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LYOPHILIZATION / FREEZE DRYING – A REVIEW

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ABSTRACT

Freeze-drying is a method of removing water by sublimation of ice crystals from frozen material. 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. Lyophilization is common, but cost intensive and hence one of the key objectives during freeze-drying process development is to minimize the drying time (mainly primary drying time, which is the longest of the three steps in freeze-drying). However, increasing the shelf temperature into secondary drying before all of the ice is removed from the product will likely cause collapse or eutectic melt. Thus, from product quality as well as process economics standpoint, it is very critical to detect the end of primary drying. This review focused on the recent advances and its targets in near future. At first, the principle, steps involved, formulation aspects and importance of lyophilization, methods of lyophilization with detection of end point in lyophilization was explained.On 21st century, in pharmaceutical field lyophilization has become important subject to ongoing development and its expansion. Lyophilization is common, but cost intensive. In old days process optimization was focused only on drying rather than lyophilization. But lyophilization was more (or) equally important for the process of pharmaceuticals. This review focused on the recent advances and its targets in near future. At first, the principle, steps involved, formulation aspects and importance of lyophilization was explained.

KEYWORDS: End point of freeze-drying, Freeze drying, Freeze drying methods, Lyophilization.

INTRODUCTION

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). Freeze drying is a process of drying in which water is sublimed from the product after it is frozen.[1] 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]

The term 'lyophilization' describes a process to produce a product that 'loves the dry state.' However, this term does not include the freezing process. Therefore, although lyophilization and freeze-drying are used interchangeably, freeze-drying is a more descriptive term.[3] Lyophilization is the most common method for manufacturing parenterals 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] Freeze-drying or lyophilization is an effective way of drying materials without harming them. It makes use of the physical phenomenon of sublimation, which involves the direct transition between the solid state and the gaseous state without passing through the liquid phase. To achieve this, the frozen product is dried under vacuum, without being allowed to thaw out. The process of freeze-drying has taken on greater prominence in the parenteral industry, due to the advent of recombinant DNA technology. Proteins and peptides must be freeze-dried for clinical and commercial use. There are other technologies available to produce sterile dry powder drug products besides freeze-drying, such as sterile crystallization or spray-drying and powder filling. However, freeze-drying is the most common unit process for manufacturing drug products too unstable to be marketed as solutions.[6]

PRINCIPLE

The main principle involved in freeze drying is a phenomenon called sublimation, where water passes directly from solid state (ice) to the vapor state without passing through the

liquid state. Sublimation of water can take place at pressures and temperature below triple point i.e. 4.579 mm of Hg and 0.0099 degree Celsius.[7] 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 vapor between the drying front and condenser is the driving force for removal of water during lyophilization.[8]

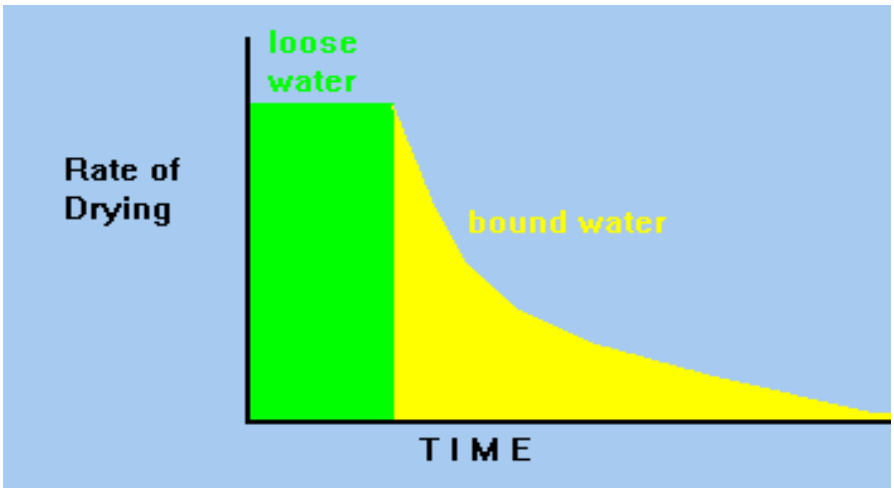

Figure 1: Rate of drying of water

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 (Figure.1).

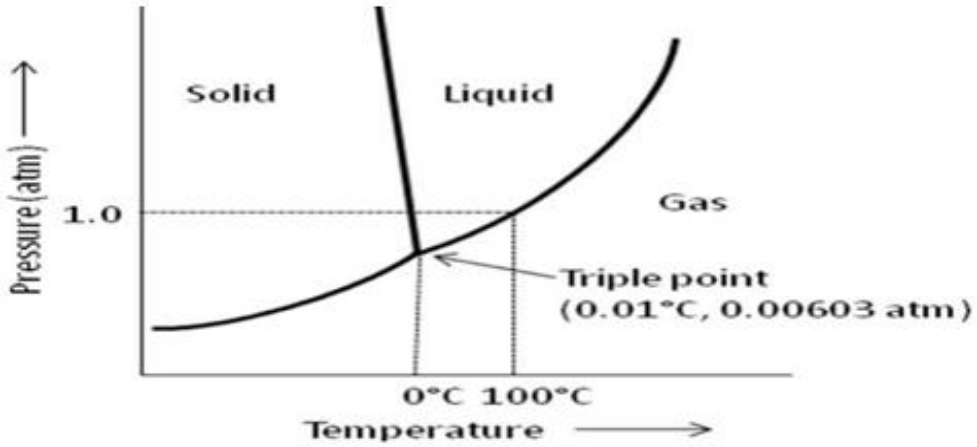

Figure 2- Phase diagram of water

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 (Table 1).

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.5 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 vapor between the drying front and condenser is the driving force for removal of water during lyophilization. To extract water from formulation, the process of lyophilization consists of :

1. Freezing the formulation so that the water in the food become ice.
2. Under a vacuum, sublimating the ice directly into water vapour.
3. Drawing off the water vapour.
4. Once the ice is sublimated,the foods are freeze dried and can be removed from the machine.[9]

Table 1: Ice vapor pressure data

Temperature (°C)	Vacuum (mbar)	Temperature (°C)	Vacuum (mbar)	Temperature (°C)	Vacuum (mbar)	Temperature (°C)	Vacuum (mbar)	Temperature (°C)	Vacuum (mbar)
0	6.110	-16	1.510	-34	0.250	-54	0.024	-70	0.0026
-1	5.620	-17	1.370	-35	0.220	-55	0.021	-71	0.0023
-2	5.170	-18	1.250	-36	0.200	-56	0.018	-72	0.0019
-3	4.760	-19	1.140	-37	0.180	-57	0.016	-73	0.0017
-4	4.370	-20	1.030	-38	0.160	-58	0.014	-74	0.0014
-5	4.020	-21	0.940	-39	0.140	-59	0.012	-75	0.0012
-6	3.690	-22	0.850	-40	0.120	-60	0.011	-76	0.0010
-7	3.380	-23	0.770	-41	0.110	-61	0.009		
-8	3.010	-24	0.700	-46	0.060	-62	0.008		
-9	2.840	-25	0.630	-47	0.055	-63	0.007		
-10	2.560	-28	0.470	-48	0.050	-64	0.006		
-11	2.380	-29	0.420	-49	0.045	-65	0.0054		
-12	2.170	-30	0.370	-50	0.040	-66	0.0047		
-13	1.980	-31	0.340	-51	0.035	-67	0.0047		
-14	1.810	-32	0.310	-52	0.030	-68	0.0035		
-15	1.650	-33	0.280	-53	0.025	-69	0.003		

Process to produce a product that "loves dry state"

Freeze drying also known as lyophilization, is widely used for pharmaceuticals to improve the stability and long term storage of labile drugs. Lyophilization or Freeze-drying fills an important need in pharmaceutical manufacturing technology by allowing drying of heat-sensitive drugs and biologicals at low temperature under conditions that allow removal of

water by sublimation, or a change of phase from solid to vapor without passing through the liquid phase.[10] The most common application of pharmaceutical freeze drying is in the production of injectable dosage forms, the process is also used in the production of diagnostics and, occasionally, for oral solid dosage forms where a very fast dissolution rate is desired.[11]

Lyophilization or freeze drying is a process in which water is removed from a product after it is frozen and placed under a vacuum, allowing the ice to change directly from solid to vapor without passing through a liquid phase.[12]

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 thermolabile 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.[13] The concentration gradient of water vapor between the drying front and condenser is the driving force for removal of water during lyophilization. The vapor 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.[14]

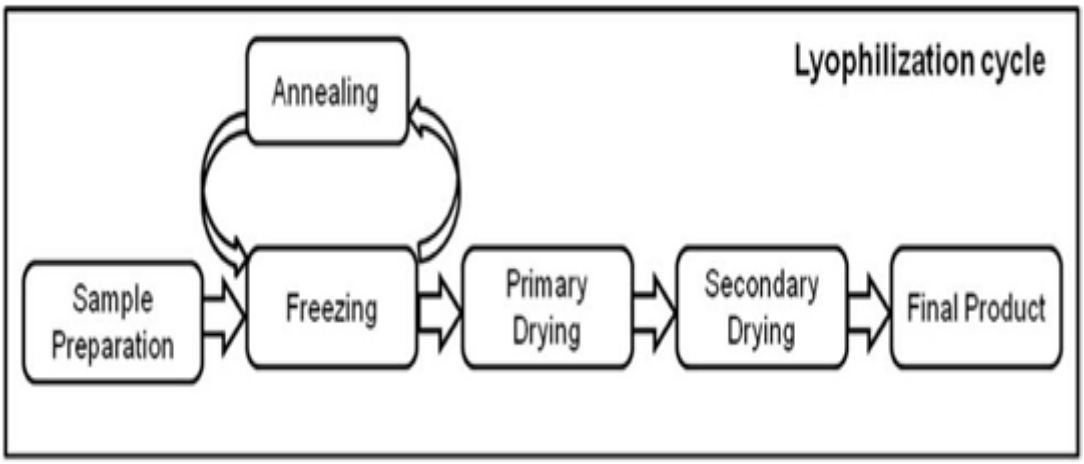

Figure 3: Steps involved in lyophilization from sample preparation to final product formation

ADVANTAGES

- Oxidizable substances are well protected under vacuum conditions.
- Long preservation period owing to 95%-99.5% water removal.
- Loading quantity accurate and content uniform.
- Little contamination owing to aseptic process.
- Minimal loss in volatile chemicals and heat-sensitive nutrient and fragrant components.
- Minimal changes in the properties because microbe growth and enzyme effect can not be exerted under low temperature.
- Transportation and storage under normal temperature.
- Rapid reconstitution time.
- Constituents of the dried material remain homogenously dispersed.
- Product is process in the liquid form.
- Sterility of product can be achieved and maintained.

DISADVANTAGES

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

APPLICATIONS

Pharmaceutical and biotechnology

Pharmaceutical companies often use freeze-drying to increase the shelf life of products, such as vaccines and other injectables.[15] By removing the water from the material and sealing the material in a vial, the material can be easily stored, shipped, and later reconstituted to its original form for injection.

Food Industry

Freeze-drying is used to preserve food and make it very lightweight. The process has been popularized in the forms of freeze-dried ice cream, an example of astronaut food.

Technological Industry

In chemical synthesis, products are often freezedried to make them more stable, or easier to dissolve in water for subsequent use. In bioseparations, freeze-drying can be used also as a

late-stage purification procedure, because it can effectively remove solvents. Furthermore, it is capable of concentrating substances with low molecular weights that are too small to be removed by a filtration membrane.[16]

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

TRADITIONAL LYOPHILIZATION TECHNOLOGY

Traditional lyophilization is a complex process that requires a careful balancing of product, equipment, and processing techniques. For nearly 30 years, lyophilization has been used to stabilize many types of chemical components. In their liquid form, many such biochemicals and chemical reagents are unstable, biologically and chemically active, temperature sensitive, and chemically reactive with one another. Because of these characteristics, the chemicals may have a very short shelf life, may need to be refrigerated, or may degrade unless stabilized. When performed properly, the process of lyophilization solves these problems by putting reagents into a state of suspended activity.[17] Lyophilization gives unstable chemical solutions a long shelf life when they are stored at room temperature. The process gives product excellent solubility characteristics, allowing for rapid reconstitution. Heat- and moisture-sensitive compounds retain their viability. Most proteins do not denature during the process, and bacterial growth and enzyme action, which normally occur in aqueous preparations, can be eliminated. Thus, lyophilization ensures maximum retention of biological and chemical purity.[18]

PROCESSING

There are four stages in the complete drying process:

- Pretreatment
- Freezing

- Primary drying
- Secondary drying

Freeze-drying process

Freeze drying is mainly used to remove the water from sensitive products, mostly of biological origin, without damaging them, so they can be preserved easily, in a permanently storable state and be reconstituted simply by adding water.[19] Examples of freeze dried products are: antibiotics, bacteria, sera, vaccines, diagnostic medications, protein containing and biotechnological products, cells and tissues, and chemicals. The product to be dried is frozen under atmospheric pressure. Then, in an initial drying phase referred to as primary drying, the water (in form of ice) is removed by sublimation; in the second phase, called secondary drying, it is removed by desorption. Freeze drying is carried out under vacuum.[20]

Pretreatment

Pretreatment includes any method of treating the product prior to freezing. This may include concentrating the product, formulation revision (i.e., addition of components to increase stability and/or improve processing), decreasing a high vapor pressure solvent or increasing the surface area. In many instances the decision to pretreat a product is based on theoretical knowledge of freeze-drying and its requirements, or is demanded by cycle time or product quality considerations.[21] Methods of pretreatment include: Freeze concentration, Solution phase concentration, Formulation to Preserve Product Appearance, Formulation to Stabilize Reactive Products, Formulation to Increase the Surface Area, and Decreasing High Vapor Pressure Solvents. Traditionally, lyophilization cycle design has been divided into three parts[22]:

1. 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.
2. 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.
3. Secondary drying, wherein most of the remaining water is desorbed from the glass as the 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 (ca. 10 m2/g).[23]

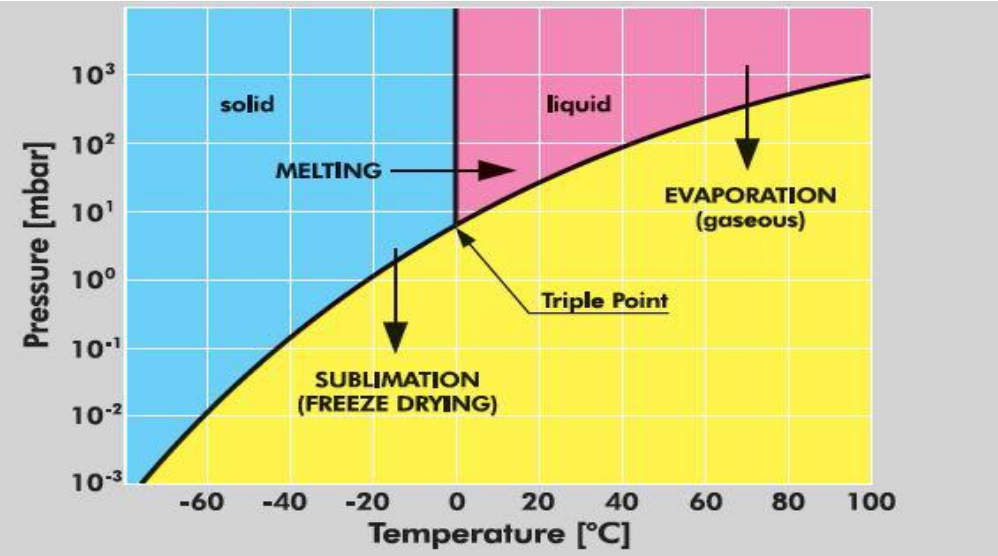

Figure 4: Freeze drying process

LYOPHILIZATION EQUIPMENT

There are essentially three categories of freezedryers: the manifold freeze-dryer, the rotary freezedryer and the tray style freeze-dryer. Two components are common to all types of freezedryers: a vacuum pump to reduce the ambient gas pressure in a vessel containing the substance to be dried and a condenser to remove the moisture by condensation on a surface cooled to −40 to −80°C (−40 to −112°F). The manifold, rotary and tray type freeze-dryers differ in the method by which the dried substance is interfaced with a condenser. In manifold freeze-dryers a short usually circular tube is used to connect multiple containers with the dried product to a condenser.[24] The rotary and tray freeze-dryers have a single large reservoir for the dried substance. Rotary freeze-dryers are usually used for drying pellets, cubes and other pourable substances. The rotary dryers have a cylindrical reservoir that is rotated during drying to achieve a more uniform drying throughout the substance.[25] Tray style freeze-dryers usually have rectangular reservoir with shelves on which products, such as pharmaceutical solutions and tissue extracts, can be placed in trays, vials and other containers. Manifold freeze-dryers are usually used in a laboratory setting when drying liquid substances in small containers and when the product will be used in a short period of time.[26] A manifold dryer will dry the product to less than 5% moisture content. Without heat, only primary drying (removal of the unbound water) can be achieved. A heater must be added for secondary drying, which will remove the bound water and will produce lower moisture

content. Tray style freezedryers are typically larger than the manifold dryers and are more sophisticated. Tray style freeze-dryers are used to dry a variety of materials. A tray freezedryer is used to produce the driest product for long-term storage. A tray freezedryer allows the product to be frozen in place and performs both primary (unbound water removal) and secondary (bound water removal) freeze-drying, thus producing the driest possible end-product. Tray freeze-dryers can dry products in bulk or in vials or other containers.[27] When drying in vials, the freeze-dryer is supplied with a stoppering mechanism that allows a stopper to be pressed into place, sealing the vial before it is exposed to the atmosphere. This is used for long-term storage, such as vaccines. Improved freeze drying techniques are being developed to extend the range of products that can be freeze dried, to improve the quality of the product, and to produce the product faster with less labor. A lyophilizer consists of a vacuum chamber that contains product shelves capable of cooling and heating containers and their contents. A vacuum pump, a refrigeration unit, and associated controls are connected to the vacuum chamber.[28] Chemicals are generally placed in containers such as glass vials that are placed on the shelves within the vacuum chamber. Cooling elements within the shelves freeze the product. Once the product is frozen, the vacuum pump evacuates the chamber and the product is heated. Heat is transferred by thermal conduction from the shelf, through the vial, and ultimately into the product.[29]

Lyophilization Container Requirements

The container in which a substance is lyophilized must permit thermal conductivity, be capable of being tightly sealed at the end of the lyophilization cycle, and minimize the amount of moisture to permeate its walls and seal.[30] The enclosed reagents can only remain properly lyophilized if the container in which they are processed meets these requirements.

Lyophilization Heat Transfer

Successful lyophilization is heavily dependent on good thermal conductivity. For this, containers used in the lyophilization process must be capable of meeting a number of heat-transfer requirements. Such containers should be made of a material that offers good thermal conductivity; should provide good thermal contact with the lyophilizer shelf, which is the source of heat during processing; and should have a minimum of insulation separating the source of heat from the product requiring heating. Poor thermal conductivity often results from the use of containers made of materials with low coefficients of heat transfer. It can also be caused by the shape, size, or quality of the container.[31] It may come from thermal

barriers, such as excessive amounts of material, which can act as insulation, preventing energy from being transferred to the point at which the frozen ice and dried product interface.[32]

FREEZE DRYER DESIGN

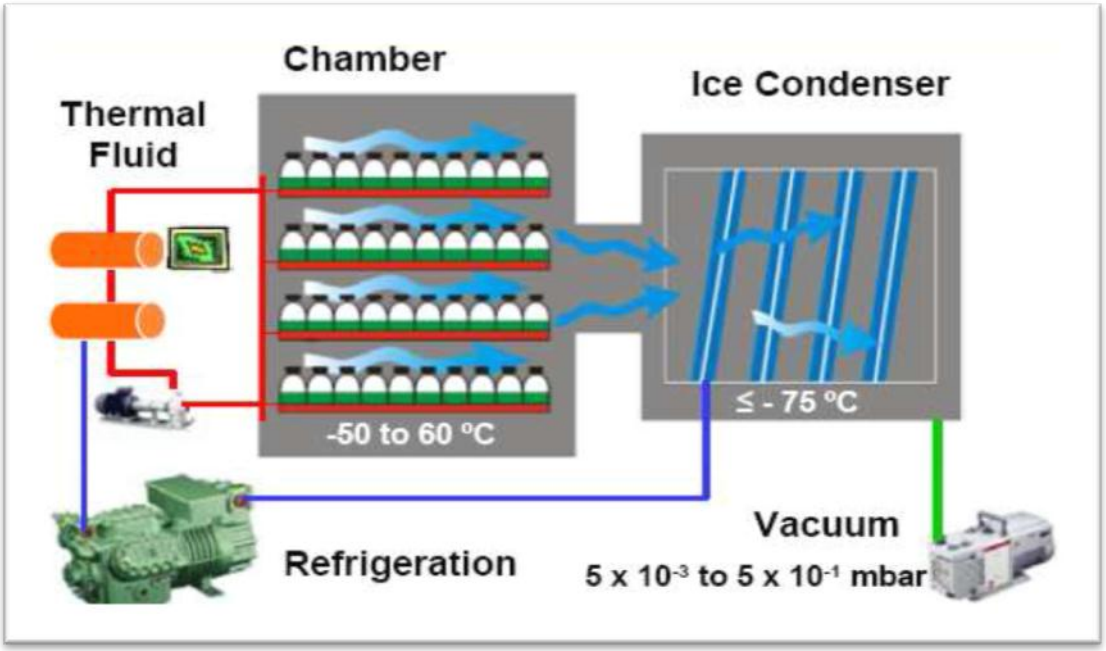

Figure 5: Lyophilizer Design

Essential Components Chamber

This is the vacuum tight box, sometimes called the lyophilization chamber or cabinet. The chamber contains shelf or shelves for processing product. The chamber can also fit with a stoppering system. It is typically made of stainless steel and usually highly polished on the inside and insulated and clad on the outside.[33] The door locking arrangement by a hydraulic or electric motor.

Shelves

A small research freeze dryer may have only one shelf but all others will have several. The shelf design is made more complicated because of the several functions it has to perform. The shelf act as a heat exchanger, removing energy from the product during freezing, and supplying energy to the product during the primary and secondary drying segments of the freeze drying cycle. The shelves will be connected to the silicone oil system through either fixed or flexible hoses. Shelves can be manufactured in sizes up to 4 m2 in area.[34]

Process Condenser

The process condenser is sometimes referred as just the condenser or the cold trap. It is designed to trap the solvent, which is usually water, during the drying process. The process condenser will consist of coils or sometimes plates which are refrigerated to allow temperature. These refrigerated coils or plates may be in a vessel separate to the chamber, or they could be located within the same chamber as the shelves. Hence there is designation "external condenser" and "internal condenser". Physically, the external condenser is traditionally placed behind the chamber, but it may be at the side, below or above.[35] The position of the condenser does not affect trapping performance. For an internal condenser the refrigerated coils or plates are placed beneath the shelves on smaller machines, and behind the shelves on larger machines, but again there is no performance constraint, only the geometry of the chamber.

Shelf fluid system

The freeze-drying process requires that the product is first frozen and then energy in the form of heat is applied throughout the drying phases of the cycle. This energy exchange is traditionally done by circulating a fluid through the shelves at a desired temperature [36]. The temperature is set in an external heat exchange system consisting of cooling heat exchangers and an electrical heater. The fluid circulated is normally silicone oil. This will be pumped around the circuit at a low pressure in a sealed circuit by means of a pump.

Refrigeration system

The product to be freeze dried is either frozen before into the dryer or frozen whilst on the shelves. A considerable amount of energy is needed to this duty. Compressors or sometimes-liquid nitrogen supplies the cooling energy. Most often multiply compressors are needed and the compressor may perform two duties, one to cool the shelves and the second to cool the process condenser.

Vacuum system

To remove solvent in a reasonable time, vacuum must be applied during the drying process. The vacuum level required will be typically in the range of 50 to 100μ bar. To achieve such a low vacuum, a two stage rotary vacuum pump is used. For large chambers, multiple pumps may be used.

Control system

Control may be entirely or usually fully automatic for production machines. The control elements required are as mentioned above, shelf temperature, pressure and time. A control program will set up these values as required by the product or the process. The time may vary from a few hours to several days. Other data such as a product temperatures and process condenser temperatures can also be recorded and logged.[37]

FREEZE DRYING PROCESS

The freeze drying process consists of three stages:

1. Freezing,
2. Primary drying, and
3. Secondary drying.

FREEZING

Since freeze drying is a change in state from the solid phase to the gaseous phase, material to be freeze dried must first be adequately prefrozen. The method of freezing and the final temperature of the frozen product can affect the ability to successfully freeze dry the material. Rapid cooling results in small ice crystals, useful in preserving structures to be examined microscopically, but resulting in a product that is more difficult to freeze dry. Slower cooling results in larger ice crystals and less restrictive channels in the matrix during the drying process. Products freeze 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. When the aqueous suspension is cooled, changes occur in the solute concentrations of the product matrix. And as cooling proceeds, the water is separated from the solutes as it changes to ice, creating more concentrated areas of solute. These pockets of concentrated materials have a lower freezing temperature than the water. Although a product may appear to be frozen because of all the ice present, in actuality it is not completely frozen until all of the solute in the suspension is frozen. The mixture of various concentration of solutes with the solvent constitutes the eutectic of the suspension. Only when all of the eutectic mixture is frozen is the suspension properly frozen. This is called the eutectic temperature. It is very important in freeze drying to prefreeze the product to below the eutectic temperature before beginning the freeze drying process. Small pockets of unfrozen

material remaining in the product expand and compromise the structural stability of the freeze dried product.

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.

The freezing point can be determined by means of,

- Theoretical thermodynamic value
- Cryo-microscope
- DSC (Differential Scanning Calorimetry)
- Measurement of temperature and resistance during the freezing phase

The electric resistance of the product being dried almost always rises dramatically with the transfer from the liquid to the solid state due to the reduced mobility of the ions and electrons. This means that by measuring the product temperature and electrical resistance at the same point it is possible to determine the freezing point. Because there is usually a very abrupt rise in resistance, the intersection of the Rx- and T-curves can be taken as the freezing point with a very high level of accuracy. This has been confirmed by numerous measurements with various solutions.

PRIMARY DRYING

After the freezing step has been completed, the pressure within the freeze-dryer is reduced using a vacuum pump. Typical chamber pressures in the lyophilization of pharmaceuticals range from 30 and 300 mTorr and depend on the desired product temperature and the characteristics of the container system. The chamber pressure needs to be lower than the vapor pressure of ice at the sublimation interface in the product to facilitate sublimation of ice and transport of water vapor to the condenser where it is deposited as ice. Very high chamber pressures decrease the sublimation rate by reducing the pressure gradient between sublimation interface and chamber, thereby mitigating the driving force for sublimation and continuing removal of ice. If the chamber pressure exceeds the vapor pressure at the sublimation interface, no mass transfer is possible. On the other hand, very low pressures (< 50 mTorr) are also counter productive for fast sublimation rates since they greatly limit the rate of heat transfer to the product. The ice at the sublimation interface shows a vapor

pressure that is directly correlated to the product temperature (Table 1). Once the chamber pressure decreases below the vapor pressure of ice in the product, sublimation can occur, i.e. ice is removed from the top of the frozen layer and directly converted to water vapor. Water vapor is transported to the ice condenser and deposited onto the coils or plates which are constantly cooled to a temperature associated with very low vapor pressure of the condensed ice. 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.[38] Low product temperature and the corresponding low vapor pressure of ice result in extensive primary drying times. It has been reported that elevation of product temperature by 1°C can reduce the overall primary drying time by as much as 13%, which offers enormous potential of saving process time and manufacturing costs when administering more aggressive product temperatures.[39] However, an increase of product temperatures to temperatures above the "critical formulation temperature" which refers to the eutectic melting temperature, TE, for crystalline and to Tc or Tg for amorphous materials, mostly leads to loss of cake structure. If the critical temperature is exceeded, the dried pore structure close to the sublimation front that still contains high amounts of water can undergo viscous flow, resulting in fusion of pores and formation of holes in the cake structure. This occurrence is associated with a reduction of inner surface area as well as elevated moisture contents with potentially detrimental effects on reconstitution time and completeness as well as API stability.[40] Most importantly, the cake shows shrinkage or may fully collapse, making the product unsuitable for sale and application in patients due to the lack of elegance. The critical formulation temperature can be determined using Freeze-Dry Microscopy (FDM) which allows observation of the drying cake structure under vacuum at varying temperatures.[41] Once the collapse temperature is reached it is possible to observe formation of holes in the dried cake structure. Since the sample is being dried during the experiment, the conditions are more similar to lyophilization than alternative methods, making the results more representative for a vial freeze-drying process.[42] A different approach to determine the critical formulation temperature is Differential Scanning Calorimetry (DSC) which measures the heat flow and thermal properties of the frozen sample. This way it is possible to determine the glass transition temperature of the maximally freeze-concentrated solute, Tg, which is indicative for molecular mobility in the amorphous matrix.[43] Since no removal of

water is involved, the critical temperature is not as representative for vial freezedrying as the collapse temperature determined using FDM. It is possible to increase the critical temperature by crystallizing salts (i.e. buffers etc.) quantitatively during freezing, or by adding amorphous excipients with high Tg' values such as dextran or cyclodextrines.[44] If formulations with high contents of crystallizing solutes are lyophilized, a crystalline lattice is formed that is stable up to product temperatures equivalent to the eutectic melting point TE which is much higher than common Tg' values. Therefore it is possible to create formulations with a high ratio of crystallizing substances and freeze-dry at temperatures above the Tg' of the amorphous ingredients which then collapse onto the crystalline matrix. Thus no global loss of structure occurs and the cake appearance is still elegant. It is important to pay close attention to API stability and choice of stabilizers to obtain a product stable over the shelf life when following such an approach, but it offers huge benefits for process optimization.[45]

SECONDARY DRYING

After primary freeze-drying is complete, and all ice has sublimed, bound moisture is still present in the product. The product appears dry, but the residual moisture content may be as high as 7-8% continued drying is necessary at warmer temperature to reduce the residual moisture content to optimum values. This process is called 'Isothermal Desorption' as the bound water is desorbed from the product.[46] Secondary drying is normally continued at a product temperature higher than ambient but compatible with the sensitivity of the product. In contrast to processing conditions for primary drying which use low shelf temperature and a moderate vacuum, desorption drying is facilitated by raising shelf temperature and reducing chamber pressure to a minimum. Care should be exercised in raising shelf temperature too highly; since, protein polymerization or biodegradation may result from using high processing temperature during secondary drying. Secondary drying is usually carried out for approximately 1/3 or 1/2 the time required for primary drying. The general practice in freeze-drying is to increase the shelf temperature during secondary drying and to decrease chamber pressure to the lowest attainable level. The practice is based on the ice is no longer present and there is no concern about "melt track" the product can withstand higher heat input.[47] Also, the water remaining during secondary drying is more strongly bound, thus requiring more energy for its removal. Decreasing the chamber pressure to the maximum attainable vacuum has traditionally been thought to favor desorption of water.

EXCIPIENTS IN LYOPHILIZED FORMULATION

The design of aq 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. Excipients may be characterized as buffers and pH adjusters, bulking agents, stabilizers, and tonicity modifiers.[48]

Buffers

Buffers are required in pharmaceutical formulations to stabilize pH. In the development of lyophilized formulations, the choice of buffer can be critical. Phosphate buffers, especially sodium phosphate, undergo drastic pH changes during freezing. A good approach is to use low concentrations of a buffer that undergoes minimal pH change during freezing such as citrate and histidine buffers.

Bulking agents

The purpose of the bulking agent is to provide bulk to the formulation. This is important in cases in which very low concentrations of the active ingredient are used. Crystalline bulking agents produce an elegant cake structure with good mechanical properties. However, these materials often are ineffective in stabilizing products such as emulsions, proteins and liposomes but may be suitable for small chemical drugs and some peptides. If a crystalline phase is suitable,mannitol can be used. Sucrose or one of the other disaccharides can be used in a protein or liposome product.

Stabilizers

In addition to being bulking agents, disaccharides form an amorphous sugar glass and have proven to be most effective in stabilizing products such as liposomes and proteins during lyophilization. Sucrose and trehalose are inert and have been used in stabilizing liposome, protein, and virus formulations. Glucose, lactose, and maltose are reducing sugars and can be reduce proteins by means of the mallard reaction.

Tonicity adjusters

In several cases, an isotonic formulation might be required. The need for such a formulation may be dictated by either the stability requirements of the bulk solution or those for the route of administration. Excipients such as mannitol, sucrose, glycine, glycerol, and sodium chloride are good tonicity adjusters. Glycine can lower the glass transition temperature if it is

maintained in the amorphous phase. Tonicity modifiers also can be included diluent rather than the formulation.

FREEZE DRYING METHODS

Three methods of freeze drying are commonly used:

1. Manifold drying,
2. Batch drying, and
3. Bulk drying.

Each method has a specific purpose, and the method used depends on the product and the final configuration desired.

MANIFOLD METHOD

In the manifold method, flasks, ampules or vials are individually attached to the ports of a manifold or drying chamber. The product is either frozen in a freezer, by direct submersion in a low temperature bath, or by shell freezing, depending on the nature of the product and the volume to be freeze dried. The prefrozen product is quickly attached to the drying chamber or manifold to prevent warming. The vacuum must be created in the product container quickly, and the operator relies on evaporative cooling to maintain the low temperature of the product. This procedure can only be used for relatively small volumes and products with high eutectic and collapse temperatures. Manifold drying has several advantages over batch tray drying. Since the vessels are attached to the manifold individually, each vial or flask has a direct path to the collector. This removes some of the competition for molecular space created in a batch system, and is most ideally realized in a cylindrical drying chamber where the distance from the collector to each product vessel is the same. In a "tee" manifold, the water molecules leaving the product in vessels farthest from the collector experience some traffic congestion as they travel past the ports of other vessels. Heat input can be affected by simply exposing the vessels to ambient temperature or via a circulating bath. For some products, where precise temperature control is required, manifold drying may not be suitable. Several vessels can be accommodated on a manifold system allowing drying of different products at the same time, in different sized vessels, with a variety of closure systems. Since the products and their volumes may differ, each vessel can be removed from the manifold separately as its drying is completed. The close proximity to the collector also creates an environment that maximizes drying efficiency.

BATCH METHOD

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

BULK METHOD

Bulk drying is generally carried out in a tray dryer like batch drying. However, the product is poured into a bulk pan and dried as a single unit. Although the product is spread throughout the entire surface area of the shelf and may be the same thickness as product dried in vials, the lack of empty spaces within the product mass changes the rate of heat input. The heat input is limited primarily to that provided by contact with the shelf as shown in Figure 6.

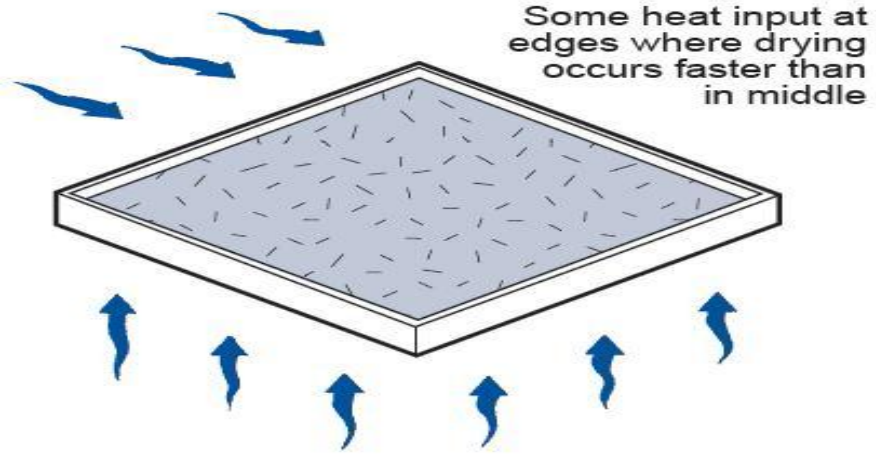

Figure: 6-bulk drying, heat is provided primarily through conduction from shelf

Bulk drying does not lend itself to sealing of product under controlled conditions as does manifold or batch drying. Usually the product is removed from the freeze dry system prior to

closure, and then packaged in air tight containers. Bulk drying is generally reserved for stable products that are not highly sensitive to oxygen or moisture.[49]

DETERMINATION OF END POINT OF FREEZE-DRYING PROCESS

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 H2O 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)

COMPARATIVE PRESSURE MEASUREMENT (I.E., PIRANI VS. CAPACITANCE MANOMETER)

During the drying step, the chamber pressure is controlled using a capacitance manometer, which measures the absolute pressure in the drying chamber. However, the Pirani vacuum gauge works on the principle of measuring the thermal conductivity of the gas in the drying chamber.[50] The Pirani gauge reads about 60% higher than the capacitance manometer (i.e., MKS Baratron) during primary drying when essentially all of the gas in the chamber is water vapor. This is because the thermal conductivity of water vapor is ~1.6 times the thermal conductivity of nitrogen. With this inherent property, the Pirani vacuum gauge can be used to detect the end of primary drying. The point where the Pirani pressure starts to sharply decrease (i.e., onset) indicates that the gas composition is changing from mostly water vapor to nitrogen; i.e., sublimation is “essentially” complete (Figure.7).

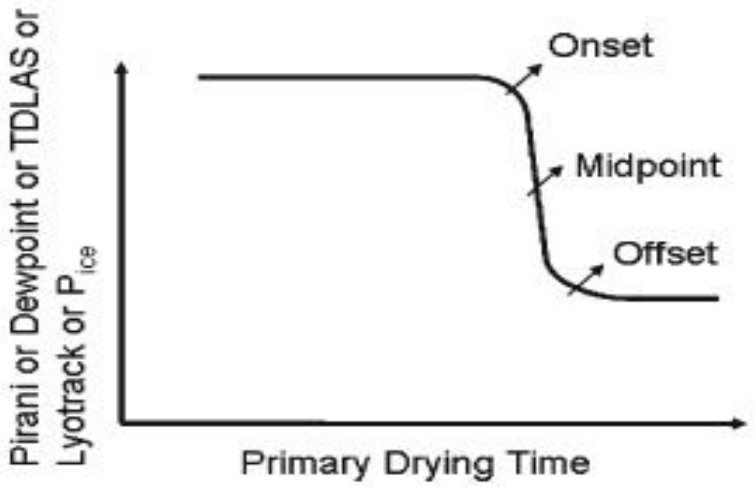

Figure 7: Pirani pressure, dew point, TDLAS (process [H2O]), Lyotrack (gas composition), and ice(vapor pressure of ice from pressure rise test) profile during primary drying

DEW POINT

An electronic moisture sensor can be used to measure the frost point, which is the temperature at which ice has an equilibrium vapor pressure equal to the measured partial pressure of water. The measurement is based on the principle of changes in the capacitance of a thin film of aluminum oxide arising from adsorption of water at a given partial pressure. Similar to the Pirani, the point where "dew point" starts dropping indicates that the sublimation is "essentially" complete, i.e. gas composition is changing from mostly water vapor to nitrogen (figure 7).[51]

PROCESS H2O CONCENTRATION VIA TDLAS

Tunable diode laser absorption spectroscopy (TDLAS) directly measures the water vapor concentration (molecules/ cm3) in the duct connecting the chamber and the condenser. The TDLAS unit is commonly installed with two laser beams, one directed with and the other directed against the vapor flow. TDLAS works on basic spectroscopic principles measuring absorption of radiation by water vapor to monitor the trace concentration of water vapor in real time. A laser beam is passed through a gas mixture containing a quantity of the target gas, and the beam's wavelength is tuned to one of the target gas's absorption lines to accurately measure the absorption of that beam from which one can deduce the average concentration of target gas molecules integrated over the beam's path length. the sublimation rate can be determined from the gas flow velocity and concentration of water vapor. The

point where water concentration starts decreasing sharply (i.e., onset) indicates that the gas composition is changing, and hence sublimation is "essentially" complete (Figure.7).[52]

LYOTRACK (GAS PLASMA SPECTROSCOPY)

This method is the latest addition to the online monitoring devices for freeze-drying and is manufactured by Alcatel Vacuum Technology, France. Lyotrack is based on optical emission spectroscopy and measures water vapor concentration during the drying process. It consists of a plasma generator and an optical spectrometer. Lyotrack gas composition signal was sensitive to gas composition in the chamber as well as the duct but not in the condenser. The wavelengths of the emitted light are the characteristic signatures for the identification of the atom or molecule. The point where water vapor concentration starts sharply decreasing (i.e., onset) indicates that the gas composition is changing, and hence sublimation is "essentially" complete (Figure.7).[53]

PRODUCT TEMPERATURE DURING PRIMARY DRYING

The end point of primary drying can also be determined from the product thermocouple response, assuming the vials containing the thermocouples are representative of the batch as a whole.[54] Product temperature approaching the shelf temperature set point (i.e., "offset" in Figure.8) is commonly taken as an indication of the end of primary drying.

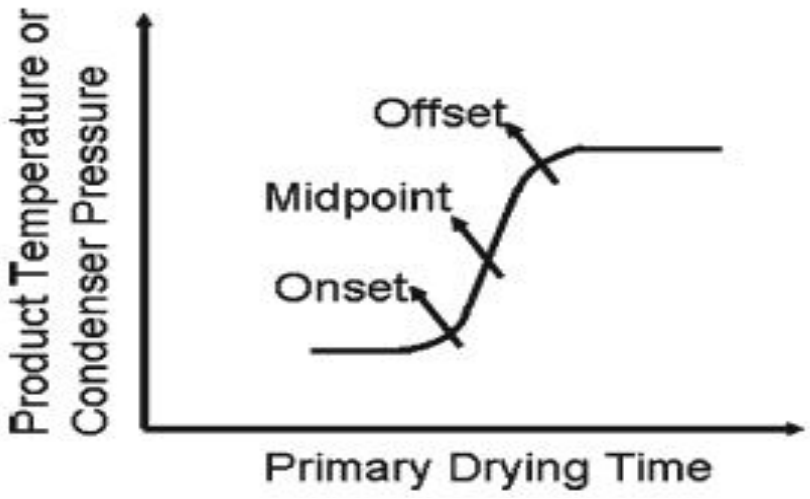

Figure 8: Product temperature and condenser pressure profile during primary drying

CONDENSER PRESSURE DURING PRIMARY DRYING

Yet another indicator of the end point of primary drying is the condenser pressure. During primary drying, most of the gas in the chamber is water vapor, and because the total vapor

flux is high, a high ΔP (difference between chamber and condenser pressure) develops to remove the water from the chamber. However, once primary drying is over, ΔP decreases (i.e., condenser pressure (Pcond increases since chamber pressure (Pc) is held constant). The point where condenser pressure starts increasing (i.e., onset) indicates that the sublimation is "essentially" over since the high mass transfer portion of the process (i.e., sublimation) is largely over (Figure.8). A capacitance manometer installed in the condenser reads the condenser pressure.

PRESSURE RISE TEST

MTM is a procedure to measure the product temperature during primary drying by quickly isolating the chamber from the condenser for a short time (≈25 s) and analyzing the pressure rise during this period. This analysis yields vapor pressure of ice at the sublimation interface, the product temperature, and the mass transfer resistance of the dried product.[55] However, the data obtained measure the vapor pressure of ice accurately only as long as the system remains in primary drying. At the end of primary drying, there is little or no pressure rise because all ice is gone, and hence the calculated "vapor pressure of ice" becomes equal to the chamber pressure (Figure.7). Thus, a close approach of the calculated vapor pressure of ice to the chamber pressure forms the basis of the criterion for end of primary drying.

STABILITY OF FREEZE DRIED PRODUCTS

Several factors can affect the stability of freeze dried material. Two of the most important are moisture and oxygen. All freeze dried products have a small amount of moisture remaining in them termed residual moisture. The amount of moisture remaining in the material depends on the nature of the product and the length of secondary drying. Residual moisture can be measured by several means: chemically, chromatographically, manometrically or gravimetrically. It is expressed as a weight percentage of the total weight of the dried product. Residual moisture values range from <1% to 3% for most products. By their nature, freeze dried materials are hygroscopic and exposure to moisture during storage can destabilize the product. Packaging used for freeze dried materials must be impermeable to atmospheric moisture. Storing products in low humidity environments can reduce the risk of degradation by exposure to moisture. Oxygen is also detrimental to the stability of most freeze dried material so the packaging used must also be impermeable to air. The detrimental effects of oxygen and moisture are temperature dependent. The higher the storage temperature, the faster a product degrades. Most freeze dried products can be maintained at

refrigerator temperatures, i.e. 4-8°C. Placing freeze dried products at lower temperatures extends their shelf life. The shelf life of a freeze dried product can be predicted by measuring the rate of degradation of the product at an elevated temperature. This is called accelerated storage. By choosing the proper time and temperature relationships at elevated temperatures, the rate of product degradation can be predicted at lower storage temperatures.

CONCLUSION

The lyophilization technique proved to be an advantage for development of stable injectable dosage form as the moisture content of the formulation is greatly reduced thus enhancing the stability of the product, ease of handling, rapid dissolution because of porous nature of the cake and easier transport of the material during shipping. About 50% of the currently biopharmaceuticals are lyophilized, representing the most common formulation strategy. In the freeze dried solid state, chemical or physical degradation reactions are inhibited or sufficiently decelerated, resulting in an improved long term stability.The awareness of the complexity of the freezing process and its consequences on product quality and process performance is essential for successful lyophilization. The knowledge of how to control, or atleast manipulate, the freezing step will help to develop more efficient lyophilization cycles and biopharmaceutical products with an improved stability.

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