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REVIEW ON LYOPHILIZATION TECHNIQUE

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ABSTRACT

While arriving a new century, lyophilization in the pharmaceutical field has been subjected to ongoing development and steady expansion. The objectives of the review is to highlight recent advances but also to discuss further challenges in lyophilization. At first, the three major steps of a typical lyophilization process, namely freezing, primary drying, and secondary drying, are described in brief. Additionally, a general description of the essential components and their function in a lyophilizer is given. The principle of lyophilization; heat transfer and mass transfer also described. With respect to recent advances in lyophilization, the expanded range of pharmaceutical applications based on lyophilization is brief. Moreover, novel formulation aspects and novel container systems are discussed and the importance of the freezing step is outlined. Furthermore, the dogma of—never lyophilizing above the glass transition temperaturlre is argued and recent insights into novel stabilization concepts are provided. Process analytical technology (PAT) and quality by design (QbD) are now leading issues and the design of the lyophilization equipment might have to be reconsidered in the future. Nowadays, lyophilization has also gained importance for the preservation and stabilization of biological products, hormones, proteins, nucleic acid based pharmaceuticals.

Key Words: Lyophilization, Recent trend, novel formulation, novel container.

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INTRODUCTION [1-3]

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. 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. Freeze drying has been used in a number of applications for many years, most commonly in the food and pharmaceutical industries. 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. Freeze-drying or lyophilisation 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. The steps involved in the formulation of freeze dried product are depicted in below figure.

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 cannot 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.

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

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. 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 foods, the process of lyophilization consists of:

1. Freezing the food 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 [4, 5].

**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. 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. 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. 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. 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.

![Phase diagram showing the triple point of water at 0.01°C, 0.00603 atm.](image)

**Fig. 2:** Phase diagram showing the triple point of water at 0.01°C, 0.00603 atm.

Lyophilization is carried out below the triple point to enable conversion of ice into vapor, without entering the liquid phase (known as sublimation).

![Steps involved in lyophilization from sample preparation to final product formation](image)

**Fig. 3:** Steps involved in lyophilization from sample preparation to final product formation

Formulations, excipients are included to improve the functional properties and stability of the lyophilized product. The International Pharmaceutical Excipients Council has defined excipients as "substances other than the pharmacologically active drug or prodrug which are included in the manufacturing process or are contained in a finished pharmaceutical product dosage form”

**The Fundamental Process Steps**

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. Resulting product has a very large surface area thus promoting rapid dissolution of dried product.

Applications

Pharmaceutical and biotechnology:

Pharmaceutical companies often use freeze-drying to increase the shelf life of products, such as vaccines and other injectables. 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 freeze-dried 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.

Other Uses:

Organizations such as the Document Conservation Laboratory at the United States National Archives and Records Administration (NARA) have done studies on freeze-drying as a recovery method of water-damaged books and documents. In bacteriology freeze-drying is used to conserve special strains.

FREEZE DRYING PROCESS [5, 6]

The freeze drying process consists of three stages:

1. Freezing,

2. Primary drying, and

**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 concentrations 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 pre-freeze 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 [7-10]:**

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 vapour pressure of ice at the sublimation interface in the product to facilitate sublimation of ice and transport of water vapour 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 vapour 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 vapour pressure that is directly correlated to the product temperature (Table 1). Once the chamber pressure decreases below the vapour 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 vapour. Water vapour is transported to the ice condenser and deposited onto the coils or plates which are constantly cooled to a temperature associated with very low vapour 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. Low product temperature and the corresponding low vapour 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. 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. 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. 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 representatives for a vial freeze-drying process. 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. Since no removal of water is involved, the critical temperature is not as representative for vial freeze drying 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. 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.

**Secondary Drying [11-16]:**

In the area where the ice has already been removed, desorption of water from the cake occurs; this process is referred to as secondary drying and already starts in the primary drying phase. Once all ice has been removed from all product containers, the shelf temperature is
elevated and typically maintained at a temperature between 20°C and 40°C for several hours. The rate of desorption and the obtainable moisture level is controlled by diffusion within the solute phase and desorption from the surface and therefore depends mostly on product temperature; further reduction of chamber pressure is not required. The ramp rate to the secondary drying temperature needs to be moderate (0.1°C/min to 0.3°C/min) for amorphous substances to avoid surpassing the glass transition of the lyophilized cake and pertaining cake shrinkage. Secondary drying times are usually designed to achieve a reduction of moisture content within the cake to less than 1%. For most lyophilized API’s the stability increases with the reduction of moisture, so it is beneficial to reduce the residual moisture as much as possible. However, thermal stresses to the API due to the elevated product temperature need to be considered. Especially for proteins it is necessary to determine optimal secondary drying conditions which result in an optimum moisture content without detrimental effects from heating. For some protein formulations, the stability optimum has been found at intermediate moisture contents, i.e. between 1-3% RM. Targeting of such moisture contents for all vials in the batch is often difficult and hard to monitor.

**REFERENCE**


