LYOPHILIZATION - PROCESS AND OPTIMIZATION FOR PHARMACEUTICALS

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REVIEW ARTICLE

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ABSTRACT

In this 21st century, Lyophilization emerges to be a novel trend for the drying of pharmaceuticals and biological that are thermolabile or are unstable in aqueous form but stable for longer periods in their dried form. This article provides an overview on the process of lyophilization, how the freeze drying cycle is designed, discussing several important parameters which are important for understanding of this process as well as their role in the designing of an optimized freeze drying cycle, so that a robust and economical process of lyophilization can be developed which does not impact the product quality. It also describes the use of this process in various industries.

Keywords: Lyophilization, Stages of lyophilization, 1°drying, 2°drying, Excipients used for the process, applications.

INTRODUCTION

Lyophilization or freeze drying or cryodessication is a process that is widely used for pharmaceuticals or food products, which improves the stability as well as long term storage stability of thermolabile drugs.(1, 2) It is a stabilizing process in which substance is first frozen and then quantity of solvent is reduced first by sublimation (1° drying) and then by desorption (2° drying) to such a level that final product produced, will no longer support biological growth or any chemical reactions. It is a process that is applicable for manufacture of certain pharmaceutical products that are heat sensitive or unstable in aqueous solutions but are stable for a long period in their dry state. The term Lyophilization or freeze drying describes a process to manufacture a product that "loves the dry state".(3-5)

PRINCIPLE OF FREEZE DRYING PROCESS

The process of lyophilization involves a phenomenon called sublimation in which water is directly passes from solid state (ice) to vapor state without converting to the liquid state. The product that has to be dried is first frozen and then it is subjected to heat (by conduction or radiation or both) under a very high vacuum, so that frozen liquid sublimes and only dried solid components of original liquid remains. The driving force for the process of sublimation of ice during lyophilization process is the pressure difference between the vapor pressure of ice and partial pressure of water in the chamber.(6-8)

Advantages :

Apart from reduction in thermal inactivation of drugs it has several advantages as mentioned below :

- Content of water in final drug product can be reduced to very low levels so that it is stable for longer periods.
- Chemical decomposition of the product can be minimized.
- The product is sealed under vacuum or inert gas such as nitrogen, so chances of oxidation of product is minimized. (9,10)
- Water can be removed without excessive heating as in certain dryers.
- As it operates in a controlled environment, there will be less chances of contamination.

Type of materials that can be Lyophillized

There are certain materials onto which the process of lyophilization is applied which are :

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- Non-biologicals where sole aim of this process is to concentrate the product.
- Non living bio-products – It covers a broad area and includes enzymes, hormones, antibiotics, vitamins, blood products, bone and other body tissues for medical use, industrially used bioproducts.
- Living organisms where reconstituted cells after drying must be able to grow and multiplies to form new progeny. Ex- bacteria and fungi used as seed cultures or attenuated viral vaccines.

Others – It includes books damaged by floods or museum artifacts etc.

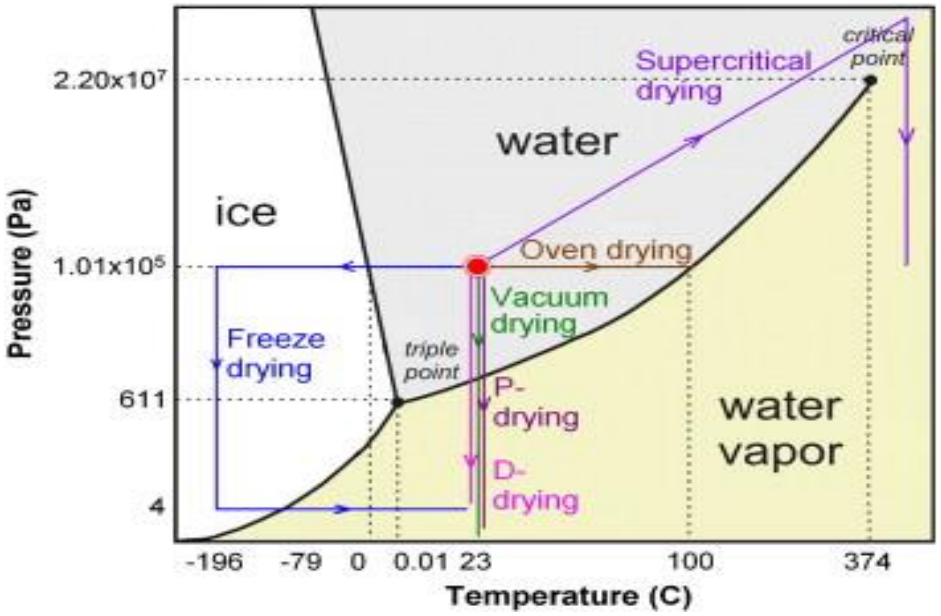

Figure. 1: Phase diagram of water showing triple point of water

Figure 2: A bench top freeze dryer, front view, showing control wizard, shelf and internal condenser

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DESIGN OF FREEZE DRYER

The general layout of a freeze dryer is given in figure, it consists of following essential components:

Chamber

The chamber performs two main functions :

- To provide a safe environment for the product during the entire process, and
- To provide the necessary temperature and pressure to conduct each step of the process.

It contain shelves for processing product. It is a metal vessel, made up of stainless steel and usually highly polished on the inside and well insulated. (11) The chamber door is either fabricated from stainless steel or from clear plastic. The door is fitted with an elastomer gasket to form a vacuum seal with the drying chamber.

Shelves

For R&D purposes, it contains only one or two shelves, but for production purposes it may contain several shelves. The design of shelf is made much more complicated as it performs several functions firstly it act as heat exchanger so that it removes the energy from the product during freezing and secondly it supplies energy to the product during primary and secondary drying segments of freeze drying cycle. Shelves are available in various sizes upto $4m^2$ in area.(12)

Shelves must be:-

- Flat and level to ensure that there is a proper contact with product containers.
- Constructed in such a way that shelves must be heavy enough to ensure that a level surface is maintained.
- Strong enough to support the forces of stoppering system.

Shelves are of hollow construction so as to ensure serpentine flow of heat-transfer fluid which is usually Sillicone oil.

Condenser System

The main function of condenser chamber is to house condenser surface for removal of water during drying process. It consists of coils or plates, which are refrigerated by the direct expansion of a refrigerant. There are physically two types of condensers one is 'Internal condenser' which is housed in the drying chamber and the other is 'External condenser' which is placed behind the chamber. However the position of condenser does not affects the trapping performance. (13)

Vacuum System

Along with condenser system it provides necessary pressure for conducting primary and secondary drying processes. The low pressure (200mTorr) is achieved by two stage rotary vacuum pump but for large chambers multiple vacuum pumps may be used. Vacuum pumps which are used in freeze dryers are either mechanically oil-lubricated or oil-free pumping system. It compresses the non-condensable gases that pass through the condenser chamber and discharge these gases directly into the atmosphere.

Sensors

It is important to measure the temperature and pressure during the entire process.(14) Shelf temperature and chamber pressure are the controlled parameters while the product temperature and temperature of the condenser has to be measured during the entire process. The temperature measuring devices used are Resistance thermometers or Thermocouples (normally type T) while the vacuum or pressure sensors includes Capacitance manometers (frequently used) Thermoelectric or Pirani gauges.

Control System

Control may be entirely manual or automatic. The control elements are shelf temperature and chamber pressure plus time. A control programme is needed to set up these values as required by the product or by the process which can vary from hours to days. Data such as product temperature and condenser temperature can also be recorded and logged. (15-17)

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Preparation And Pre-treatment

It includes dissolving drugs and Excipients in the solvent, then sterilized the bulk solution by passing it through bacteria retentive filter and then filling of this bulk solution into the container. Pre-treatment includes any method of treating the product prior to freezing. This includes any method of treating the product, changes in formulation (i.e. inclusion of several components to increase stability of final product and or to improve the ease of processing), decrease in vapour pressure or increase in the surface area. It is done to reduce the time of freezing drying cycle and done on basis of theoretical knowledge of freeze drying cycle or product quality consideration. Method of pre-treatment includes freeze concentration, solution phase concentration, change in formulation variables such as use of high vapour pressure solvents to stabilize reactive products.

STAGES OF LYOPHILIZATION

Lyophilization is the most common method for manufacturing of pharma products that have to be dried thoroughly in order to ensure stability. It is a process that requires input of energy for a certain period of time ranging from days to even weeks, which depends whether the cycle is optimized or not. (1,5,18). The stability of the drug during the process and storage (19) and duration of the cycle are two ajor considerations for optimization of freeze drying process. Basically, the process of lyophilization consists of three stages:

- Freezing
- Primary Drying
- Secondary Drying

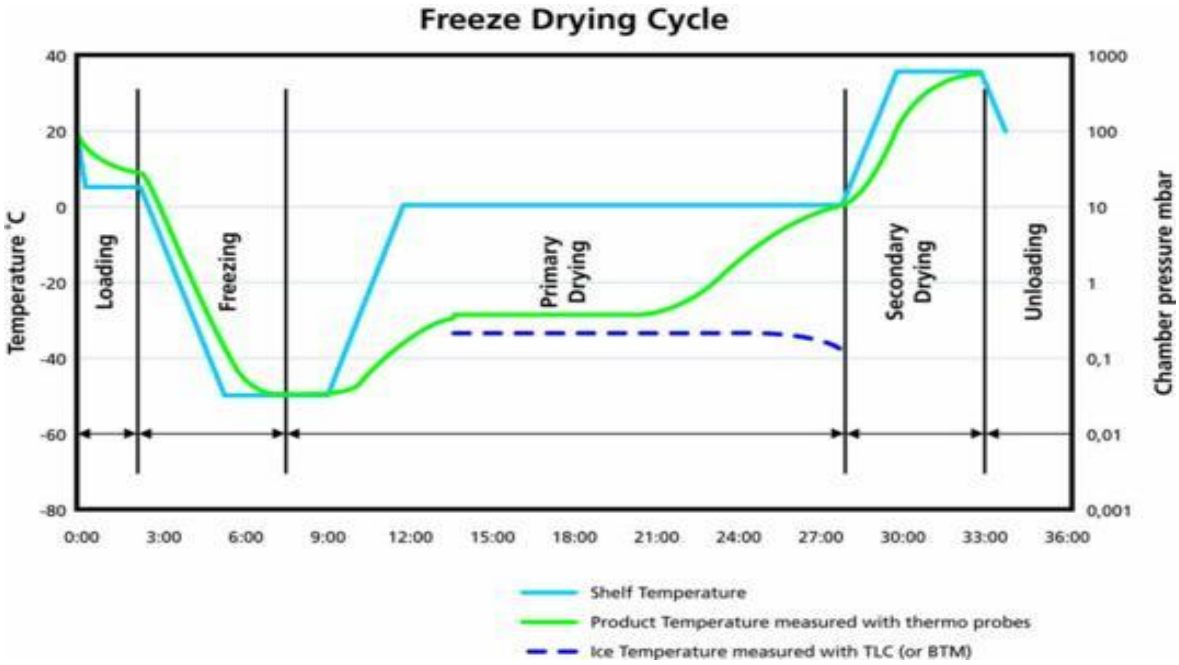

Figure 3: A Lyophilization cycle showing shelf temperature, product temperature measured with thermo probes, at different time under pressure in mbar.

FREEZING

This is an important step, since microstructures formed during freezing determines the quality of final product and rates of primary and secondary drying. The main function of freeze drying is to separate the solvent from the solute, to minimize thermal degradation in the product, to prevent product foaming when vacuum is applied. It is the stage where most of water is removed from drug and Excipient, the system separates into multiple phases and the interfaces between ice and drug phases form. The formulation must be frozen to a lower temperature so as to solidify the product. The temperature necessary to achieve complete

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freezing of formulation will depend on the nature of solvent and Excipients. (20)

The process of freezing induces many stresses which includes increased chances of aggregation, pH changes due to crystallization of buffer salts, reduced hydrophobic interaction, formation of larger ice-aqueous interfaces and increase in ionic strength. The pH drift can be minimized by optimal choice of buffers (i.e. avoid use of succinate, phosphate or tartarate) or by reducing the concentration of buffer. (21,22)

Rate of Cooling In freezing Step – One approach for the stabilization of formulation is to minimize the surface area of ice by growing large ice crystals which can be achieved by reduced supercooling. Rapid cooling results in smaller ice crystals useful in preserving structures to be examined microscopically, but resulting in a product that is difficult to freeze dry, while slower cooling results in formation of larger ice crystals. Slower cooling (of about 0.5°C/min) causes larger supercooling effects than the precooled shelf method, while precooled shelf method gave larger heterogenicity between vials which is highly undesirable.(23) Slower freezing has the potential to increase the protein damage in systems prone to phase separation.(24) A cooling rate of about 1°C/min yields moderate supercooling with moderate ice surface area and a reasonably fast freezing rate and produces uniform ice structures.

Freezing Temperature And Time – Critical formulation properties that have to be considered during freezing stage is Collapse temperature (T_c) of the formulation (as determined by DSC process) which is the temperature above which the freeze dried product loses macroscopic structure and collapses during drying. T_c is usually kept 2°C higher than the Glass transition temperature (T_g) for amorphous or Eutectic temperature(T_{eu}) if solutes are crystallized in frozen solution. It requires the shelf temperature for freezing stage to be set below the *T*g or *T*eu, and must be kept at that temperature long enough so that all the solution has been changed into the solid. As there is limited thermal conductivity between vials and shelf, complete freezing of solution requires significant time. Freezing time also depends on fill volume i.e. vials containing larger fill volume takes longer time to freeze. (1,3)

ANNEALING

It is an optional step in which the product is hold at a temperature above the final freezing temperature for a defined period to crystallize the potentially crystalline components (usually crystalline bulking agents such as mannitol or glycine) in the formulation during freezing stage. If bulking agent is not crystallized during freezing stage, then in primary drying vial breakage may occur.(25) Hence completion of crystallization may be facilitated by annealing. The temperature for annealing should be between the *T*g of amorphous phase and *T*eu of bulking agent to give a high crystallization rate and complete crystallization. Annealing conditions can be studied using either frozen solution X-ray diffraction or DSC procedures to evaluate the development of crystallinity. (26) Annealing above the glass transition temperature causes growth of ice crystals, and results in shorter time for primary drying (27,28)

PRIMARY DRYING

In this stage, pressure inside the chamber is reduced and heat is applied to initiate the process of sublimation of ice crystals formed during the freezing stage. The application of vacuum allows the free migration of the water vapour from the frozen mass, which may be regarded as a diffusion process which is the reason for its relatively slow process. The solvent vapours resulting from the process of sublimation passes through the opening in the closure. As the sublimation process proceeds, frozen mass changes into a cake type structure. As there is loss of latent heat during the process of sublimation, heat must be applied to the product throughout primary drying.

The basic idealogy of primary drying is to first choose the optimum target product temperature (T_p), then bring the product T_p quickly and hold the product at T_p roughly constant throughout all of the primary drying.

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Target Product Temperature (T_p)

The product temperature (Tp) depends on the properties of formulation, temperature of the shelf and pressure within the chamber of lyophilizer, which cannot be controlled directly. Hence product temperature should always be several degrees below T_c in order to obtain a dried product with acceptable appearance. The temperature difference between product temperature and collapse temperature is called as the temperature safety margin. Higher product temperature yields a faster process, with each 1°C increase in product temperature decreasing the primary time by about 13%. The target product temperature should be as close as possible to Tc, but the risk of collapse is high if product temperature is too close to Tc. Tp should not be higher than −15°C or the heat and mass transfer capabilities of the lyophilizer may be overloaded.

There are only two process parameters that can be controlled during the primary drying step which are:

- Chamber pressure
- Shelf temperature

Chamber Pressure

Primary drying is carried out at low pressure to improve the rate of ice sublimation. It affects both heat and mass transfer and is an important parameter. It should be below the ice vapor pressure at $T_p$so that ice is transferred from the product to the condenser by process of sublimation and crystallizes onto the coils or plates. The rate of sublimation is defined as the mass of ice sublimed (in g) per unit time (hour), represented by equation:

$$dm/dt = P_i - P_c/R_{dl}+R_s$$

where, *dm*/*dt* is ice rate of sublimation (g/hour per vial), while P_i is the equilibrium vapor pressure of ice at the sublimation interface temperature (in Torr), and R_{dl} and R_s are the dry layer and stopper resistance, respectively.

At a given product temperature, the smallest chamber pressure gives the highest rate of sublimation of ice while at very low chamber pressure, it may poses problems, such as contamination of the product with the volatile stopper components or pump oil and also produces larger heterogeneity in heat transfer between vials, thereby giving larger product temperature heterogeneity between vials. In most of the practical applications, the chamber pressure varies from 50 to 200 m Torr. (29)

Shelf Temperature

The most important and time consuming part of producing an optimized freeze-drying process is the determination of the shelf temperature: time profile that efficiently achieves the target product temperature (T_p) during 1˚ drying stage. The T_p during the primary drying stage typically ranges from 5 to 40°C lower than the shelf temperature and changes with change in chamber pressure, shelf temperature, rate of heat transfer between the vials, and even with the model of the freeze dryer.

Shelf temperature is an important parameter as it affects the rate of drying by controlling the T_p through uniform heat transfer and or by providing the necessary energy for sublimation. The shelf temperature should be optimum to enable the process of sublimation, if it is too low, then there may not be sufficient sublimation due to a minimum difference between the ice vapour pressure at the T_p and the chamber pressure, while if the shelf temperature is set to too high, the condenser may not be able to handle all of the sublimed water vapour.

Product temperature during 1˚ drying can be measured by various methods including thermocouples or by Resistance Thermometer Detectors (RTDs) in selected product vials, and by pressure rise measurement (Manometric Temperature Measurement). (30,31) Manometric temperature measurement (MTM) is a procedure to measure the T_p at the sublimation interface during the primary drying stage, this method has advantage of minimal human intervention as well as it yields accurate product resistance which is used to describe the heat and mass transfer during primary drying stage. (31,32)

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SECONDARY DRYING

This is the last stage of freeze drying in which water that did not get freezed, is removed by the process of desorption from the solute phase. The objective of secondary drying is to reduce the unbound water (moisture) to a level that is optimal for stability (less than 1%) of final product. The shelf temperature in secondary drying is kept much higher than that used for primary drying so that desorption of water may occur at a practical rate. This process is also known as 'Isothermal Desorption'.(33)

Rate of Heating and Chamber Pressure

The shelf temperature for secondary drying should be increased slowly because a fast temperature ramp might causes the collapse of amorphous products because of the presence of high residual moisture content that is present in the amorphous product during secondary drying and thus low glass transition temperature (T_g) which has the potential for collapse of the product during the secondary drying. As crystalline products, does not shows any potential for collapse during secondary drying, a higher ramp rate is suggested for these products. The rate of desorption does not depends on chamber pressure till it is less than 200 m Torr (100-150mTorr), but rate of desorption is very sensitive to product temperature (T_p).(32,34)

Shelf Temperature and Drying Time

The products should be kept at higher temperature for a period that would be sufficient to allow the desorption of water. It is better to run a cycle of higher shelf temperature for a shorter time period rather than a cycle of lower temperature for a longer period, as rate of desorption decreases with time at a given temperature and after a certain time, it causes little changes in the moisture content level. (36) Amorphous products are more difficult to dry than crystalline products thus higher temperatures and longer times are needed to remove the absorbed water.

The secondary drying conditions also depends on the concentration of the solute. At higher concentration of solute, the dried product has smaller specific area and it becomes more difficult to remove the absorbed water thus taking longer times or higher temperatures for secondary drying. The optimum time for secondary drying can be determined by the in-situ residual moisture measurement during secondary drying by extracting samples from the freeze dryer without interrupting the freeze drying and measuring their moisture content using Karl Fischer titration (KFT), thermal gravimetric analysis (TGA), or near IR spectroscopy.(32) The real-time moisture content can also be measured by a modified MTM method.(35)

EXCIPIENTS USED FOR THE PROCESS OF LYOPHILIZATION

Excipients are the inert substances, other than the pharmacologically active drug which are included in the manufacturing process or are contained in a pharmaceutical product dosage formed. The main purpose of Excipients to use in freeze drying process is that they improve the cake structure as well as Excipient inclusion also helps in development of robust and economical freeze drying process. Various Excipients are used for the process which are:

- Bulking agent: These agents provides bulk to the lyophilized product and provides a well adequate structure to the final cake. Bulking agent is used in the formulation especially when the content of total solid is less than 2%. Examples of commonly used buffering agents includes Mannitol, Glycine, Dextran, sucrose etc(2,37-38). The nature of final cake depends on the ratio of drug as well as on the bulking agent, shows as the amount of bulking agent was increased, there are more chances of crystallization. Korey and Schwartz studied freeze drying process for various active ingredients and found that the degree of crystallization gets increased as the amount of Excipients gets increased. At lower value, the cake of Cephazolin sod. was found to be amorphous while as the amount of Excipient was increased there was complete crystallization of cake which was desired for this antibiotic as

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amorphous product shows melt back phenomena. (2,39)

- Tonicity modifiers: The main purpose of adding tonicity modifiers to the formulations is to make the drug isotonic with the blood. There are so many buffers are used which includes sodium chloride, dextrose, glycerol etc., but addition of these can increase the primary drying time owing to their lower collapse temperature which may complicate the process of formulation development. An approach to sort out this problem is to incorporate the tonicity modifier to the reconstitution diluent rather than to the freeze dried product. (40)

- Buffering agents: Buffering agents are used for control of pH, so as to avoid degradation of during formulation, storage and reconstitution. Buffering agents used for the formulation should have high collapse temperature(T_c) which facilitates faster 1˚ drying, high glass transition temperature (T_g) which ensures that the product remains stable during the storage, as well as it should be non volatile which helps to prevent any pH shift. (41,42) Commonly used buffering agents includes citrate, phosphate, succinate etc. Shalave et al studied effect of citrate, succinate and tartarate buffer on crystallization behaviour as well as on pH and found that citrate was most as it remained amorphous as well as change in pH was minimum as compared to succinate and tartarate. (22)

- Collapse temperature (T_c) modifiers: The 1˚ drying temperature for amorphous materials should be below the T_c of the formulation, while some Excipients have very low values of T_c which increases the 1˚ drying time hence to reduce the 1˚ drying temperature Collapse temperature modifiers are used which increases the T_c of the formulation, making the process economical without compromising the quality of product. (43) Examples of collapse temperature modifiers includes dextran which have a collapse temperature of -9°C, hydroxyethyl starch having a collapse temperature of -5°C etc. (44)

Methods Of Freeze Drying

There are two methods of freeze drying which are used commonly, these are :

- Manifold Method - In this method, product container was directly attached to the ports of lyophilizer. Then the product was freezed at low temperature which depends on the nature as well as volume of the product to be freeze dried. Then this freezed product was heated at higher temperature as well as the pressure is reduced to maintain lower temperature of the product. This procedure is generally used for products having small volumes or having high eutectic and collapse temperatures. (3,13,45,46)

- Batch Method –In batch method, large numbers of similar sized vials or ampoules containing product are placed together on the shelves in the lyophilizer. The product is then freezed on the shelf of the lyophilizer by applying low temperature. Then it was heated at high temperature (below T_c) and the vacuum was reduced for primary drying. Precise control of the product temperature (T_p) and the amount of heat applied to the product during drying has to be maintained during the entire process. There may be slight differences in the heat input from the shelf which is different in different areas, which can results in smaller differences in the amount of residual moisture.

The main advantage of batch drying is that it allows closure of all vials in a lot at the same time, under the same atmospheric condition. These vials can be stoppered in a vacuum, or after backfiling with inert gas such as nitrogen. (9,10) Stoppering of all the vials at the same time ensures a uniform environment in each vial and uniform product stability during the storage. This process is widely used to prepare large numbers of ampoules or vials of one product and is commonly used in the pharmaceutical industry.

SCOPE OF LYOPHILIZATION PROCESS

There are certain applications of Lyophilization process in the various industries which includes:

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- Pharmaceutical And Biotechnological Products – This process is widely used for manufacturing of pharmaceuticals such as chemical compounds, parenteral formulations, vaccines and also in diagnostic products. It also includes biotechnology products that are mainly of protein-based products. Example of various pharmaceutical products are :

Product	Active Ingredient	Drug category
Zinecard®	Dexrazoxane	Cardio-protective agent
Nitroprusid-Na®	Nitroprusside	Vasodilator
Azaran®	Ceftriaxone	Antibiotic
Zyprexa®	Olanzapine	Antipsychotic

- Food Industry - This process is also used to preserve food. Examples of food products that has been freeze dried includes coffee, freeze dried ice cream which is an example of Astronaut food, freeze dried fruits etc.

- Microbiology - In bacteriology, freeze-drying is used to conserve special strains. (47)

- Other Applications - It includes the lyophilization of floral products and taxidermy, but there is a limited success with the floral products as only the tile portion of flower responds to the process. Along with this it has been applied to the taxidermy of small mammals like squirrels and racoons etc but again the process has limited success. It has also been used by organisation such as Document Conservation Laboratory at the United States National Archives and Records Administration (NARA) as a recovery method for water damaged books and documents.

CONCLUSION

Freeze Drying process is widely used for the manufacturing of pharmaceutical products at conditions of low temperature and pressure so that they remain stable for longer periods in their dried form. Lyophilization basically consists of mainly three stages i.e. Freezing in which the water present in the formulation is frozen, then Primary drying in which the water is removed from the frozen mass by the process of lyophilization, and then Secondary drying in which the bound moisture present in the formulation is removed by the process of desorption.

Now a days, trend of freeze dying is booming for manufacturing of pharmaceuticals, biologicals as well as for the biotechnological products due to advantage that in solid state, chemical or physical degradation reactions are inhibited or minimum resulting in long term stability of products. Along with stability, it also provides an easy handling of products during shipping and storage as they are light and in weight compared to similar liquid formulations. The knowledge of the freeze drying process as well as various parameters are essential for successful designing of freeze drying cycle which makes the process more efficient and less complicated so that product gets dried in shorter time retaining its properties so that operating costs and other inputs can be minimized and in return it will maximize the capitals.

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CONFLICT OF INTEREST

Author declares that there are no conflicts of interest.

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