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Theory and Practice of Freeze Drying in Pharmaceuticals

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ABSTRACT

Lyophilization or freeze drying is widely acknowledged as one of the most effective methods for ensuring the long-term stability of a variety of pharmaceutical and medical products. Lyophilization is a dehydration (or "desiccation") method used in the food, chemical, pharmaceutical, and biotechnology sectors. It has been used to preserve foods, pharmaceuticals, and a broad variety of other things. The process takes time and variety of freezing and drying conditions. This review gives a clear idea of each and every step of freeze-drying and the merits, demerits, and limitation of the process.

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INTRODUCTION:

Freeze-drying is a technique for dehydrating frozen materials using a high-vacuum sublimation process. Freeze drying is also called as lyophilization. [1]

In 1906, Jacques-Arsene d'Arsonval of the College of France in Paris created freeze-drying. In 1938, the first freeze-dried coffee was manufactured, which led to the development of powdered food products. During World War II, freeze drying advanced significantly. [2] Blood plasma and penicillin were required to treat the injured in the field, and many serum supplies decayed before reaching their intended users due to a lack of refrigerated conveyance. The freeze-drying method was developed as a commercial method of preserving the chemical stability and viability of blood plasma and penicillin without the need of refrigeration. [3] In the 1950s and 1960s, freeze drying became popular as a multi-purpose method for pharmaceuticals and food. The use of freeze-dried foods in astronaut and military rations became common place. By simplifying the process of rehydrating freeze-dried meals with water for astronaut crews, tubed meals and freeze-dried snacks that were difficult to rehydrate in space were transformed into hot meals. Freeze-drying is being used to preserve foods, pharmaceuticals, and a broad variety of other things. [4]

Because of the mild temperatures at which the process takes place, lyophilization creates high-quality products, both food and pharmaceuticals. This contributes to the development of highly porous solids that maintain scent, colour, and flavour.

Lyophilization is a heat-mass transfer process in which the frozen solvent, usually water, is removed first by sublimation under decreased pressure and sub-zero temperatures, and then by desorption under reduced pressure and above zero temperatures to produce a dry product. [5]

The lyophilization process includes the following steps to extract water from a formulation:

1. Freezing the product until the water in the product turns to ice.
2. In a vacuum, sublimate the ice directly into water vapour.
3. Removing the water vapour.

4. The products are freeze dried and can be taken from the machine once the ice has been sublimated.

STABILTY OF SOLUTION AND LYOPHILIZED FORMS: [5]

Table 1: Comparison of stability of solution and lyophilized forms.

Product	**Solution Form**	**Lyophilized Product**
Caspofungin/Cancidas	1 hr at RT	2 years
Fosaprepitant/Emend	24 hr at RT	More than 2 years
Gemcitabine/Gemzar	24 hr at RT	More than 2 years
Ixabepilone/Ixempra	1 hr at RT	More than 2 years
Infliximab/Remicade	3 hr at RT	More than 2 years
Asoarginase/Erwinase	4 hr at RT	More than 2 years

ADVANTAGES: [5]

1. Aqueous Stability - To ensure that less than 10% of the product degrades in the first 2-4 years. To put it another way, the storage period should be extended.

2. Some APIs are moisture sensitive and can't be found stable in water.

3. Thermal stability is required for thermolabile compounds and temperature-sensitive APIs.

4. Product qualities has improved.

5. Freeze-dried foods have a greater quality than other methods of dehydration. The lack of a liquid phase, as well as the low temperature of the process, contribute to its high quality.

6. Shipping advantage - lyolight weight mass- low weight and leakage can be overcome since the product is in dry form.

7. Dosage specificity - how easily complex formulations can be filled as a solution.

8. Nitrogen, argon, and vacuum are used to create a sterile and stable headspace.

9. It is simple to use because it is automated.

10. Freeze-drying keeps the flavour, colour, and appearance of the food while reducing thermal damage to heat-sensitive nutrients. Furthermore, because the process occurs in a solid form, the texture is highly retained. Freeze-dried foods are frequently crisper than air-dried meals and have four to six times higher rehydration ratios.

DISADVANTAGES: [5]

1. Costly and complex equipment that requires more maintenance has a high energy consumption and high operating and maintenance costs. Freeze-drying requires nearly double the amount of energy as traditional air drying.

2. Increased handling and processing time, e.g., 7-day cycles.

3. Reconstitution necessitates the use of sterile diluents.

4. Scaling-up and transfer issues.

PHASE DIAGRAM OF WATER [6]**:**

A phase diagram is a graphical representation of several phases of a material or mixture of substances that exist in thermodynamic equilibrium and change phase under various operating conditions such as temperature, pressure, or volume.

The three stages of the water system are ICE (S), WATER (L), and WATER VAPOR (G).

Because water is the only chemical compound present, it is a one-component system.

Depiction in Phase Diagram of Water:

A phase diagram displays the preferred physical states of matter at various temperatures and pressures. Water is a liquid at normal room temperatures and pressures, but it solidifies (i.e.,

ice) when the temperature drops below 273 K, and becomes gaseous (i.e., steam) when the temperature rises over 373 K. Each line depicts the conditions that exist while two phases coexist; however, a change in temperature or pressure can instantly shift the phases. When three lines come together, there is a 'triple point,' where three phases coexist but can instantly and completely shift into each other due to a change in temperature or pressure. Four lines cannot cross at the same time. The point at which the qualities of two phases become indistinguishable from one another is known as a 'critical point.'

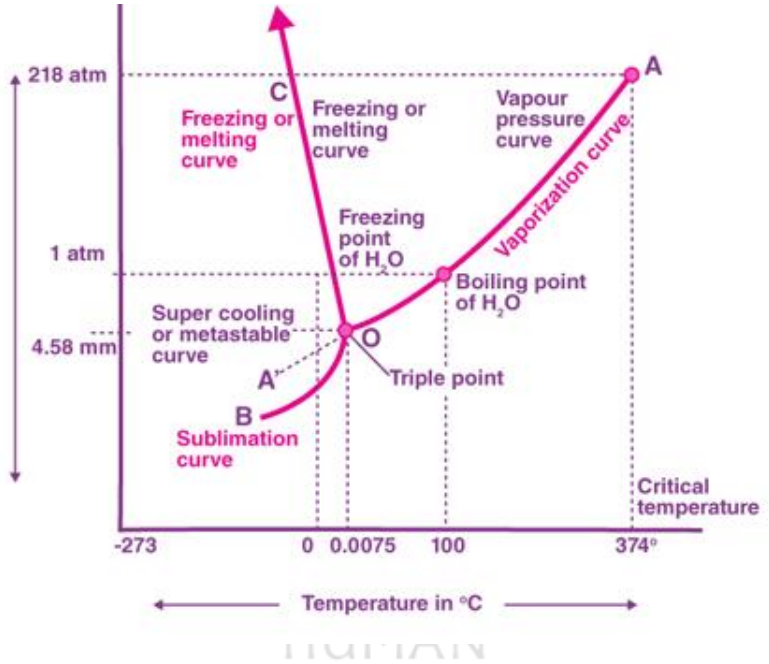

Figure 1: Phase diagram of water.

Curves:

1. OA- Water & Vapor

The letter OA stands for the vaporisation curve. The OA curve comes to an end at A. It has a critical pressure of 218 atm and a temperature of 374°C. It displays the vapour pressure of a liquid at various temperatures. Along the curve, two phases of water and water vapour are in equilibrium. The vapour pressure is one atmosphere. The equivalent temperature in degrees Celsius is the boiling point of water, which is 100°C.

2. OB- Ice and Vapor

3. The OB curve is a sublimation curve. The curve OB comes to an end at B, the absolute zero temperature of -273°C. The vapour pressure of solid ice is shown at various temperatures. Solid ice and water vapour coexist in a state of equilibrium.

4. OC- Ice & Water

The OC curve is the fusion curve. The OC curve comes to a standstill at C, the critical pressure. Solid ice and liquid water live in a healthy balance. As the chart shows, the melting point of ice decreases as pressure increases. At 0°C and one atm, the line intersects the curve.

Areas:

The areas between the curves AOC, AOB, and BOC reflect the temperature and pressure parameters under which a single phase, such as ice, water, and water vapour, can remain indefinitely.

1. The solid phase is denoted as BOC-Ice.
2. The liquid phase is denoted by COA-Water.
3. The gaseous phase is denoted as AOB- Vapour.

Triple point: Where the three curves, OA, OB, and OC, connect is the triple point, which is the point at which all three phases, solid, liquid, and vapour, are in simultaneous equilibrium. At 0.0075°C and 4.58 mmHg pressure, the triple point is reached.

Critical temperature: The critical temperature (TC) of a substance is the maximum temperature at which it may exist as a liquid.

The critical point is defined as the intersection of the critical temperature and pressure. Water particles in the gas phase travel extremely quickly at 373.99°C. The gas phase cannot liquefy at any temperature higher than that, regardless of how much pressure is applied to it.

SUBLIMATION: [7]

Sublimation is the transformation of a solid (ice) into a vapour without passing through a liquid (water). Sublimation occurs only at low pressures, as shown in the phase diagram for water. Sublimation is a phase transition in which heat energy is added to a frozen product.

In the freeze-drying process, sublimation can be simply defined as:

1. Freeze: The product is fully frozen in a vial, flask, or tray.

2. Vacuum: The product is then placed under a high vacuum, well below the triple point of water.

3. Dry: The product is then provided heat energy, causing the ice to sublime.

HEAT AND MASS TRANSFER PHENOMENON:

1. For the sublimation process, heat energy is provided.

2. If more heat is delivered than is required for phase shift, the excess heat will be used to raise the product's temperature and finally melt the ice.

3. The heated shelves in the lyophilizer chamber are the source of heat (ambient heat, IR, etc).

To accelerate the pace of sublimation, do the following:

1. Increase the amount of heat available (shelf temperature)

2. Raise the temperature of the product.

3. The rate of drying doubles for every 10 degrees Celsius increase in product temperature.

4. Heat transfer is the process of transferring heat into a vial.

5. Mass transfer refers to the movement of water vapour from the vial.

6. To keep the product frozen and the sublimation process going, the two transfer procedures must be equivalent.

Flow of heat transfer:

1. In freeze drying, this is the rate limiting phase.

2. Heat is transferred from the heat medium to the shelf surface.

3. Three paths for heat transmission to the vial's bottom.

i. Heat transfer is handled by a gas that exists between the shelf surface and the vial.

ii. Heat transfer at the vial's bottom surface where it comes into contact with the shelf.

iii. Heat is transmitted using radiant heat from the lyophilizer's walls.

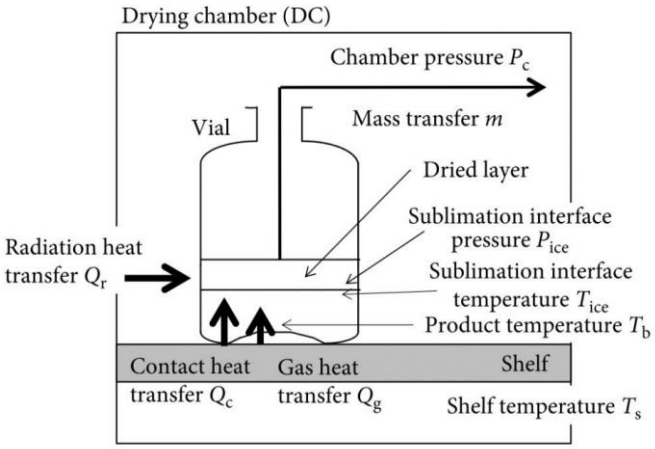

Figure 2: Schematic diagram of heat transfer. [8]

4. The sublimation contact receives heat from the bottom of the vial.

5. This heat was converted to latent heat of sublimation and consumed.

6. Ice is converted to vapour through heat transmission.

7. The formation of a dry layer occurs.

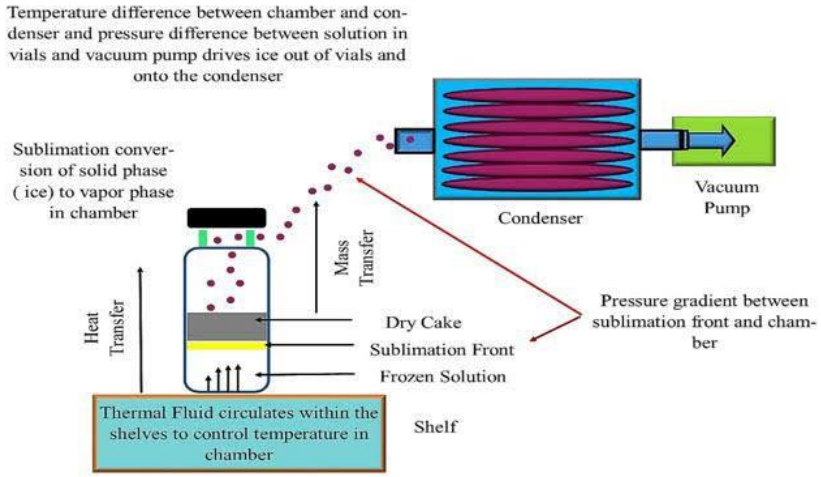

Figure 3: Schematic diagram of heat and mass transfer in drying chamber. [9]

8. The water vapour from the vial is ejected.

9. The water vapour exits from the chamber and condenses in the condenser, where it freezes on the condenser plates' cold surfaces.

10. To avoid a pressure rise that would inhibit sublimation, the vapour generated must be continuously evacuated.

STEPS IN LYOPHILIZATION:

1. Pre-treatment
2. Freezing and Annealing
3. Primary Drying
4. Secondary drying
5. Sealing lyophilized products

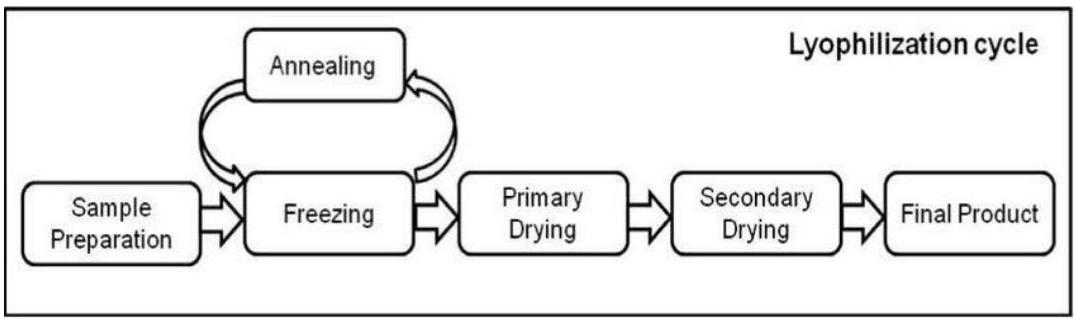

Figure 4: Steps involved in lyophilization. [10].

1. PRETREATMEMT:

Any procedure of "improving" the product prior to freezing is considered pre-treatment. This could include things like:

1. Increasing product concentration.
2. Adding water to the product.
3. Revisions to the formulation, such as the incorporation of components to improve stability, appearance, or processing.
4. Lowering the vapour pressure of a solvent with a high vapour pressure.

5. Increasing the area of the surface.

In many cases, the decision to pre-treat a product is based on theoretical knowledge of Freeze Drying and its needs, which are influenced by cycle time or product quality concerns. [11]

2. FREEZING:

Prior to pulling a vacuum and beginning the drying process, it is critical that the sample be thoroughly frozen. When placed under a vacuum, unfrozen product may expand outside of the container. [12] The freezing process is crucial since it controls the ice morphology and distribution of pore sizes. Rapid cooling produces little ice crystals, which are useful for conserving features for microscopy but make it more difficult to freeze dry the product. The freezing temperature is between -50ºC and -80ºC. The freeze dryer acts like a freezer during pre-freezing because no vacuum is applied. Separate from the dryer, pre-freezing could be done. The freezing capability of more advanced shelf freeze dryers is incorporated into the product shelf, allowing the product to be frozen inside the freeze dryer. Product is either pre-loaded into vials and then placed to the shelf, or it is loaded directly onto a product tray in bulk form. Shelf freeze dryers enable for precise cooling rate control, which has an impact on product freezing rates and crystal size. Because of the bigger vapour routes left behind in the dried portion of the product as the ice crystals are sublimated, larger ice crystals improve the speed of the freeze-drying process. Because some biological products are sensitive to large ice crystals, they must be freeze dried using smaller ice crystals. The majority of freeze-dried samples are eutectics.

EUTECTIC POINT:

1. The eutectic temperature is the lowest melting point imaginable.

2. If the temperature of the frozen sample rises over the eutectic point, it will melt.

3. Before initiating the freeze-drying process, it is critical to pre-freeze the product to temperatures below the eutectic temperature.

ANNEALING:

When amorphous products (such as mannitol or glycine) are frozen, they create a metastable glass with partial crystallisation. A heat treatment method, also known as annealing, can

benefit these products. To produce more thorough crystallisation, the product temperature is cycled during annealing (for example, from -40ºC to -20ºC for a few hours and then back to -40ºC). Thermal stress is reduced [13].

3. PRIMARY DRYING:

During the primary drying process, all of the free ice crystals are sublimated in a vacuum, removing the majority of the water from the product. During primary drying, organic solvents are also eliminated. Chamber pressure is 40 to 400 Torr and the shelf Temperature is around -30ºC to -10ºC.

The ideal primary drying period is 10 hours, with an additional hour or two added if necessary to achieve thorough drying. The product is kept in a solid state below its eutectic melting point or collapse temperature to dry it while keeping the structure produced during the freezing stage. To avoid melting the frozen cake, which would result in its collapse and incorrect drying, the primary drying temperature should be kept several degrees below this temperature. The temperature of both the product and the shelf within the drying chamber must be carefully monitored during primary drying in order to determine when the product's temperature equals the shelf temperature, signifying the end of this phase.

The rate of effective heat transmission between the shelf and the vials carrying the product is determined by the degree of vacuum in the primary drying chamber, which must be constantly monitored. A main drying phase under higher vacuum will allow more effective heat transfer, speeding up drying and resulting in a more cost-effective process if the vacuum can be increased gradually without harming the API. [14]

One of numerous mechanisms allows heat to enter the product:

1. By putting the container in direct touch with the shelf.

2. The sublimation interface is reached through conduction across the container base and then through the frozen substance.

3. By gaseous convection between the product and the chamber's leftover gas molecules.

4. By radiation.

Determination of the end of Primary Drying

There are several analytical methods for identifying whether primary drying is complete. The most basic way is to use a thermocouple probe to measure the product temperature. Because the heat from the shelf is used for the sublimation phase shift, the recorded product temperature will be lower than the shelf temperature set point during active primary drying. When the ice crystals have completely sublimated, the product temperature will rise until it reaches the shelf temperature. When the product temperature reaches the same level as the shelf temperature, primary drying is accomplished. [15]

4. SECONDARY DRYING

A significant number of water molecules are attached to the product, in addition to the free ice that is sublimed during primary drying. During secondary drying, this is the water that is eliminated (desorbed). Due to the removal of all free ice during primary drying, the product temperature can now be increased significantly without concern of melting or collapse. Secondary drying begins during the main phase, however desorption occurs considerably more quickly at higher temperatures (usually in the 30ºC to 50ºC range). The temperature of the product affects secondary drying rates. In comparison to the vacuum employed in primary drying, a higher degree of vacuum in the secondary drying phase can permit faster water desorption and speed up the secondary drying process. Secondary drying is carried out until the product's moisture content is suitable for long-term storage. Moisture level in thoroughly dried items ranges from 0.5 percent to 3 percent, depending on the use. In most circumstances, the longer the shelf life of a product, the more dry it is. Certain complex biological materials, on the other hand, may become excessively dry for optimal storage results, necessitating careful regulation of the secondary drying process. A "sample thief" mechanism may be employed to periodically retrieve vials from the freeze drier for residual moisture content assessment during secondary drying. During the secondary drying phase, it's a good idea to take the vials out of the lyophilizer and use infrared scans to check their residual moisture content. The secondary drying process is finished when the moisture content of the cake drops below excessive levels while still keeping enough moisture to keep the product stable. [11]

5. SEALING LYOPHILIZED PRODUCTS

Before withdrawing a freeze-dried product from the ultra-dry atmosphere created at the end of the freeze-drying process, it should be sealed within its container. Because the formulation that has been through this cycle has less than 1% moisture most of the time, when it comes into touch with a moisture-containing environment, the product will strive to absorb moisture as its capacity. The product's quality will immediately deteriorate. The lyophilized product's chemical performance, shelf life, and quick reconstitution qualities, which are required after freeze drying, may be jeopardised. If the product absorbs moisture again, it will result in product loss, incorrect results, product failure, and product recalls.

All four stages of the lyophilization process (freezing, primary drying, secondary drying, and sealing) are equally important for the proper performance of any lyophilization process, so it can generate a dried and stable product for long-term storage. [13]

CRITICAL PARAMETERS OF LYOPHILIZED PROCESS: [13]

Table 2: Critical parameters of lyophilized process.

STEPS	CRITICAL PARAMETERS OF PROCESS
STEP 1- FREEZING	RAMP Freezing temperature and time Annealing
STEP 2- PRIMARY DRYING	RAMP Target product temperature Shelf temperature Primary drying end point Chamber pressure
STEP 3- SECONDARY DRYING	Heating rate Chamber pressure Shelf temperature
FINAL PRODUCT	Physical appearance Residual moisture

MERITS OF LYOPHILIZATION CYCLE:

1. Because drying occurs at such low temperatures, chemical degradation, especially hydrolysis, is limited.

2. The solution is frozen in the same volume as the original, resulting in a light and porous product.

3. Because of the porosity nature of the substance, it is easily soluble.

4. Prior to drying, there is no concentration of solution. As a result, unlike other drying processes, salts do not concentrate and denature proteins.

5. Because the procedure is carried out under high vacuum, there is limited contact with air, which reduces oxidation.

6. This procedure is used to dry things that can't be dried any other way. Antibiotics, blood products, vaccinations, enzyme preparations, and microbiological cultures are examples of biological products.

DEMERITS OF LYOPHILIZATION CYCLE:

1. The substance is extremely hygroscopic due to its porosity, rapid solubility, and total dryness. Packing necessitates particular circumstances unless things are dried in their final container and sealed in place.

2. The procedure is extremely sluggish and necessitates the use of a complex and expensive plant.

LIMITATIONS OF LYOPHILIZATION CYCLE:

1. Although freeze drying products like blood plasma is straightforward in theory, it has a number of practical issues: - Because the presence of dissolved solutes lowers the freezing point, the solution must be chilled below the standard freezing temperature for pure water (-10-30).

2. Sublimation is a slow process that can only happen on a frozen surface (1mm thickness of ice per hour). As a result, the surface area must be expanded, and

3. To lower the thickness of ice to be sublimated, the liquid thickness previous to freezing should be reduced.

4. Large amounts of water vapour are created at low pressure and must be evacuated to keep the pressure from climbing above the triple point pressure.

5. The dry material must frequently be sterile, and it must be kept from regaining moisture before being packaged.

CONCLUSION:

The process of creating a successful lyophilized product can be time and energy consuming. High concentration formulations are more resistant to freezing and lyophilization in general. Given the lyophilization process's and formulation's complexity, design, production of a lyophilized formulation with a high concentration and quick reconstitution. Individual process parameter optimization and a thorough understanding of the process take time and variety of freezing and drying conditions. This review gives a clear idea of each and every steps of lyophilization and merits, demerits and limitation of lyophilization cycle.

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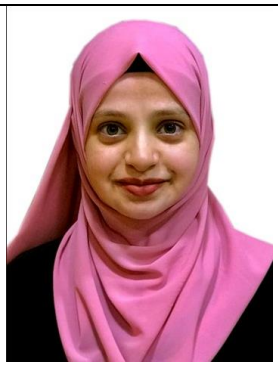	***Author Name – Heena Tajir (Corresponding Author)*** *Author Affiliation – Research Student* *Author Address/Institute Address – H. K. College of Pharmacy, Oshiwara, Jogeshwari west, Mumbai – 400102.*
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