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A REVIEW ON: LYOPHILIZATION

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Abstract:

In current scenario Pharmaceutical industries have different modified method for drying and other process. Lyophilization is one of the most promising methods for Drying. Lyophilization or freeze drying is a process in which water is frozen, followed by its removal from the sample, initially by sublimation (primary drying) and then by desorption (secondary drying). In this review we focused on basic principles of lyophilisation, process in detail along with advantages and disadvantages.

Keywords: Lyophilization, Freeze Drying, Sublimation, Desorption

INTRODUCTION:

The coining of the term Lyophilization is generally attributed because of porous nature of the dried product & its "lyophil" characteristics to rapidly reabsorb the solvent & restores the substance to its original state. The lyophilization equated with freeze drying, the latter term has become more common because it is applicable to both aqueous & non aqueous systems.[1]

In simplest form Lyophilization is defined as a stabilizing process in which the substance is first frozen & then quantity of the solvent is reduced first by sublimation (primary drying) & then by desorption (secondary drying). To values that will no longer support biological growth or chemical reactions.[2]

Lyophilization or freeze drying is a process in which water is frozen, followed by its removal from the sample, initially by sublimation (primary drying) and then by desorption (secondary drying).

The lyophilization means to make solvent loving. Operationally freeze-drying defines as a controllable method of dehydrating labile products by vacuum desiccation. Technically, freeze-drying may be defined as:

- Freezing of the liquid sample, followed by the conversion of water into ice and crystallization of crystallizable solutes as well as formation of an amorphous matrix comprising non-crystallizing solutes associated with unfrozen moisture.
- Sublimation of freezed ice under vacuum.
- Evaporation of moisture from the amorphous matrix.

The steps involved in the formulation of freeze dried product are depicted in below figure:

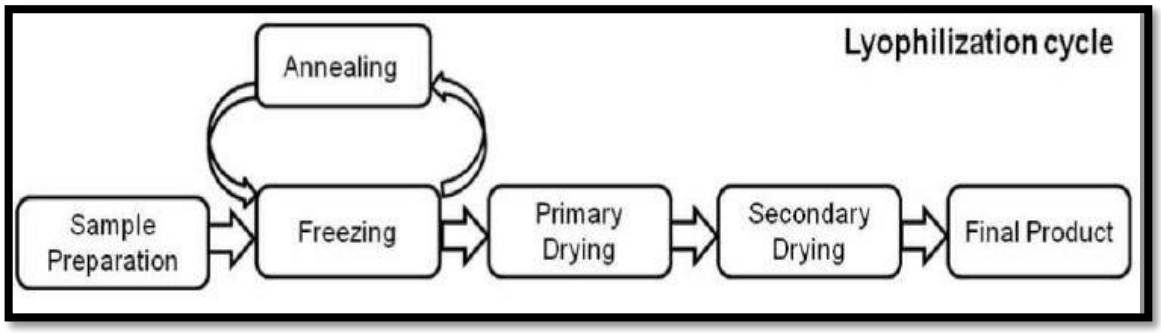

Fig 1.Steps involved in lyophilization from sample preparation to final product formation [3]

Lyophilization is the most common method for manufacturing parenteral when aqueous solution stability is an issue. It is central to the protection of materials, which require low moisture content (less than 1%) in order to ensure stability and require a sterile and gentle preservation process[4]. Freeze drying has been used in a number of applications for many years, most commonly in the food and pharmaceutical industries. There are, however, many other uses for the process including the stabilization of living materials such as microbial cultures, preservation of whole animal specimens for museum display, restoration of books and other items damaged by water, and the concentration and recovery of reaction products [5].

Principle in Lyophilization:

At atmospheric pressure (approx. 1,000 mbar) water can have three physical states
Solid;
Liquid;
Gaseous.

Below the triple-point (for pure water: 6.1 mbar at 0°C), only the solid and the gaseous states exist

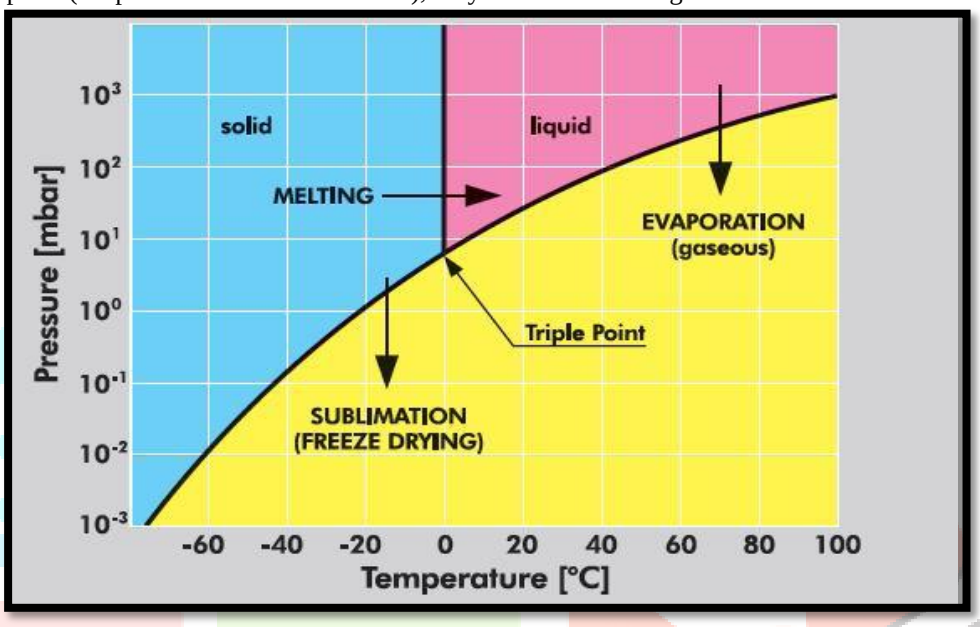

Fig 1.4- Phase diagram of water

The principle of freeze/sublimation-drying is based on this physical fact. The ice in the product is directly converted into water vapor (without passing through the "fluid state") if the ambient partial water vapor pressure is lower than the partial pressure of the ice at its relevant temperature.

The main principle involved in freeze drying is a phenomenon called sublimation, where water passes directly from solid state (ice) to the vapour state without passing through the liquid state. Sublimation of water can take place at pressures and temperature below triple point i.e. 4.579 mm of Hg and 0.0099 degree Celsius. [6]The material to be dried is first frozen and then subjected under a high vacuum to heat (by conduction or radiation or by both) so that frozen liquid sublimes leaving only solid, dried components of the original liquid. The concentration gradient of water vapour between the drying front and condenser is the driving force for removal of water during lyophilization. Lyophilization is performed at temperature and pressure conditions below the triple point, to enable sublimation of ice. The entire process is performed at low temperature and pressure, hence is suited for drying of thermo labile compounds.

Advantages of Freeze-Dried Products

1. Product is stored in dry state-few stability problems
2. Product is dried without elevated temperatures
3. Good for oxygen and/or air-sensitive drugs
4. Rapid reconstitution time
5. Constituents of the dried material remain homogenously dispersed
6. Product is process in the liquid form
7. Sterility of product can be achieved and maintained

Disadvantages of Freeze-Dried Products

1. Volatile compounds may be removed by high vacuum
2. Single most expensive unit operation
3. Stability problems associated with individual drugs
4. Some issues associated with sterilization and sterility assurance of the dryer chamber and aseptic loading of vials into the chamber.

Desired Characteristics of Freeze-Dried Products

- Intact cake
- Sufficient strength
- Uniform color
- Sufficiently dry
- Sufficiently porous
- Sterile
- Free of pyrogens
- Free of particulates
- Chemically stable

Steps involved in lyophilisation:

Basic steps involved in lyophilisation are as follows:

- Freezing
- Primary drying
- Secondary drying

1. Freezing:-

The principle involved in the freezing step is to separate the solvent from solute.During freezing solvent generally water freezes o ice. The ice crystals form which was entrapped the solute in ice crystal matrix.A product freezes in two ways, depending on the makeup of the product. The majority of products that are subjected to freeze drying consist primarily of water, the solvent, and the materials dissolved or suspended in the water, the solute. Most samples that are to be freeze dried are eutectics which are a mixture of substances that freeze at lower temperatures than the surrounding water.

The second type of frozen product is a suspension that undergoes glass formation during the freezing process. Instead of forming eutectics, the entire suspension becomes increasingly viscous as the temperature is lowered. Finally the product freezes at the glass transition point forming a vitreous solid. This type of product is extremely difficult to freeze dry.[7]

Product can be frozen by two methods;

1. Immersion Freezing:-[8]

In this method, when a container is immersed in a cold gas or liquid, ice will form normal to the wall of the container result in the formation of layers of interstitial material perpendicular to the flow of the water vapour from the container.

2. Snap freezing:-[9]

In this method product is placed in the chamber. Chamber is evacuated and pressure is reduced by degassing. Heat of evaporation and heat of sublimation reduces the temperature of the matrix to less than collapse temperature, in this the ice crystals form at the top of the formulation rather than at bottom.

Limitation of this method is evaporation of the water will result in a change in the concentration of product. For reduction of temperature less than chamber temperature,sublimation of ice must occur but the sublimation occurs above the chamber temperature.

Super cooling: - [10, 11, 12].

This refers to the temperature difference at which nucleation of ice crystals occurs. Nucleation proceeds through hydrogen bonding at triple point i.e 0°c.

Modes of supercooling:-

i. Homogeneous nucleation:-

In this mode nucleation occurs through critical random aggregation of ice crystals. It occurs at temperature less than -15°c.

ii. Heterogeneous nucleation:-

In this mode ice crystals form on foreign substances like wall of container, particulate matter, proteins etc. it occurs temperature more than-15°c.

Mechanism for heterogeneous nucleation is adsorption of layers of water on the surface or on the particles and because of temperature of the water is below the triple point, these layers have structure with ice-like water cluster, serve's as nuclei for the formation of ice crystals.

Effect of supercooling:-

An increase in the degree of supercooling could produce a dense cake structure that could impede the transport of water vapour from the product.The decrease in supercooling may result in the formulation of a crust or glaze on the top surface which could also impede the secondary drying process.

Annealing:-

Annealing is holding the product at a temperature above the final freezing temperature for a defined period to crystallize the potentially crystalline component (eg. Bulking agent) in the formulation during freezing stage. Annealing temperature should be between glass transition temperature of amorphous phase and eutectic temperature of bulking agent to give high crystallization rate.

Normal freezing or fast freezing produces small crystals that are hard to dry; annealing produces large crystals that can be easily dried.

2. Primary Drying:-[13]

After the freezing of product, pressure is reduced and heat is applied to the formulation to initiate the sublimation process of ice crystals.As the sublimation of the ice crystals precedes the ice-gas interface recedesthrough cake.Completion of primary drying achieved by complete removal of ice crystals from the cake.

The volume occupied by the cake after primary drying is equals to the frozen matrix. The sublimation of water from the product requires energy (temperature-dependent, around 670 cal/g), leading to cooling of the product. The energy for continuing sublimation of ice needs to be supplied from the shelves that are heated to a defined higher temperature. The product temperature is in general the most important product parameter during a freeze drying process, in particular the product temperature at the sublimation interface during primary drying.

3. Secondary Drying:-[13]

Finally all ice has already been removed, desorption of water from the cake occurs; this process is referred to as secondary drying and already starts in the primary drying phase. Once all ice has been removed from all product containers, the shelf temperature is elevated and typically maintained at a temperature between 20°C and 40°C for several hours. The rate of desorption and the obtainable moisture level is controlled by diffusion within the solute phase and desorption from the surface and therefore depends mostly on product temperature; further reduction of chamber pressure is not required.

Completion of secondary drying can be identified by measuring the pressure.At the end of the secondary drying partial pressure of nitrogen was increases and partial pressure of water vapour decreases. Thermal conductivity gauge and capacitance manometer gauge were used for pressure measurement.

Determination of End Point of Freeze-Drying Process: [14, 15.]

The following are the techniques used for determination of end point of primary drying process,

Techniques based on gas composition in the product chamber:

1. Comparative pressure measurement (i.e., Pirani vs. capacitance manometer)
2. Dew point monitor (electronic moisture sensor)
3. Process H_2O concentration from tunable diode laser absorption spectroscopy (TDLAS)
4. Lyotrack (gas plasma spectroscopy)

Others:

5. Product thermocouple response
6. Condenser pressure
7. Pressure rise test (manometric temperature measurement (MTM) or variations of this method)

Properties of Lyophilized Materials:-[16]

Stability:

The principle purpose for conducting a lyophilization process is to enhance the stability of a formulation (Slow down kinetic clock for degradation or loss in potency of the active constituents).The dried formulation is considered stable as long as its activity or its potency remains within a given range of values. The expiration date of product will be determined from the length of time that all the lyophilized formulations remain within the specified potency limits. There are two basic methods for determination of the stability of lyophilized products accelerated & long term or real time stability studies.

Cosmetics properties:

Cosmetic properties or appearance of a lyophilized product is dependent, in varying degrees, on each step of the lyophilization process. The uniform cake structure is a result of ice structure that formed during the freezing process. The condition under which primary drying is conducted can result in partial collapse of cake structure. The presence of such collapsed is generally associated with the primary drying process when the ice product matrix is not in a completely frozen state. Melt back is result of the presence of liquid state in the ice product matrix.

Moisture:

The main function of the lyophilization has been defined as enhancing the stability of a formulation primarily by the reduction of solvent system, primarily water, to quantities that will no longer support biological growth or chemical reactions. The quantity of water remaining in the product, in order to achieve the desired stability, is product dependent.

Reconstitution:

Reconstitution is the process that is used to restore the lyophilized product to its original formulation. The process involves addition of known quantity of diluent to the dried cake. The properties (potency of active constituent & pH) of the resulting solution

should be within the prescribed limits of the original formulation. Excessively long reconstitution times, loss in potency or the formation of turbid solution are indication of an improper lyophilization process or a malfunction of the freeze-drying equipment.

Product Properties and lyophilisation process:-

A. General Properties:-[17]

i. Moisture:-

Moisture present in the product will affect the stability of product. Moisture cause microbial growth in the product.

Moisture present in two forms:-

a. Free water:-

This is moisture absorbed by the cake or adsorbed on the cake surface. This water can steam either directly from the interstitial region (Final cake) or from the flow of water vapour that occurs during primary drying process.

This form of moisture should be removed from the product as this form is responsible for instability of dried formulation.

b. Bound water:-

This form of moisture is associated with stability of active constituents or excipients like constitute the hydrate form of compound or may be involved in configuration of protein molecule.Removal of this form of water may cause denaturation of proteins. Water of stability that is **surface water** a form of bound water is identified with stability of protein. It forms pentagonal rings which are associated with hydrophobic region and chain like array with hydrophilic region. This association confirms stability of proteins.

ii. Reconstitution:-

Moisture present in the product causes increase in the reconstitution time.

B. Physical Properties:-

i. Effect of temperature (Transition temperature):-[18]

Transition temperature refers to the temperature at which lyophilised cake undergoes liquefaction .As the residual water increases the transition temperature increases.

ii. Cake Volume:-[19, 20.]

Cake volume of lyophilised cake is generally proportional to the frozen matrix.

iii. Colour of cake:-[21]

In case of rapidly frozen matrix, some products like food tend to produce a light colour cake. When product is frozen at a slower rate, the resulting dried product will have darkened colour.

iv. Texture:-[22]

The lyophilised cake has highly porous, sponge like appearance due to removal of moisture.

v. Cake Density:-[23]

The cake density in the absence of collapse or melt back during primary process is an indication of self-supporting cake. For lyophilised product cake density is low.

vi. Shrinkage or collapse:-[24]

Shrinkage occurs when cake volume is less than that of the original frozen matrix. Shrinkage occurs if water is present in interstitial region. PVP at high concentration is used to prevent collapse.

vii. Pores:-[23]

Due to the primary drying process porous lyophilised mass is formed as moisture is completely removed.

viii. Cake strength:-[25]

Strength of the lyophilised cake depends on efficacy of freezing step. Quickly frozen products have higher cake strength than that of slowly frozen product. NaCl and PVP used to improve strength of product. PVP has capability to increase the strength of cake due to its elastic and thread-like binding properties arising from polymeric characteristics.

C.Thermal Properties of products:-

i. Collapse Temperature:-

It is the temperature which referred to the sublimating interface of the matrix. At this temperature the mobility of the water in the interstitial region of the matrix becomes significant. If mobile water present then the interstitial region cannot be completely frozen.

ii. Interstitial melting temperature:-

The interstitial melting temperature is defined as the temperature at which there is known liquid state present in interstitial region of the matrix. It cause meltback or collapse during primary drying process.

iii. Ice melting temperature:-

Ice melting temperature is onset temperature required for the melting of ice in the matrix. These thermal properties not have measure effect on lyophilisation process.

Parameters affected by lyophilization:-[26]

i. Potency:-

In case of biological products such as vaccines, the freezing process may cause loss in the titer of the vaccines.

ii. Froth and foam:-

Froth or foam form because of two reasons. First, if the vials are filled with the formulation containing proteins froth or foam present in final cake. And secondly if formulation contains significant quantities of dissolved gases, particularly CO_2, the gas will be concentrate in the interstitial region is still in liquid state.As the gas pressure increasesmatrix get deformed release of gas occurs and foam will form. These can be reduced by sparging with gas that has low solubility like NO_2, He.

iii. Crust or Glaze:-

It depends on the nature of freezing process. Formation of large ice crystals due to heterogeneous matrix. Large ice crystals that tend to concentrate unfrozen formulation and push the solute at the top surface.

iv. Melt back:-

This refers to the extreme case of collapse in which liquid state exists in the interstitial region during primary drying process. Thus interstitial region no longer supporting and collapse occurs.

Melt back can be occurs in two ways:

a. Partial melt back:-

It occurs when the temperature of shelf surface is increased to commence the secondary drying process prior to completion of primary drying.

b. Total melt back:-

It occurs through the entire batch and all vials have higher product temperature than that of chamber.

v. Browning:-[29]

It occurs when cake having carbohydrates & ascorbic acid as constituents are exposed to the excessive heat during secondary drying process the result is browning or discoloration.

vi. Puffing: [30, 31.]

Puffing refers to cake defect in which upper surface of cake expand during the interstitial portion of the primary drying process. This occurs due to incomplete freezing prior to lowering chamber pressure.

vii. Mushy state:-[32, 33.]

This refers to the observation of chimney like structure in the middle of the cake. This occurs because freezing start from the bottom of the container and mushy state consisting of a mixture of solid and liquid phase.

Reconstitution of lyophilized cake:-[34]

Reconstitution refers to rehydration of material to its original state by the addition of suitable diluent. Sterile Water for injection, NaCl solution and dextrose solution is used as diluent.A sterile solution of diluent with respect to the volume of cake is inserted by needle through the closure. Reconstitution time may be from few seconds to one hour.

Properties of Diluents:-

1. pH of diluents: pH between 5 – 7 is acceptable from stability point of view.

2. Temperature: Diluent temperature is between 20 - 25◦c. Warm or boiling water is not tolerated by cake.

3. Particulate matter: Diluent should be free from particulate matter to comply with USP specifications.

Relationship between lyophilized process and reconstitution time is summarized in following table:

Table 1. Relationship between lyophilized process and reconstitution time

Sr no.	Lyophilization process		Impact on Reconstitution time
1.	**Freezing**	Homogeneous cake	Rapid reconstitution time
		Heterogeneous cake	Extension of reconstitution time
2.	**Primary drying**	Collapse	Extension of reconstitution time
		Puffing	Extension of reconstitution time
		Meltback	Extension of reconstitution time
3.	**Secondary drying**	Complete removal of moisture in protein	Extension of reconstitution time

Conclusion:

The lyophilized technique is used in the development of stable injectable dosage form for a drug those having poor self-life and stability problem degraded in the presence of moisture content present in the formulation. By using freeze drying technique the stability and self-life of the product are enhanced. The dry pharmaceutical product is mainly prepared by lyophilization. Approximately 50% of biopharmaceuticals products are lyophilized, it representing the most common formulation technique.

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