International Journal of Pharmaceutical and Medicinal Research

Journal homepage: **www.ijpmr.org**

Review Article

A REVIEW ARTICLE ON LYOPHILIZATION TECHNIQUES USED IN PHARMACEUTICAL MANUFACTURING

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ARTICLE INFO:

Article history:
Received: 05 November, 2017
Received in revised form: 21 November , 2017
Accepted: 28 November, 2017
Available online: 30 December , 2017

Keywords:
Freeze drying
Cycle optimization
Parenterals Lyophilization
Stability

ABSTRACT

Lyophilization processes are widely employed in the industrial field to preserve several kinds of substances, such as foods and drugs. In some cases, e.g. during the process optimization, the real-time measurement of mass and temperature is important to monitor drying rate and to prevent product damages because of unsuitable temperatures. In these cases sensors and measurement instruments have to be installed inside the vacuum chamber of the freeze dryer, where temperature and pressure can reach -60 ^{0}C and 1 Pa respectively. This paper deals with the problems related to the measurement of mass and temperature inside freeze dryers and describes a measurement system that has been specifically designed to monitor lyophilization processes.

1. Introduction

Lyophilization is the most general technique for formulating Parenterals products when stability in aqueous solution 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. Lyophilization produces excellent quality products, both foodstuff and pharmaceuticals, due to the moderate temperatures at which the process takes place, contributing to the formation of highly porous solids that retain aroma, colour, and flavour[1,2]. Vacuum lyophilization takes place at very low pressures so that the operation occurs below the triple point of water, leading to high investment and operating costs[2,3]. Lyophilization is a technology, method, process by a product is frozen (converting all mater to solid state) and then water removed by sublimation (primary drying) of the freezed water molecule (solid particles) i. e. Ice. The complete process of freeze drying requires steps; freezing of the molecule in which water is there by nucleation and many other method; after that second step is main drying (MD) by this sublimation of the ice molecules happens; then secondary drying (SD) in this step desorption of the water molecule which is bounded to the solid particle or particles, and in the last packing should be done in the vails/containers to prohibit reabsorption of water and/or oxygen from the atmosphere. With the help of freeze-drying a product unstable/less stable/ decomposable/degradative in the presence of moisture is converted into a dried and stable formulation[4]. Development of this technique to comply four demands on the Rinsed product: its volume should remain in the frozen state, the structure and the biological activity of the dried solid and the original substance should be same as far as possible, the dried product remains stable during storage in room temperature. It is possible that the product can be stable at temperatures up to 40 ^{0}C during storage for up to 2 years and when water is added then the lyophilized product is quickly reconstituted[5].

2. Sublimation

Sublimation is when a solid (ice) changes directly to a vapour without first going through a liquid (water) phase. Thoroughly understanding the concept of sublimation is a key building block to gaining knowledge of freeze drying. As shown below on the phase diagram for water, low pressures are required for sublimation to take place. Sublimation is a phase change and heat energy must be added to the frozen product for it to occur. Sublimation in the freeze drying process can be described simply as:

- FREEZE - The product is completely frozen, usually in a vial, flask or tray.
- VACUUM - The product is then placed under a deep vacuum, well below the triple point of water.
- DRY – Heat energy is then added to the product causing the ice to sublime.

The steps required to lyophilize a product in a batch process can be summarized as follows:

- Pre-treatment / Formulation
- Loading / Container (Bulk, Flask, Vials)
- Freezing (Thermal Treatment) at atmospheric pressure
- Primary Drying (Sublimation) under vacuum
- Secondary Drying (Desorption) under vacuum
- Backfill & Stoppering (for product in vials) under partial vacuum

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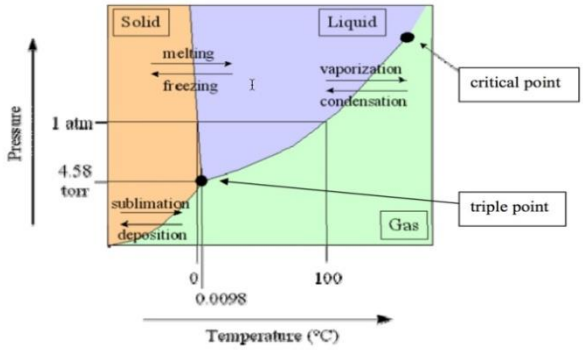

Figure No. 1: Phase diagram for freeze drying

2.3 Advantages & disadvantages of lyophilization

2.3.1 Advantages

- Processing a liquid with ease (and thereby simplifying aseptic handling).
- Enhancing the stability of a dry powder as well as the product stability in a dry state
- Removing water without having to heat the product excessively.
- Dissolution of reconstituted product (rapidly and easily).

2.3.2 Disadvantages

- Handling and processing time increases
- Sterile diluents needed upon reconstitution
- Equipment becomes costly and complex
- Expensive
- Timely
- Requires large capital investment
- More of an art than a science
- One of the biggest problems in lyophilization is the mess it can create," said Robinson.

The filling of vials that are to be lyophilized can also be challenging, specifically having an open system from filling all the way through the end of the process (and the resulting potential for contaminants to enter the vials).

At present, 80 percent of the market lyophilizes in vials. "Consider using a closed tray in lyophilization chambers. Using a closed tray can prevent product flyout during the filling, transportation, and lyophilization process, which reduces dryer cleaning time and validation of cleaning procedure," said Robinson

2.4 Lyophilization

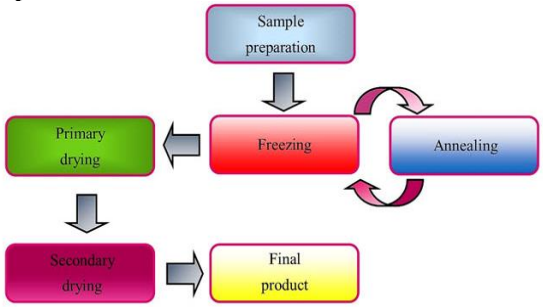

Figure No. 2: Lyophilization process flow

Typically, lyophilization occurs in following stages:

- Pre Freezing
- Freezing
- Primary drying
- Secondary drying
- Sealing of lyophilised product

"Freezing takes place in stage one of the lyophilization processes. It can take place in the freeze dryer. Some customers freeze in a freezer instead. Freezing temperatures are around -40°C, Robinson explained.

There is no thawing in the second stage, she adds. "The product goes from frozen state to dry powder through the process of sublimation."

"Think of lyophilization as basically freeze-drying - it is a dehydration process typically used to preserve a perishable material or make the material more convenient for transport. Freeze-drying works by freezing the liquid material and then reducing the surrounding pressure to allow the frozen water in the material to sublimate directly from the solid phase to the gas phase. This leaves us with a dry powder."

Depending on the type of product and quantity, it can take 12-72 hours to go through all of these stages.

As to the lyophilization process, Robinson broke it down into the following steps:

- Fill tray with liquid solution
- Carry trays over to dryer and load
- Lyophilization (including those three stages).

"Lyophilization is ideally introduced in the R&D phase, but more commonly in Phase 1 of drug development and follows along with the drug development cycle," said Robinson. "End users perform lyophilization in pilot scale in house freeze dryers. At some point, likely around Phase 3, the end user transfers the lyophilization to CMOs unless they have large scale freezer dryers in house."

3. Recipe for freeze drying

Lyophilization in a shelf freeze dryer requires the design of a working process or cycle which is sometimes referred to as a "recipe". Typically, there are multiple steps involved for both freezing and drying of the product. Individual temperature, pressure and time settings need to be determined for each step. Each specific product or formulation that is lyophilized requires the development of a freeze drying process that is based on the unique characteristics of the product, the amount of product and the container used. There is no universal "safe" recipe that will work with every product.

3.1 Pre-freezing

In lyophilization method and final temperature affect ability to successfully freeze dry the material. In lyophilization freezing step mainly affected by cooling rate. Rapid cooling rate mainly used for preserving stature to be examined in the microscopically but product is more difficult to lyophilized. Slow cooling rate can use to bigger ice crystal but in the case of human or plant cell larger crystal can rupture and product is

less restriction channels in the matrix to freeze dry[5]. Product can be freeze by two ways. Product consists of primarily of water, solvent material dissolved/ material suspended in the water or solute. Most sample are eutectics which freeze at lower temperature than surrounding water. In pre freezing step on cooling pocket are formed, ice contain pockets in which solute is present and have lower freezing temperature than water. Product looks as frozen but it is not frozen completely till solute in the suspension is completely frozen. Only when all of mixture (eutectic mixture) is frozen then only product fitly frozen. This temperature is called the eutectic temperature. Pre freezing of the formulation is performed below this temperature (eutectic temperature) before freezing step is performed because small pockets of unfrozen suspension leftover in the product amplify and degrade the structure stability of the lyophilized product. In other way formulation that bear glass formation during freeze drying process. The complete suspension as the temperature lower the formulation becomes more viscous. At last the product freezes at the point forming called as glass transition point when the formulation becomes a viscous solid. This type of product is very difficult to lyophilized[7-12].

3.2 Freezing

While in simple terms, the freezing step is first step in processing and apparently the most complicated step in freeze drying process. A phase stated the "freeze-concentrate" when water freezes the soluble particles in the product remain in the remaining liquid. At which point ice formation became maximal, that make up the lattice when the freeze concentrate solidifies between the ice crystals. At the stage of primary drying, ice is formed during freezing is which is the crystalline and it's removed by sublimation. That's why, the vapour pressure of the chamber is decreased below the ice vapour pressure, and the temperature of shelf is increased to supply the heat removed by sublimation of ice[13].

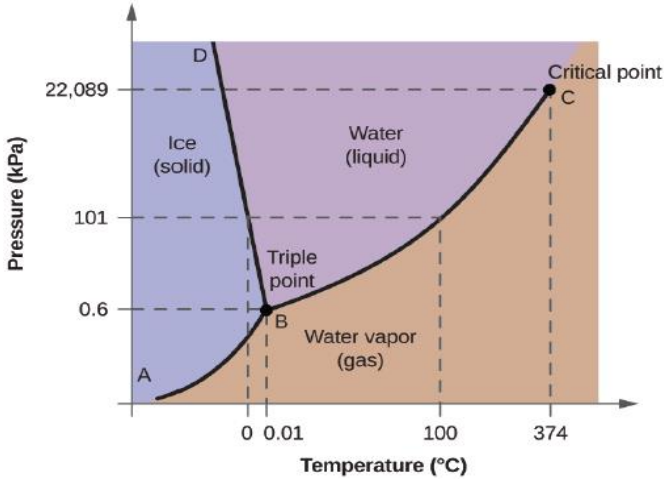

Figure No. 3: Phase diagram of water-ice-vapour system

As per Fig. 2 (phase diagram of water)[14], most product are frozen well below their eutectic/glass transition point (A). Temperature is decreased to just below critical temperature (B). No matter what type of freeze drying system is used condition must be crated to encourage the free flow of water molecule. Therefore vacuum pump is an essential component of a freeze drying system and is used to lower the pressure (C). the molecule have a natural affinity to move towards the collector chamber because it's vapour pressure is lower than that of the product. Therefore the collector temperature (D). must be significantly lower than the product temperature. So freezing can be defined as it is a process when ice crystallization occurs from super cold water. In simple, in freezing process first Cooling of solution that will lead to "nucleation" (nucleation is a process in which small nuclei is formed in solution or saturated solution). The degree of super cooling is mainly responsible for the rate of ice growth, number of ice nuclei formed, and the ice crystal's size[16].That will lead to growing of Ice crystals at a defined rate, freeze concentration of the solution will be formed, a process that can result in crystalline and amorphous solids, or in mixtures of amorphous & crystalline[17,18]. The freezing rate of a formulation is not necessarily related to its cooling rate[18]. Because the cooling rate is defined "as the rate at which a solution is cooled & the freezing rate is the rate of post nucleation ice crystal growth, which is largely determined by the amount of super cooling prior to nucleation"[17,19]. Ice nuclei getting more time to grow when providing slow rate of freezing, the ice will grow and form the ice crystals and solution which is present in between the crystal becomes more concentrated. But in the case rapid freezing will leads to grow small and the remaining solution will become so much vicious. As the viscosity increased, water molecule becomes a part of concentrated liquid (glass) in between ice crystals and not able to diffuse[17]. The cryoprotective effect of four carbohydrates (glucose, fructose, mannose and maltose) on paradodecanoyl-calixarene based SLNs (solid lipid nanoparticles) has been investigated by PCS (photon correlation spectroscopy) and these four carbohydrates have been shown to act as good cryoprotectants, allowing reconstitution of the suspensions after the freeze-drying process[6,20]. If other solid/ dissolved substances are present the freezing behaviour of water changes differently, e.g. cryoprotective agents (CPAs). They all protect quality of the product to be lyophilized in one way or another; they can be used in alone or in combination. Lyophilization of liposomes without special protectants, cryoprotectors, causes them to coalesce and aggregate. The included water-soluble drug can also leak out[6].

There are several freezing method are describe[6,22].

- Shelf-ramped freezing
- Pre-cooled shelf method
- Annealing
- Quench freezing Quench
- Directional freezing
- Ice fog technique
- Electro-freezing
- Ultrasound-controlled ice nucleation
- High-pressure shift freezing or depressurization technique
- Vacuum-induced surface freezing
- Non-aqueous co-solvents
- Addition of ice nucleating agents

3.3 Primary drying

Primary drying is also known as main drying because in this phase of Lyophilization sublimation occurs. Sublimation occurs when a frozen solvent passes to gaseous phase without passing through liquid phase. The crystals of ice by using a

special freezing method will grow extremely uniformly. The ice sublimes and the remaining solids show their original structure after freezing. In the process of sublimation the ice temperature at the sublimation front (Tice) should be done at well below the collapse temperature (Tc)[17]. The end of the sublimation phase corresponds to the decrease in the moisture sensor signal down to a low constant value[23]. The vapour transportation of vapour and supply of heat to the condenser which is shown Fig. 4 is most important parameter during primary drying. That's why the operation pressure is a very mandatory tool to control Tice, if the temperature of shelf is conserved constant and the temperature of condenser is always down to a maximum, which depends on the design of the plant and water vapour pressure in the chamber. Sublimation is a process when matter directly converts from solid state to vapour state without melting (liquid Phase). Sublimation occurs at a controlled environment for a particular substances at define range of pressures and temperatures[24]. The phase diagram of pure water shows that sublimation of water ice can be done when the temperature and vapour pressure are below the triple point of water - i.e., below 0.010C and 611.73 Pa, accordingly. In the end of sublimation process most of the water which is present in the form of moisture removed from formulation[17]. In lyophilizer, vapour is formed after sublimation process in lyophilization chamber goes to condenser. Condenser continuously remove it[25]. When main drying MD completed the most of the ice is sublimated. And the standard deviation of Tice above the measured Tice decreases during MD. This parameter can be utilized to change continuously from second stage to third stage that is main drying MD to secondary drying (SD), e.g. if the average becomes 2-30C above than measured Tice the during main drying (MD)[17].

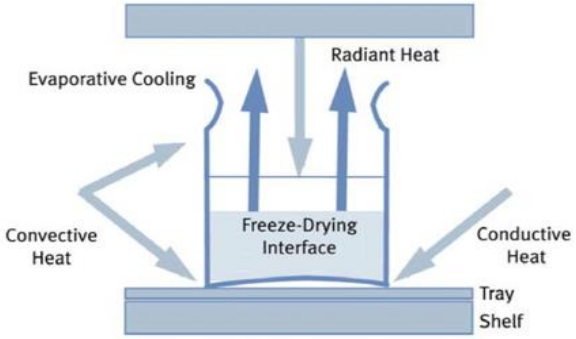

Figure No. 4: Heat transfer in a shelf freeze dryer

3.4 Secondary drying

After primary drying all ice will be sublimated but moisture will be present in form of bound to the solid particle pf formulation. The formulation seems to be dried but it's not because of bound residual moisture can be present as high as 7-8%, so it is mandatory to drying at higher temperature. This will leads to reduce the moisture. When the bound water is desorbed from formulation then it's called as Isothermal Desorption. Secondary drying carried out at high vacuum and moderate temperature (20–60°C). The dryer loses shelf control for 30 mins during secondary drying as a result of a brief power outage. This results in the shelf temperature being maintained at 5°C cooler than the set point of 25°C. In the freeze drying process the third stage secondary drying is used for desorption of water which is present in the bound form until target residual water content is achieved. At this stage, the drying temperature is more important determinant of the amount of moisture with drying time[26].

3.5 Sealing Lyophilized Products

A freeze dried product should be closed within its container before removing it from the ultra-dry atmosphere present at the end of the freeze drying process. The formulation which has been go through this cycle most of the time it contains less than 1 % of moisture, so when it will come contact with moisture containing environment, product will try to take moisture as its capacity. The quality of the product will be degraded immediately. Enhanced chemical performance, increased shelf life, and rapid reconstitution properties required by lyophilized product after freeze drying, will be compromised. If moisture is again taken by product then loss of product, false result, product failure and product recalls will be consequences. To use of packages that can't be sealed inside the lyophilizer before to re-pressurization that is the most common mistake by companies. For example, the manufacturing process for some diagnostic products can require lyophilizing the product inside a large number of screw-top tubes. Sealing of these tubes inside of a lyophilizer before terminating the batch, there is no practical way, that"s why the company will gather a large production staff to apply manually to a room which is incompatible with freeze drying process. Recently, stable chemistry will be endangered by the risk of unacceptable high and variable moisture levels during manual sealing process. So exposing lyophilized material to atmospheric moisture in this way may result in an unstable product[27]. For the successful performance of any lyophilization process all four stages of the lyophilization process (freezing, primary drying, secondary drying and sealing) carrying equally importance , So it can produce a dried and stabilized product for storage at long-term. Subsequent steps, final moisture level, or quality of overall product can largely affected by any change in one step of any stage of lyophilization cycle. The basic principles of lyophilization must be understood and then it should apply to lyophilization process and individual product. To ensure proper "validation proper process qualification and continuous process monitoring" should be performed. For scrutinizing the process is under control or not, process validation of lyophilization process should include the validating process variables of formulation. This process should take consideration of critical variables a. e. Final mixing, filtration, filling, partial stoppering of three batch after that stoppering sealing sand packing.

4. Cycle optimization

In addition to designing a recipe that successfully dries a product, it is also extremely valuable to optimize (shorten) the length of the cycle, especially if there is potential for process repetition or scale-up for production. Freeze drying can be a multi-day process. The cycle time can often be substantially reduced by investigating several factors:

- Freezing and annealing – maximize crystal size and crystallization to increase drying rates.
- Thickness of product - water vapour molecules experience resistance as they exit from the dried portion of the product. Thinner samples yield less resistance to vapour flow and lead to faster drying. Shell freezing can help when drying bulk product in flasks.
- Critical Collapse Temperature – this is the most important piece of information for cycle optimization. The ability to run primary drying at higher product temperatures greatly reduces drying time by creating a larger pressure differential between the vapour pressure over ice in the product and the pressure at the condenser.

Cycle optimization using eutectic/collapse temperature information requires an iterative approach of taking real-time measurements of the product temperature during primary drying and then making corresponding adjustments to the shelf temperature settings. This can be accomplished manually using product thermocouples or, if drying in vials, an automated smart system can be used.

5. Evaluation parameters

5.1 In process evaluation parameters

1. Leak Test of vials
2. Description Testing In Process
3. Water content
4. Average Weight of Cake
5. Dosage form Uniformity
6. Constituted solution
7. Description of solution
8. Reconstitution time
9. Particulate matter in the solution
10. pH of the bulk
11. Bacterial Endotoxin limit
12. Sterility
13. % Assay
14. Relative substance

5.2 Evaluation parameters at end of stage

1. Description of formulation
2. pH after reconstituted
3. % Assay of API
4. Filtration
5. Sterility Test of the final formulation
6. Filling of Bulk
7. Filled Volume of Bulk
8. % Assay at Bulk
9. Lyophilization
10. Water Content in %
11. Sealing of Vials (Full Stoppered)

6. Freeze drying equipment

The main components of freeze drying equipment are:

- Refrigeration System
- Vacuum System
- Control System
- Product Chamber or Manifold
- Condenser

The refrigeration system cools the (ice) condenser located inside the freeze dryer. The refrigeration system can also be employed to cool shelves in the product chamber for the freezing of the product.

The vacuum system consists of a separate vacuum pump connected to an airtight condenser and attached product chamber.

Control systems vary in complexity and usually include temperature and pressure sensing ability. Advanced controllers will allow the programming of a complete "recipe" for freeze drying and will include options to monitor how the freeze drying process is progressing. Choosing a control system for the freeze dryer depends on the application and use (i.e. lab vs. production).

Product chambers are typically either a manifold with attached flasks, or, a larger chamber with a system of shelves on which to place the product.

The purpose of the condenser is to attract the vapours being sublimed off of the product. Because the condenser is maintained at a lower energy level relative to the product ice, the vapours condense and turn back into solid form (ice) in the condenser. The sublimated ice accumulates in the condenser and is manually removed at the end of the freeze drying cycle (defrost step). The condenser temperature required is dictated by the freezing point and collapse temperature of the product. The refrigeration system must be able to maintain the temperature of the condenser substantially below the temperature of the product.

In shelf freeze dryers, the condenser can be located inside the product chamber (internal condenser) or in a separate chamber (external condenser) connected to the product chamber by a vapour port.

Manifold freeze dryers rely on ambient conditions to provide the heat of sublimation to the product. This heat input does not melt the product because an equivalent amount of heat is removed by vaporization of the solvent. Advanced shelf freeze dryers can provide a heat source to control/expedite the drying process and they can also employ the refrigeration system to allow freezing of product inside the unit.

Freeze dryers can be informally classified by the type of product chamber: (1). Manifold dryers where the product is typically pre-frozen & in flasks (2). Shelf dryers where the product is placed in a tray or directly on a shelf (3). Combination units with both drying options.

7. Conclusion

Lyophilization technique is used in the development of stable injectable doses form for a drug those having poor shelf life and stability problem degraded in the presence of moisture content present in the formulation. By using freeze dry technique the stability and shelf life of the product are enhanced. The dry pharmaceutical product mainly prepare by

lyophilization. There are following steps involved in the lyophilisation: Freezing, primary drying and secondary drying. In freezing process, the solvent generally water is frozen in

to form ice. The solute is present in the interstitial space between the ice crystals. Primary drying mainly involve sublimation of ice that transfer in to vapour phase without going through to the liquid phase. The vapours formed during sublimation are removed through the condenser plate in lyophilisation chamber. In the secondary drying water content of product is low enough that there are no chemical reaction or biological grow occurs. Therefore secondary dry is done for reduce the water level and remove the residual water and ensure the stability of final product.

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Source of support: Nil, Conflict of interest: None Declared