

FREEZE DRYING: A REVIEW**Jyoti Shravan Gangurde* , K. B. Erande and L. M. Shevale**Department of Pharmaceutical Quality Assurance, M.G.V.'S Pharmacy College, Panchavati,
Nashik-3, India.Article Received on
08 Jan. 2019,Revised on 29 Jan. 2019,
Accepted on 18 Feb. 2019

DOI: 10.20959/wjpr20193-14335

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ABSTRACT

Freeze-drying is a method of removing water by sublimation of ice crystals from ice-cold material. Suitable parameters of process application allow us to obtain best quality products compared to products dried with traditional methods. Very good physical and chemical properties of food and biotechnological products make this method the best for drying exclusive products. The moisture content value decreased after freeze dry processing whereas original color and the shape of the sample is maintained. This review contains history, principle, methods, and advantages of lyophilization.

KEYWORDS: Lyophilization, Freeze drying, Freeze drying method.**HISTORY**

The process of freeze-drying was invented in 1906 by Arsened Arsonval and his assistant Frederic Bordas at the laboratory of biophysics of College de France in Paris. In 1911 Downey Harris and Shackle established the lyophilization process of preserving live rabies virus which eventually led to development of the first antirabies vaccine. Freeze drying was first actively established during WORLD WAR II transport of serum. The main purpose was to store the products without refrigeration and to eliminate moisture from thermolabile compounds. Shortly thereafter, the freeze-dry method was applied to bone and penicillin, and lyophilization became recognized as an important technique for preservation of biologicals. Atlas in 1961 built six production freeze drying cabinet for Nestle group in Germany, Holland.^[1]

INTRODUCTION

Freeze drying is a widely used method for the stabilization of easily degraded substances like microorganism, foods, biological products and pharmaceutical products. Freeze drying is the process of removing moisture from a frozen product using vacuum. Lyophilization means freeze drying. The term “lyophilization” defines a process to produce a product that “loves the dry state”. This method includes three steps: freezing, primary drying (main drying), and secondary drying (Final drying). The most common method of sterile parenteral powder is lyophilized powder or freeze dried.

Principle

The main principle involved in freeze drying is sublimation, where water passes directly from solid state (ice) to the vapor state without passing through the liquid state. Sublimation of water can take place at pressures and temperature below triple point i.e. 4.58 mm of Hg and 0.0098 degree Celsius. The material to be dried is first frozen and then subjected under a high vacuum to heat so that frozen liquid sublimates leaving only solid, dried components of the original liquid. The concentration gradient of water vapor between the condenser and drying front is the driving force for elimination of water during lyophilization. Sublimation arises between the solid and the vapor phase regions. Since only two phases are present solid ice and the vapor ice are in equilibrium. The diagram also says that once Temp of ice is fixed, the vapor pressure over ice is automatically fixed, and vice-a-versa. Lyophilization is carried out below the triple point to enable conversion of ice into vapor, without entering the liquid phase (known as sublimation). Annealing is an optional step, occasionally used to crystallize the formulation component. If the solute separates out in crystalline form, it is called as the eutectic temperature.

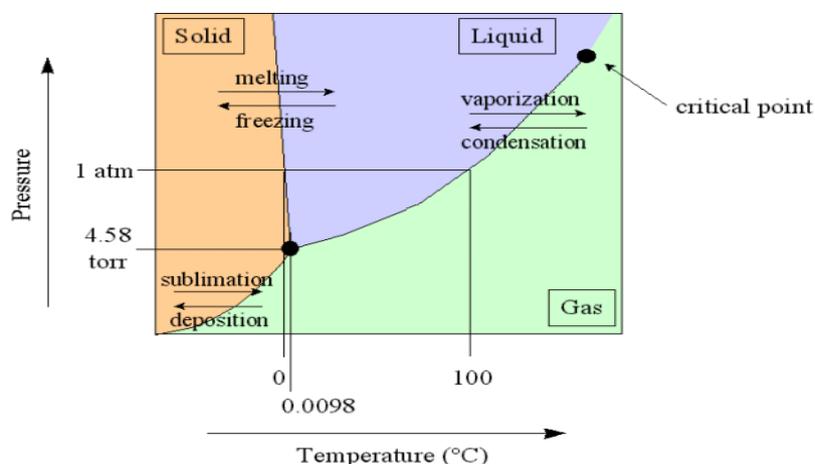


Fig:1 Phase diagram of water.

In contrast, if an amorphous form is there, then temperature is stated as the glass transition temperature (T_g). Determination of this critical temperature is significant for development of an optimized lyophilization cycle. During primary drying temperature should not exceed the critical temperature, which otherwise leads to 'meltback' or 'collapse' phenomenon. In the majority of lyophilized formulations, excipients are included to improve the functional properties and stability of the lyophilized product.

Lyophilization consists of

1. Formulation containing water it converts into ice in freezing step.
2. Under a vacuum condition, the ice directly converted into water vapour, is known as sublimation.
3. Drawing off the water vapour.
4. When sublimation of ice is completed, then the sample are freeze dried and can be removed from the machine.^[2,3]

STAGES OF LYOPHILISATION

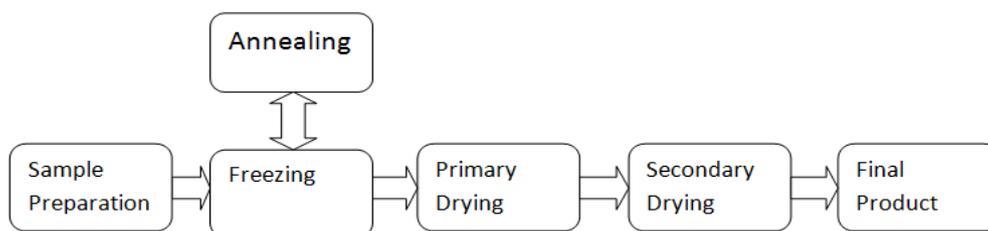


Fig:2 Lyophilization cycle.

1. Freezing:- At low temperature the sample gets frozen then it will reach to vitreous state where pure water has crystallized and the amorphous content remains in interstitial region.
2. Primary drying:- In the second stage, the product undergoes sublimation at a temperature below the glass transition or collapse temperatures and crystallized water is sublimed.
3. Secondary drying:- Finally, secondary drying is undertaken at a higher temperature to remove absorbed water from the interstitial region^[3,8]

LYOPHILISATION PROCESS

Step 1:- The drug and excipients are dissolve in a suitable solvent, generally water for injection (WFI) is used.

Step 2:-The bulk solution is sterilized by using bacteria-retentive filter, generally it will pass through a 0.22 micron.

Step 3:-Under aseptic condition the sample is filling into individual sterile containers and partially stoppered it.

Step 4:-Under aseptic condition transport the partially stoppered containers to freeze dryer and loading into the chamber.

Step 5:- The solution gets freeze by placing the partially stoppered containers on cooled shelves in a freeze-drying chamber.

Step 6:- Then Applying a vacuum to the chamber and heating the shelves for evaporation of the water from the frozen state.

Step 7:-Complete stoppering of the vials usually by hydraulic or screw rod stoppering mechanisms installed in the freeze dryer.

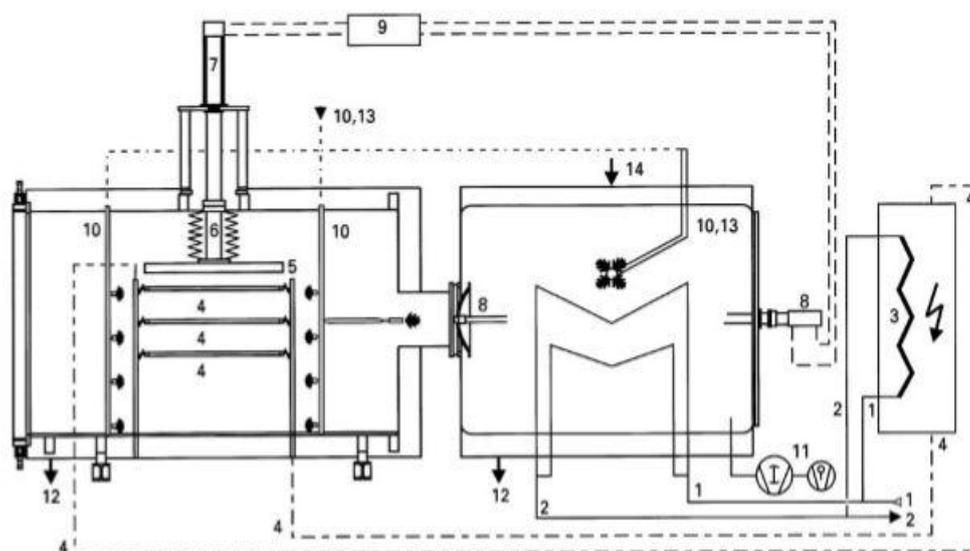


Fig 3: Schematic diagram of Lyophilizer.

Freeze-drying plant condensers and shelves cooled with liquid nitrogen. Clean in place system in chamber and condenser.

1.Liquid nitrogen inlet to condenser and heat exchanger; 2.nitrogen outlet from the condenser and heat exchanger; 3.heat exchanger for the brine in the shelves; 4.brine to and from the shelves; 5.pressure plate for the vials; 6.piston rod with bellows; 7. Hydraulic piston for pressure plate for the vials and piston rod with bellows; 8.hydraulically operated valve; 9.hydraulic system; 10 and 13. Water and steam inlet; 11.Pumping system; 12.water outlet.^[7]

Products that can be freeze dried are

1. Non-biological products:- Heat-labile chemicals
2. Non-living bio products (majority): Hormones, enzyme, pharmaceutical bones and body tissues for surgical/medical use, vitamins, blood products, antibodies, vaccines, foodstuffs, antibiotics.
3. Living organisms

After lyophilization material should be in the following state as

1. Dried to the right moisture content
2. Ethically acceptable
3. Cosmetically Refined
4. Active
5. Stable
6. Cake like
7. Clean

ADVANTAGES**Table 1: Advantages of Lyophilization.**

1.Economic safe	6.Rapid rehydration
2.Good stability	7.Retain flavor, color, shape
3.Long shelf life	8.No damage to the nutrition
4.High activity rate	9.Remain biological activity
5.Low moisture	10.Remain chemically active

Case1- After the rehydration of the freeze-dried indomethacin nanocapsules that were stored at ambient temperatures, losses of 8.5%, 26% and 50.5% indomethacin were found after 2, 4, 6 months respectively. But when freeze-dried nanocapsules were stored at 4 °C, the drug loss was only 9.3% at the end of 12 months.^[9]

Case 2-Dehydroemetine microparticle for treating visceral leishmaniasis have been freeze-dried using glucose 5% as cryoprotectant. These freeze-dried microparticles could be stored at -20 degree celsius for 24 months without any modification of their size or the level of drug binding. Finally, freeze-drying with trehalose was a good alternative to stabilize solid lipid microparticle loaded with azidothymidine (AZT) without any modification of their size or their drug content, because the storage of these microparticles at both 37 degree celsius or 4 degree celsius induces an increase in particle size and loss of AZT.^[9]

Case 3-Structural changes, the sought after freeze-drying product are porous that maintain their volume, can have fast and nearly complete rehydration when water is added and do not shrink. However some freeze-dried products undergo undesirable structural changes. Microscopy can be used to find a relationship to some physical properties and to study structural changes in freeze-dried fruits or material.^[6]

Case 4-One of the oldest methods of food preservation is dehydration. Dehydration is used for preserving foods in a safe and stable condition as it reduces water activity and extends self-life much longer than the food in fresh condition.^[6]

Table 2: Consensus attribute before and after freeze-drying.

Sample	Attribute	Before	After
Brown rice milk	Color	Brown and clean	Brown
	Texture	Thinner in consistency than soymilk, Smoothness, heterogeneous and watery	Very easy to destroy and melted in the tongue.
	Taste	Sweet creamy thick, film and milky also has a rice flavor.	Sweet and has strong rice flavor.
	Shape	Liquid	Solid like crystal grains.

Instrumental benefits: The CONRAD™ process is fully automatic throughout and requires only minimal staff for continuous operation. All process parameters and movement are carefully controlled, monitored and logged with the most modern PLC/PC system.

1. Overall benefits

- Continuous production
- Economical operation
- 98% efficiency
- Low maintenance costs
- Low maintenance requirements

2. Space saving: The special vapor condensers are optimized and built into the side of the drying chamber.

3. More reliable: The condenser system does not rely on large external vacuum valves with pressure drops that are difficult to secure. In the internal system, de-icing is performed under vacuum avoiding the need to seal the chamber against large pressure differentials.

4. Low product loss: No product abrasion and low vapor velocities within the dryer guarantee as little as 0.1% product loss during the process.

5. Low power consumption: De-icing vacuum, rather than at atmospheric pressure, eliminates the need to establish vacuum. This combined with optional vapour flow conditions, reduces energy consumption by up to 40% compared with competing freeze drying technology.^[5]



Fig:4 Freeze Dryer (close)



Fig:5 Freeze Dryer (open)

Disadvantages

1. More complicated development.
2. More expensive manufacturing.
3. Specialized capabilities required increased handling and processing time.
4. Volatile compounds may be removed by vacuum.

Freeze Drying Methods

Three methods of freeze drying are commonly used:

- (1) Manifold drying.
- (2) Batch drying.
- (3) Bulk drying.

Above all method has a specific purpose, and the method used depends on the product and the final configuration desired

1. Manifold Method: In the manifold method, flasks, ampules or vials are individually attached to the ports of a manifold or drying chamber. The product is frozen in a freezer, by direct submersion in a low temperature bath, or by shell freezing, depending on the nature and volume of the product to be freeze dried. The pre-frozen product is immediately attached to the drying chamber or manifold to prevent warming. Then instantly vacuum must be created in the product container, and the operator relies on evaporative cooling to maintain

the low temperature of the product. This procedure can only be used for relatively small products and volumes with high collapse and eutectic temperatures. This method has several advantages over batch tray drying. Since the vessels are attached to the manifold individually, each flask or vial has a direct path to the collector. This removes some of the competition for molecular space formed in a batch system, and is most ideally realized in a cylindrical drying chamber where the distance between the collector to each product vessel is the same. In a “tee” manifold, the water molecules leaving the product in vessels farthest from the collector experience some traffic congestion as they travel past the ports of other vessels. Heat input can be affected by simply exposing the vessels to a circulating bath or ambient temperature. For some products manifold drying may not be suitable, but precise temperature control is necessary. Several vessels can be accommodated on a manifold system allowing drying of different products at the same time, in different sized vessels, with different closure systems. Since the products and their volumes may vary, each vessel can be removed from the manifold separately as its drying is completed. The close proximity to the collector also creates an environment that increases drying efficiency.

2. Batch Method: In this method, large numbers of similar sized vessels containing like products are placed together in a tray dryer. The product is pre-frozen on the shelf of the tray dryer. Specific control of the product temperature and the amount of heat applied to the product during drying can be maintained. In this process generally all vials in the batch are treated alike during the drying process, then also some variation in the system can occur. Minor differences in heat input from the shelf can be experienced in different areas. Vials are located in the front portion of the shelf may be radiantly heated through the clear door. These minor variations can result in small differences in residual moisture. Batch drying allows closure of all vials in a lot at the same time and the same atmospheric conditions. The vials can be stoppered in a vacuum, after backfilling with inert gas. Stoppering of all vials at the same time ensures uniform product stability in each vial and uniform environment during storage. Batch drying is used to prepare large numbers of vials or ampules of one product and is commonly used in the pharmaceutical industry.

3. Bulk Method: This method is generally carried out in a tray dryer like batch drying. The product is dried and poured into a bulk pan as a single unit. Although the product is spread throughout the entire surface area of the shelf and having the same thickness as product dried in vials, the lack of empty spaces within the product, as the mass changes the rate of heat

input. The heat input is limited to that provided by contact with the shelf as shown in Figure 4. Bulk drying does not lend itself to sealing of product under controlled conditions as does batch or manifold drying. Then the product is removed from the freeze dry system prior to closure, and then packaged in air tight containers. Bulk drying is generally reserved for stable products that are not highly sensitive to moisture or oxygen.^[4]

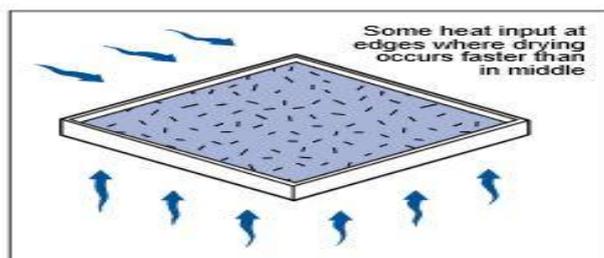


Fig: 6 Bulk drying.

Determining Drying Endpoints

The endpoint of primary drying can be determine, the drying boundary indicates in batch drying containers by moving the product to the bottom of the product container and observed ice in the product. No visible ice indicates, at the edges of the container will be dried completely and gives no indication of the conditions in the center of the product. An electronic vacuum gauge used to measure condensable gases in the system. The electronic gauge indicated the pressure, reaches the minimum pressure attainable by the system, and is determined by using a McLeod vacuum gauge or as determined previously, no more water vapor seen in the product. As the heat input to the product is increased, evaporative cooling keep the product temperature well below the temperature of its surrounding atmospheric temperature. When primary drying is completed, then product temperature rises to equal the temperature of its surrounding temperature. In tray dryers and manifold systems with external collectors, the path to the collector can be shut off with a valve and the pressure above the product measured using a vacuum gauge. If drying is still continued, then the pressure in the system increases.^[4]

Stability Of Freezedried Products

Some of the factors can affect the stability of freeze dried material. Two of the most important are oxygen and moisture. All freeze dried products have a small amount of moisture remaining in them called residual moisture. The amount of moisture remaining in the material depends on the length of secondary drying and nature of the product. Residual

moisture can be measured by several means: chemically, manometrically or gravimetrically, chromatographically. It is stated as a weight percentage of the total weight of the dried product. Residual moisture values range from <1% to 3% for most of the products. By their nature, freeze dried materials are hygroscopic and when it exposure to moisture during storage, the product can be destabilize. For freeze dried materials packaging used it must be impermeable to atmospheric moisture. When freeze dried products stored in low humidity environments, can reduce the risk of degradation by exposure to moisture. Oxygen is also affect the stability of most freeze dried material so the packaging used must be impermeable to air. The detrimental effects of moisture and oxygen are temperature dependent. The storage temperature is higher, then faster a product degrades. At refrigerator temperatures most of the freeze dried products can be maintained, i.e. 4-8°C. The freeze dried products place at lower temperatures extends their shelf life. The shelf life of a freeze dried product can be predicted by measuring the rate of degradation of the product at an elevated temperature, is called accelerated storage. By choosing the proper temperature and time relationships at elevated temperatures, the rate of product degradation can be predicted at lower storage temperatures.^[4]

CONCLUSION

Freeze-drying is the most complex and costly conservation process of all drying methods. However, it is the only way for many pharmaceutical products to maintain their original qualities for an acceptable time at room temperature and above or readily available temperatures. For cosmetic and food products it provides an opportunity to supply the customers with stable high quality products which can be easily used. For many products, e.g. some food ingredients and some antibiotics, simpler methods of preservation have been developed but in pharmaceuticals there is an increase in the number of products which have to be freeze-dried and frozen at low temperatures using tightly controlled processes. The tendency to automate the whole procedure is supported by three goals:

- (1) To have little or no personnel in the sterile areas.
- (2) To restrict the volume of sterile areas as much as possible, for example by enclosing the whole production line from vial filling to unloading from the chamber in isolators.
- (3) To exclude human error as much as possible and to have each step documented by computer.



Fig 7: Automated process.

To automate an existing process can be more difficult than to develop a new automated process. This is based on several factors. The formulation of the drug has to reflect the automation, e.g. filling and loading can require hours, during which the solution has to be stable, possibly at room temperature. Freezing of the product on the shelves and drying in the chamber have to be performed without temperature sensors in the product; for temperature control other method have to be used, tested and installed. Criteria have to be defined for the automatic change from main to secondary drying. Automatic termination of the secondary drying has to be effected when certain measurable events are accomplished. Also these main points several others have to be evaluated. More accurate and independent sensor systems will influence freezing and drying procedures. The required data processing and the actuators to fulfill the commands are available today.^[7,9]

Table 3: Example of Freeze Drying Products

Sr. No.	Brand Name	DOSE	API	Manufacturer	Use
1	Herzovir	250mg	Aciclovir Intravenous Infusion Ip	Chandra Bhagat Pharma Pvt Ltd	Antiviral
2	Kabidox tm	100MG Per Vial	Doxycycline For Injection Usp	Fresenius Kabi	Antiboitic
3	Saclist	250MG	Lyophilised Saccharomyces Boulardi Sachets	Rech Elist	Diarrhea
4	Ductaclose tm	1mg Per Vial	Indomethacin For Injection Usp	Samarth Life Sciences Pvt Ltd	Nsaid
5	Cyclophosphamide Lyophilised	1GM Per Vial	Cyclohosphamide For Injection, Usp	Baxter	Lymphoma, Breast Cancer, Small Cell Lungs Cancer

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