-dried (lyophilized) formulations, selecting the appropriate buffer is of paramount importance. Phosphate buffers, particularly sodium phosphate, experience significant pH shifts when frozen. An effective strategy involves opting for buffers that exhibit minimal pH fluctuations upon freezing, like citrate and histidine buffers, and using these buffers in low concentrations.

Stabilizers

Disaccharides serve not only as bulking agents but also play a crucial role in creating an amorphous sugar matrix, which is highly effective in preserving the stability of products like liposomes and proteins during the freeze-drying process. Sucrose and trehalose, known for their inert properties, are commonly utilized in the stabilization of formulations containing liposomes, proteins, and viruses. On the other hand, reducing sugars such as glucose, lactose, and maltose had the potential to interact with proteins through the Maillard reaction, thereby altering their structure.

Tonicity builders

For certain applications, achieving isotonicity in a formulation is crucial, either to meet the stability needs of the solution before lyophilization or to comply with requirements specific to its method of administration. Tonicity could be effectively adjusted using excipients like mannitol, sucrose, glycine, glycerol, and sodium chloride. It's important to note that glycine, when kept in its amorphous state, can reduce the glass transition temperature. Additionally, tonicity agents may be added as diluents rather than as integral components of the primary formulation.

Bulking agents

Bulking agents serve to increase the volume of a formulation, a vital function when the active

pharmaceutical ingredient (API) is present in very low concentrations. While crystalline bulking agents contribute to the formation of a structurally sound and aesthetically pleasing cake, they might not effectively stabilize formulations like emulsions, proteins, and liposomes, though they could be appropriate for certain small molecule drugs and some peptides. For formulations where a crystalline structure is desirable, mannitol is a common choice. Alternatively, for products containing proteins or liposomes, sucrose or another disaccharide might be a more suitable option.

Freeze drying process

The freeze-drying process consists of three stages:

1. Freezing,
2. Primary drying, and
3. Secondary drying.

Freezing

The material to be freeze dried must first be adequately prefrozen because the phase transition from the solid to the gaseous occurs during freeze drying. In order to successfully freeze dry a product, the freezing method and final temperature of the product are important. It is useful to freeze dry products with smaller ice crystals, since small ice crystals preserve structures for microscopic examination. As the drying process progresses, large ice crystals form and channels become less restrictive. The way in which a product freezes depends on its composition. Solutes and dissolved materials are the predominant components of products that are freeze dried. Solutes are made up of water and dissolved or suspended materials. Freeze drying typically involves eutectics, which are mixtures of substances that freeze at a lower temperature than water around them. A change in the concentration of solutes in the product matrix occurs when the aqueous suspension is cooled. In addition, the water separates from the solutes during cooling as it converts to ice, which leads to concentrations of solutes. It is below the freezing point of water in these pockets of concentrated materials. It is true that all the ice in a product may make it appear frozen, but in reality, it is not fully frosted until all the solutes in the suspension are frozen. Eutectic suspensions consist of various concentrations of solutes and solvents. A properly frozen suspension can only be achieved when the entire eutectic mixture has frozen. Eutectic temperature is the temperature at which the eutectic process takes place. Freeze drying requires products to be prefrozen to below eutectic temperatures before their freezing begins. If pockets of unfrozen material remain within freeze-dried products, they are structurally unstable.

Primary drying

After the freezing process has been completed, the vacuum pump reduces the pressure within the freeze-dryer. Pharmaceutics are typically lyophilized at a pressure between 30 and 300 mTorr depending on the temperature desired and the container characteristics.

Secondary drying

Despite the fact that all ice in the product has been sublimed and the primary freeze-drying process has been completed, bound moisture is still present in the product. As a result, continuing drying at warmer temperatures may be necessary to reduce residual moisture content from as much as 7-8% to optimum levels. Desorption of bound water from a product is called 'Isothermal Desorption'.[17] It is usually necessary to continue the secondary drying process at a higher temperature than ambient, but one that is compatible with the product's sensitivity.

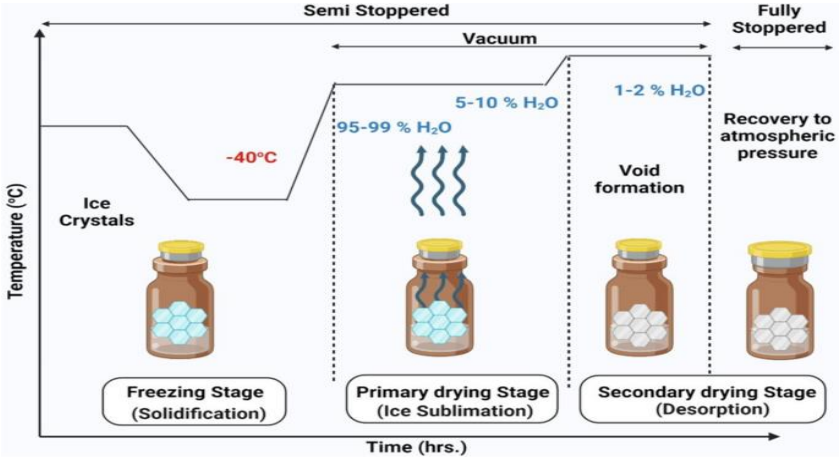

Figure 4: Stages of Freeze-drying process.

Various methods involved in freeze drying

- **Bulk drying**
- **Manifold and**
- **Batch drying**

Bulk drying

Bulk drying typically occurs in a tray dryer, following a batch drying approach, where the product is filled into a large pan and dried collectively. Despite being spread out to cover the entire shelf area and having a uniform

thickness similar to products dried in vials, the absence of gaps within the mass of the product alters the rate at which heat is introduced. Unlike manifold or batch drying, bulk drying does not easily accommodate the sealing of products under precise conditions. Instead, products are often taken out of the freeze-drying apparatus before being sealed and subsequently stored in hermetically sealed containers. This method is predominantly used for products that are stable and not overly sensitive to exposure to oxygen or moisture.[18]

Figure 5: Bulk drying process.

Manifold process

In the manifold drying technique, individual containers such as flasks, ampules, or vials are connected directly to the ports of a manifold or drying chamber. Products are pre-frozen using methods like conventional freezing, immersion in a cold bath, or shell freezing, depending on the product's characteristics and volume. These pre-frozen products are then rapidly attached to the manifold or drying chamber to avoid temperature increase. A vacuum is quickly established within the container to maintain the product's low temperature through evaporative cooling. This method is suitable for small quantities and for products that have high eutectic or collapse temperatures. Manifold drying offers several benefits over traditional batch tray drying. Each container has an unobstructed path to the moisture collector, reducing the competition for space that occurs in batch systems. This setup is especially efficient in cylindrical drying chambers, where each container is equidistant from the collector. However, in a "tee" manifold configuration, water vapor from containers farther away may encounter delays as it passes by nearer ports. Temperature adjustments can be made by exposing the containers to room temperature or using a circulating water bath, although this may not provide the precise temperature control needed for some products. The manifold system can accommodate multiple containers simultaneously, allowing for the drying of various products, in different container sizes, and with different types of closures. Given the varying nature of products and volumes, containers can be individually detached from the manifold once drying is complete. The proximity of the containers to the collector enhances drying efficiency by creating an optimal environment for moisture removal.

Batch method

In the batch drying process, numerous vessels of uniform size, all containing the same product, are arranged together within a tray dryer. Initially, the product within these vessels is pre-frozen directly on the dryer's shelf. This setup allows for the precise management of both the product's temperature and the heat applied during the drying phase. Although the drying treatment is uniform across all vessels in the batch, minor variations may occur due to slight discrepancies in heat distribution from the shelf. For instance, vessels positioned at the front of the shelf might receive additional radiant heat through the transparent door of the dryer, leading to negligible differences in the final moisture content of the product. Batch drying facilitates the simultaneous sealing of all vials within a batch under consistent atmospheric conditions. This can be accomplished either in a vacuum or following the backfilling with an inert gas, ensuring that each vial is stoppered at the same time. This simultaneous stoppering guarantees that each vial is sealed under an identical environment, promoting consistent product stability throughout its storage life. Predominantly utilized in the pharmaceutical sector, batch drying is the method of choice for processing large batches of ampules or vials containing a single product.

Applications of lyophilization technology

1) Pharmaceutical industry

- Freeze-drying is used to produce macromolecules of antibiotics and electrolytes.
- A heat-sensitive product is dried using this technique, such as antibiotics, blood products, and vaccines.
- A solid protein source utilizes this process for the development of pharmaceuticals for long-term storage.
- Nasal inserts can be lyophilized for better efficacy.

- Lyophilization is used for drying Lyosphere and micro- and nanoparticles,

2) Food industry

- Food preserved by lyophilization, makes it very lightweight and easy to carry. As an example of astronaut food, freeze-dried ice-cream has been used as part of the process. Increasing the weight of food and reconstituting with available water is also convenient for hikers since it reduces their workload.

3) Other industries

- Lyophilized product is easier to dissolve in water for later use and more stable during chemical synthesis.

4) Miscellaneous applications

- Water-damaged books and documents can be recovered by freeze-drying, a procedure studied by several institutions, including the National Archives and Records Administration (NARA).
- From sprayed slurry mists, ceramic process often uses lyophilization to create a powdered product. In this way, the particles produced by traditional hot spray drying are softer and more chemically uniform.
- Recently, some taxidermists have begun using freeze drying to preserve animals, such as pets.
- Floral preservation can also be achieved through lyophilization.[19]

Determination of Freeze-dried product's stability

The stability of freeze-dried materials is influenced significantly by moisture and oxygen content. Even after the drying process, freeze-dried products retain a small amount of moisture known as residual moisture. The level of residual moisture varies depending on the product type and the duration of secondary drying. Various methods such as chemical analysis, chromatography, manometry, or gravimetry are employed to measure residual moisture, typically expressed as a percentage of the total dried product weight, usually ranging from less than 1% to 3%. Freeze-dried materials exhibit hygroscopic properties, making them susceptible to moisture absorption during storage. Hence, it is crucial to pack freeze-dried products in impermeable containers to prevent exposure to atmospheric moisture. Storing these products in low-humidity environments further minimizes the risk of degradation due to moisture absorption. Similarly, oxygen could also compromise the stability of freeze-dried materials. Therefore, packaging must also provide an effective barrier against air permeation. Notably, the detrimental effects of both oxygen and moisture are exacerbated at higher temperatures. As such, storing freeze-dried products at lower temperatures, typically between 4-8°C, helps to prolong their shelf life. To estimate the shelf life of freeze-dried products, accelerated storage testing is often conducted. This involves subjecting samples to elevated temperatures and monitoring the rate of degradation. By extrapolating these findings to lower storage temperatures, the anticipated degradation rate at typical storage conditions can be predicted accurately.

Future prospects

The escalating prevalence of formulation stability issues associated with intricate APIs and biologics has prompted a growing reliance on lyophilization among pharmaceutical and biotech manufacturers. Over the past five years, the adoption of lyophilization in both pharmaceutical and biopharmaceutical manufacturing has exhibited a steady annual growth rate of approximately 13.5%. This trend underscores the increasing recognition of lyophilization's efficacy in addressing formulation challenges and enhancing product stability. As the pharmaceutical landscape continues to evolve with the emergence of more complex molecules, the potential benefits of lyophilization are becoming increasingly apparent. Consequently, an expanding array of products in the drug development pipeline are poised to leverage this technology. For any drug developer contemplating the adoption of lyophilization, it is imperative to ensure the availability of specialized knowledge, appropriate facilities, and state-of-the-art equipment. Moreover, collaboration with a competent Contract Development and Manufacturing Organization (CDMO) is essential. A reliable CDMO partner must offer not only the requisite equipment but also possess profound formulation and analytical capabilities, coupled with extensive experience in developing, scaling, and validating lyophilization processes. This holistic approach is paramount to maximizing the likelihood of success for lyophilization projects in the pharmaceutical and biotech industries.

CONCLUSION

Lyophilization is considered as the most popular formulation strategy for biopharmaceuticals which represents about half of the market. During the freeze-drying process, chemical and physical degradation reactions are limited or slowed down, thereby improving long-term stability. It is easy to ship and store lyophilized formulations, as they provide good stability. Understanding the freezing process and how it affects the quality of the product is crucial for successful lyophilization. Controlling, and manipulating, the steps in freezing will help in developing more efficient lyophilization cycles and biopharmaceuticals with better stability.

REFERENCES

1. Akers MJ, Fites AL, Robinson RL. Types of parenteral administration. Journal of Parenteral science and Technology, 1987; 41: 88-95.
2. Lippincolt, Williams K. Remington, The Science & practice of pharmacy, Parenteral Preparation, ISE publication, Phelabelphia, 2000; 20, 1: 804-819.

3. Akers MJ, Remington: The science and practice of pharmacy, Lippincott Williams & wilkins publisher, 2000; 21: 525.
4. Searles JA, Carpenter JF, Randolph TW. The ice nucleation temperature determines the primary drying rate of lyophilization for samples frozen on a temperature-controlled shelf. J Pharm Sci, 2001; 90(7): 860–71.
5. Flink JM. and Knudsen, An Introduction to Freeze Drying. Strandberg Bogtryk Offset, Denmark, 1983.
6. Nail SL. et al. Fundamentals of freeze-drying. Development and Manufacture of Protein Pharmaceuticals. Marcel Dekker, 2002; 281–360.
7. Remington: The science and practice of pharmacy, Gennaro RA, Lippincott Williams & wilkins publisher, 2000; 21: 1.
8. Jeff SJ. Basic Cycle Development Techniques for Lyophilized Products, 2009; 35: 126- 128.
9. Adams GD, Irons LI. Some implications of structural collapse during freeze drying using Erwinia caratovora l-asparaginase as a model. J Chem Biotechnol, 1993; 58: 71– 76.
10. Greiff D. Development of cycles for lyophilization. Dev Biol Stand, 1992; 74: 85-92.
11. Carpenter JF, Pikal MJ, Chang BS and Randolph TW. Rational design of stable lyophilized protein formulations: some practical advice. Pharm Res, 1997; 14: 969-975.
12. Yoshioka S, Aso Y and Kojima S. The effect of excipients on the molecular mobility of lyophilized formulations, as measured by glass transition temperature and NMR relaxation based critical mobility temperature. Pharm Res, 1999; 135-140.
13. Wang W. Lyophilization and development of solid protein pharmaceuticals. International Journal of pharmaceutics, 2000; 52: 1-60.
14. Cappola ML. Freeze-Drying Concepts: The Basics, in McNally EJ (ed): Technology transfer, Marcel Dekker publisher, New York, 2000; 99: 159-199.
15. Herman BD, Sinclair BD, Milton N and Nail SL. The importance of technology transfer. Pharm Res, 1994; 11: 1467-1473.
16. Wallen AJ, Nakagawa K, Hottot A. Influence of lyophilization chamber loading on homogenecity in product appearance. Jour. chem. Eng. Process, 2006; 45: 783-791.
17. Charles P, Detke HC, Pyne A. Post injection delirium/sedation syndrome in patients with schizophrenia treated with Olanzapine long-acting injection: analysis of cases. BMC psychiatry, 2005.
18. Labconco catalog, A guide to Freeze Drying for the aboratory, Labconco Corporation, An industry service publication, 2004; 8.
19. Tang X, Pikal M. Design of freeze-drying processes for pharmaceuticals: practical advice. Pharm. Res, 2004; 2: 191–200.