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REVIEW ON: LYOPHILIZATION PROCESS OF PHARMACEUTICALS.

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ABSTRACT

Lyophilization (Freeze drying) often used to prepare dry pharmaceutical formulation to achieve commercial viable shelf life. Properly freezed dried product do not need to refrigerarate it can be stored at room temperature also. Lyophilization is common but cost intensive. Suitable parameters of process application allow us to obtain best quality products compared to products dried with traditional methods. In pharmaceutical field lyophilization has become important subject to ongoing development and its expansion. Process consists of three steps: Freezing, primary drying, secondary drying. Article focused on how different factors affect lyophilization process and also gives a comprehensive list of excipients used in lyophilized formulations.

KEYWORDS: Lyophilization, Freezing, primary drying, secondary drying.

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.

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.[1]

Freeze drying is a process of drying in which water is sublimed from the product after it is frozen. It is a drying process applicable to manufacture of certain pharmaceuticals and biologicals that are thermolabile or otherwise unstable in aqueous solutions for prolonged storage periods, but that are stable in the dry state. The term "lyophilization" describes a process to produce a product that "loves the dry state".[2]

Freeze drying has used in number of applications from many years in food & pharmaceuticals however there are many other uses for process including stabilization of living materials including microbiological culture & other items damage by water. Freeze drying involve removal of water & other solvents from the frozen matrix by the process known as sublimation. Lyophilization is stabilizing process in which substance is first frozen and then quantity of solvent is reduce by sublimation then desorption which do not need refrigeration & can be stored at ambient temperature.[3]

PRINCIPLE IN LYOPHILIZATION

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.[4] 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.[5] 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. Steps involved in lyophilization start from sample preparation followed by freezing, primary drying and secondary drying, to obtain the final dried product with desired moisture content. The concentration gradient of water vapour between the drying front and condenser is the driving force for removal of water during lyophilization. The vapour pressure of water increases with an increase in temperature during the primary drying. Therefore, primary drying temperature should be kept as high as possible, but below the critical process temperature, to avoid a loss of cake structure. This critical process temperature is the collapse temperature for amorphous substance, or eutectic melt for the crystalline substance. During freezing, ice crystals start separating out until the solution

becomes maximally concentrated. On further cooling, phase separation of the solute and ice takes place.[6]

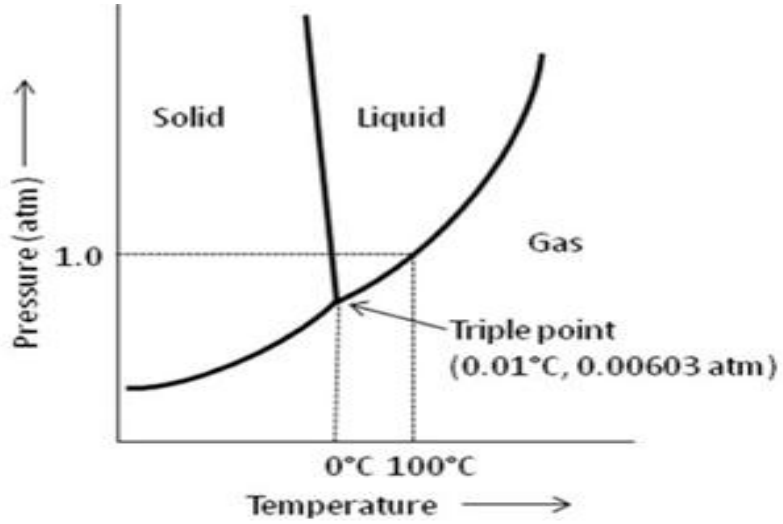


Fig1. Phase diagram showing the triple point of water at 0.01°C, 0.00603 atm

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 (Table 1).

To extract water from formulation, the process of lyophilization consists of:

1. Freezing the formulation so that the water in the product becomes ice.
2. Under a vacuum, sublimating the ice directly into water vapour.
3. Drawing off the water vapour.[7]

Temp (^{o}c)	Vacuum mmHg	Temp (^{o}c)	Vacuum mmHg	Temp (^{o}c)	Vacuum mmHg
0.0	4.5840	-15.0	1.2399	-30.0	0.2851
-0.5	4.3987	-15.5	1.1839	-30.5	0.2706
-1.0	4.2204	-16.0	1.1302	-31.0	0.2568
-1.5	4.0486	-16.5	1.0787	-31.5	0.2437
-2.0	3.8832	-17.0	1.0295	-32.0	0.2311
-2.5	3.7240	-17.5	0.98222	-32.5	0.2192
-3.0	3.5707	-18.0	0.93698	-33.0	0.2078
-3.5	3.4232	-18.5	0.89368	-33.5	0.1970
-4.0	3.2813	-19.0	0.85222	-34.0	0.1867
-4.5	3.1448	-19.5	0.81251	-34.5	0.1769
-5.0	3.0134	-20.0	0.77451	-35.0	0.1676
-5.5	2.8872	-20.5	0.7381	-35.5	0.1587
-6.0	2.7657	-21.0	0.7034	-36.0	0.1503

-6.5	2.6489	-21.5	0.6701	-36.5	0.1423
-7.0	2.5366	-22.0	0.6383	-37.0	0.1347
-7.5	2.4287	-22.5	0.6078	-37.5	0.1274
-8.0	2.3250	-23.0	0.5787	-38.0	0.1206
-8.5	2.2254	-23.5	0.5509	-38.5	0.1140
-9.0	2.1297	-24.0	0.5243	-39.0	0.1078
-9.5	2.0377	-24.5	0.4989	-39.5	0.1019
-10.0	1.9494	-25.0	0.4747	-40.0	0.09631
-10.5	1.8646	-25.5	0.4515	-45.0	0.05402
-11.0	1.7832	-26.0	0.4294	-50.0	0.02952
-11.5	1.7050	-26.5	0.4083	-55.0	0.01570
-12.0	1.6300	-27.0	0.3881	-60.0	0.008101
-12.5	1.5581	-27.5	0.3688	-65.0	0.00405
-13.0	1.4890	-28.0	0.3505	-70.0	0.00196
-13.5	1.4228	-28.5	0.3330	-75.0	0.000915
-14.0	1.3593	-29.0	0.3162	-80.0	0.00041
-14.5	1.2983	-29.5	0.3003		

Vapour pressure of water ice from 0 to -80^{o}c

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[8]

Materials that can be lyophilized

1. Non biological where process used to dehydrate or concentrate reactive or heat labile chemicals.
2. Non living bio products includes hormones, vitamins, antibiotics, enzymes, blood products, antibodies, inactivated vaccines, bones & other body tissues (surgical purpose), foodstuffs.
3. Living microorganisms where reconstituted cells after drying must be grow & able to produce new progeny.

Lyophilization is less suitable for those materials which supercool to form glasses, for products which form impervious surface skin upon cooling thereby inhibiting evolution of sublimating vapors.[9]

THE FUNDAMENTAL PROCESS STEPS IN LYOPHILIZATION

1. Freezing: The product is frozen. This provides a necessary condition for low temperature drying.
2. Vacuum: After freezing, the product is placed under vacuum. This enables the frozen solvent in the product to vaporize without passing through the liquid phase, a process known as sublimation.
3. Heat: Heat is applied to frozen product to accelerate sublimation.
4. Condensation: Low temperature condenser plates remove the vaporized solvent from the vacuum chamber by converting it back to a solid. This completes the separation process.[10]

Traditionally, lyophilization cycle design has been divided into three parts

a. Freezing, in which the liquid sample is cooled until pure crystalline ice forms from part of the liquid and the remainder of the sample, is freeze-concentrated into a glassy state where the viscosity is too high to allow further crystallization.

b. **Primary drying**, wherein the ice formed during the freezing is removed by sublimation under vacuum at low temperatures, leaving a highly porous structure in the remaining amorphous solute that is typically 30% water. This step is carried out at pressures of 10-4 to 10-5 atmospheres, and a product temperature of –45 to –20°C; Sublimation during primary drying is the result of coupled heat- and mass-transfer processes.
c. **Secondary drying**, wherein most of the remaining water is desorbed from the glass as temperature of the sample is gradually increased while maintaining low pressures.

Ideally, the final product is a dry, easily reconstituted cake with a high surface area.

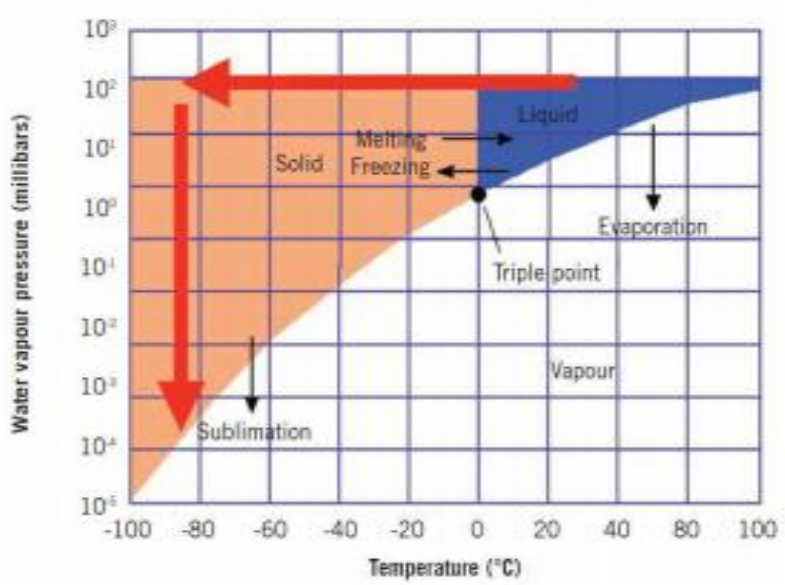


Fig 2: Freeze drying process

Freezing process

In order to successfully lyophilize a formulation, it is necessary to freeze the substance prior to start of drying process. Freezing can impact the properties of final product. All one needs for freezing is a cold (controlled &uncontrolled) environment to reduce the temperature of the formulation to obtain the matrix where there is separation of solute & solvent, to diminish the mobility of the water in the interstitial region of the matrix such that it approaches zero, & to provide a matrix structure that offers a minimum impendence to flow of water vapour during drying process.

1. **Formation of ice crystals**

First step in freezing process is formation of ice crystals. For pure ice to form solutes of the solution are pushed ahead of the freezing front & not incorporated into the ice structure. Sometimes dried cake have mushy zone in the middle of cake, which occurs during freezing

process. As ice crystal grow from the bottom of container, the system will take on phase consisting of mixture of solid & liquid phase.

2. Interstitial region

The final frozen matrix will consist of an interstitial region composed of the solutes & uncrystallized water dispersed between the ice crystals Glassy state is the most common state found in interstitial region as a result of freezing most pharmaceutical & biological formulations. For some of the formulations it has been shown that it is possible to initialize crystallization by thermally treating the frozen matrix prior to drying process.eg addition of sucrose to the formulation increases the formation of glassy state in the interstitial region.

3. Frozen matrix

While it is important to obtain completely frozen matrix prior to the start of drying process, it is equally important that the nature of this matrix not impede the flow of water vapour during either primary or secondary drying process. The morphology of frozen matrix depends not only on the composition of the formulation but also the rate at which the formulation is frozen. It is assume that when degree of crystallization approaches 1 then after removal of ice crystals by sublimation, the remaining structure will be representative of interstitial region of the matrix when in the frozen state. Time required to freeze a formulation is more dependent on the fill height than the total fill volume.

Other freezing methods

Shelf surface freezing method is most widely used method for obtaining frozen matrix.

a. Immersion Freezing

Immersion freezing is a freezing technique in which the heat transfer media, a liquid or gas is in contact with both the bottom & the wall of the container. Time to freeze the large volume was excessively long. The problem was overcome by spinning the bottles on the sides in the presence of cold blast of air. High speed was necessary because at lower speeds the ice crystals formed during supercooling would form lump & the resulting matrix would become nonuniform in nature.

b. Snap freezing

Snap freezing is a process in which the formulation is frozen by placing the product in chamber & then evacuating the chamber. The heat of evaporation & the heat of sublimation reduce the temperature of the formation to form ice crystals & then reduce the temperature of

matrix to less than collapsed temperature. This method of freezing may be applicable for thin layers of a formulation on glass or ceramic surface, where there is minimal heat transfer to the film during the freezing process. In this method sublimation of ice or primary drying occurred at temperature greater than that of collapsed temperature hence surface layer may collapsed.

Primary drying

The principle function of primary drying process is to reduce major quantity of solvent in a product while the matrix is in frozen state. (I.e. when mobility of the water in the interstitial region approaches to zero) The chamber pressure & shelf surface temperature necessary to complete the primary drying process will be determined by the thermal characteristics of the formulation, mainly collapsed temperature & eutectic temperature.

Sublimation process: Sublimation is defines as solid to gas conversion that can at pressure of 1 atm or lower than 4.58 Torr, the vapor pressure of water at triple point. Sublimation of ice occurs at gas-ice interface as the gas pressure above the gas surface interface is lowered water molecules with sufficient energy can leave the surface.

Effect of container configuration: Configuration of container affects the distribution of energy into container. This energy distribution will govern the sublimation rate at various points on the container & the configuration of the frozen structure throughout the drying process.

Configuration of frozen matrix: Because the presence of interstitial region, which eventually become the dried cake of formulation, the configuration of frozen matrix is obscured at various time during drying process.

Effect of fill height: For formulation conductance will be dependent on nature & concentration of constituents, a fill height that produces a low gas conductance cake system can become counterproductive. Not only will drying rate decreases significantly as the cake thickness increases, but chances of producing defective product will also increases. Large diameter container may produce fewer products per batch, process time will be considerably shorter & defective units are due to structural defect of container rather than manufacturing process.

Effect of cake density: Density of cake formed during primary drying will be dependent on 2 factors: Concentration of constituents in the formulation & the degree of supercooling. High solid content could result in decrease in conductance path of cake, which decrease overall conductance of cake as primary drying is proceeds & this results in increase in product temperature & decrease in drying rate. Hence in order to increase drying rate one would increase the conductance by diluting the formulation.

Impact of higher product temperature: Typically product temperature for primary drying process would be 5^0c lower than collapsed temperature. Otherwise results in collapsed or Meltback.

General relationship between P_c, T_p& T_s [Chamber pressure (P_c), product temperature(T_p) & shelf temperature(T_p)]

If any two parameters are fixed the third parameter becomes in variant. By fixing T_p& P_c, there will be only one temperature value, within limits, for T_s.

Condenser temperature plays an important role in primary drying process-the removal of water vapour from gases entering into condenser chamber. Condenser temperature not only impact primary drying process but also affects the quality of final product.

If malfunction of condenser refrigeration or leak results in an increase in Pc& there will be an increase in T_p equal to or exceed the collapsed temperature, then volume of dried cake will not be equal to frozen matrix. T_c should be sufficient to remove water vapour (T_p-$T_c \geq 20$).[11]

Process monitoring techniques

End of primary drying for formulation empirically determined when the Tp approaches that the Ts for a specified time interval. Basic problem with such a method is that Tp measures only a single vial on the shelf & other vials may not have completed primary drying. If secondary dying commences before primary drying completed then some containers may show partial meltback or collapsed. In response to this problem numbers of other techniques have been proposed for determining completion of primary drying.

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

4. Lyotrack (gas plasma spectroscopy)
5. Product thermocouple response
6. Condenser pressure
7. Pressure rise test.

Pressure rise test widely used for determination of end point of primary drying process.

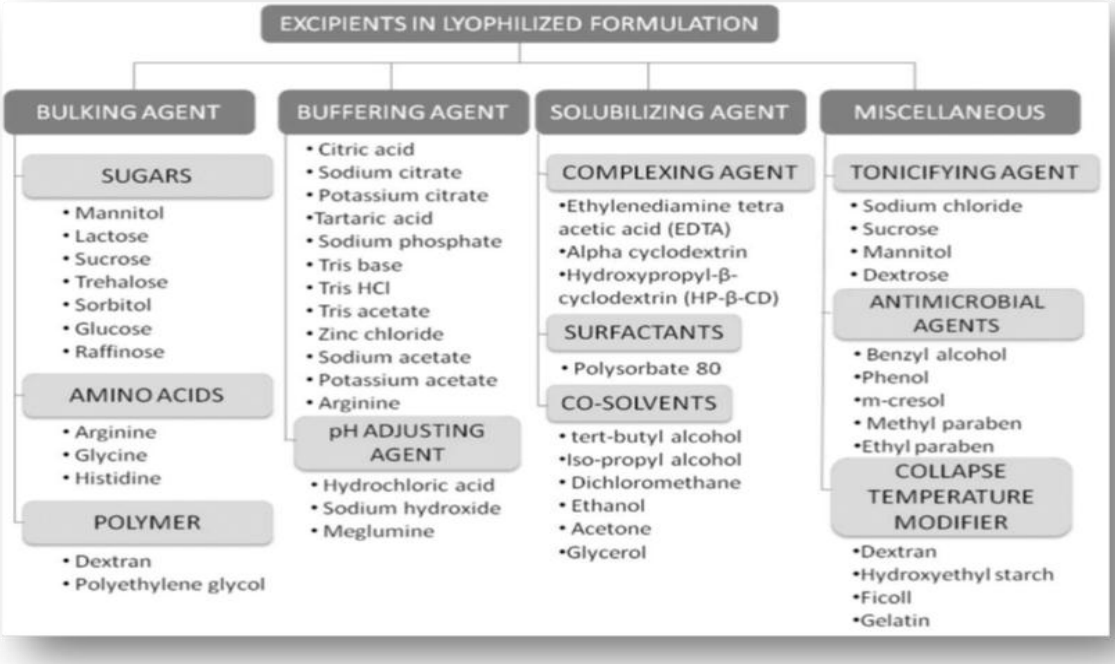
Pressure rise test: Drying chamber is isolated from the condenser chamber by means of valve. Another criterion for the use of such method is that the leak rate of chamber must be small & inconsequential for the test time period. The rate of rise in pressure will be dependent on the shelf temperature. Rapid increase in pressure will indicate that ice is still present in the chamber. If the pressure does not reach a predetermined pressure within the time restraints of the test, then primary dying is considered to be complete.[12]

SECONDARY DRYING

Principle objective of primary drying is to sublimate the solvent from the matrix, primary function of secondary drying is to reduce the residual moisture content of the product the level that will no longer support biological growth or chemical reactions. This stage of lyophilization process serves as a means for slowing the kinetic clock of active constituents. This usually accounts for long stability of lyophilized product. The basic for selecting the secondary drying parameters are (a) the quantity & nature of residual water in the product; (b) the absorption, adsorption & desorption of gases. Major increase or decrease in degree of supercooling would affect the cake structure.[11]

EXCIPIENTS IN LYOPHILIZED FORMULATION

Lyophilization is a commonly used technique for formulation development of small molecules which are unstable in aqueous medium and/or are thermolabile in nature. Lyophilization of drug alone, however, presents certain formulation development challenges, which may be overcome by incorporation of excipients (e.g. bulking agents, buffering agents, tonicifying agent, wetting agent and cosolvents, preservatives and collapse temperature modifiers) in the formulation. A need-based approach should be employed for proper selection of excipients in the formulation for lyophilization, so as to keep the formulation simple for easier processing, while simultaneously maintaining an optimal functionality. The design of aqueous lyophilized formulation is dependent on the requirements of the active pharmaceutical ingredient (API) and intended route of administration. A formulation may consist of one or more excipients that perform one or more functions.



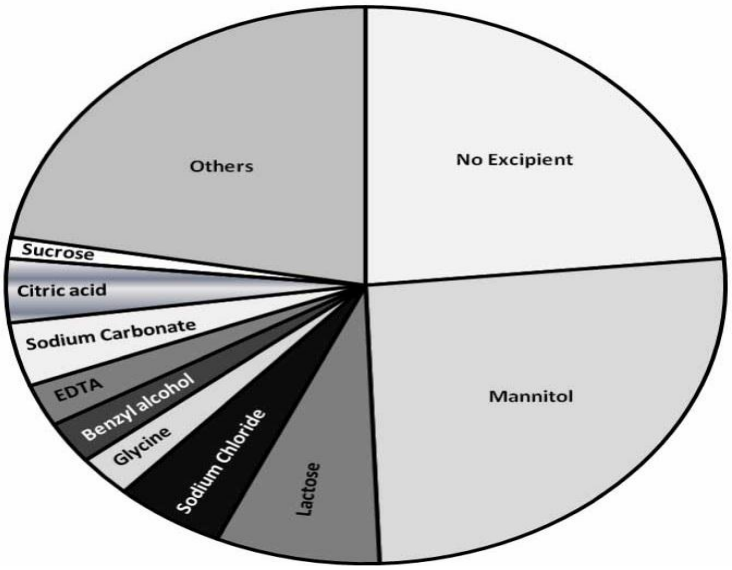


Fig: Distribution of commonly used excipients in marketed lyophilized formulations of small molecules. About 67% of marketed lyophilized formulations of small molecules contain excipients. [13]

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