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LYOPHILIZATION- A PROCESS OF OPTIMIZATION OF DOSAGE FORMS

Anjali Pande [*1], Pallavi Dhekale [2] and Vikas Thorat [1]

Department of Pharmaceutics [1], Department of Pharmaceutical Chemistry [2], PDEA's Shankarrao Ursal College of Pharmaceutical Sciences and Research Centre, Kharadi, Pune - 411014, Maharashtra, India.

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Correspondence to Author:
Ms. Anjali Pande

Assistant Professor,
Department of Pharmaceutics,
PDEA's Shankarrao Ursal College of Pharmaceutical Sciences and Research Centre, Kharadi, Pune - 411014, Maharashtra, India.

E-mail: pandeanji555@gmail.com

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 the best quality products compared to products dried with traditional methods. In the pharmaceutical field, lyophilization has become an important subject to ongoing development and its expansion. In this 21st century, Lyophilization emerges to be a novel trend for the drying of pharmaceuticals and biological those is thermolabile or are unstable in aqueous form but stable for longer periods in their dried form. This article provides an overview on the process of lyophilization, how the freeze-drying cycle is designed, discussing several important parameters which are important for an understanding of this process as well as their role in the designing of an optimized freeze-drying cycle, so that a robust and economical process of lyophilization can be developed which does not impact the product quality. This review focused on the recent advances and its targets in the near future.

INTRODUCTION: It involves the freezing, sublimation, and subsequent desorption of a solvent from a product to result in a material that is dry, stable, and readily soluble. In fact, the term lyophilization comes from the word lyophile, which means solvent loving. The principle of lyophilization or freeze-drying dates back to early Incas in South America who would freeze their potato or meat on the low lying plains overnight then carry the product up the hills in the higher altitude where the low pressure induced the sublimation of ice and resulted in a freeze-dried product.

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Freeze-drying or lyophilization is a drying process used for converting solutions of labile materials into dry solids of sufficient stability for distribution and storage [1, 2] by the end of 2003, lyophilized biological contributed to 46% of all US Food and Drug Administration (FDA) market approvals in the biopharmaceuticals category which include vaccines, proteins, and peptides. Proteins and peptides are the most rapidly growing class of drugs and had an increased growth rate globally in 2009 of 10.9%. Formulation strategies for current FDA approved vaccines, including those adopting freeze drying to overcome the cold chain, have been reviewed [1, 2, 3].

1. Sterilization Process: [3, 4] Sterile products can be manufactured by two different methods: aseptic processing or terminal sterilization. These products should be manufactured using aseptic processing only when terminal sterilization is not feasible. Therefore, when designing the manufacturing

process of a sterile drug product, the first approach should be evaluating if the product can be terminally sterilized. When aseptic processing is selected over terminal sterilization, proper scientific justification should be provided in the marketing authorization dossier. The most common and plausible reason is the degradation of the drug substance and/or drug product when exposed to terminal sterilization conditions.

1.1 Terminal Sterilization: [2, 3, 4] Terminal sterilization usually involves performing the filling and closing processes under high-quality environmental conditions (aseptic conditions are not required), in order to minimize microbial and other particulate content in the product and to help to ensure that the subsequent sterilization process is successful. Therefore, the product and the container closure system must have low bio-burden but are not sterile. The product in its final container is then subjected to a terminal sterilization process such as heat or irradiation, the method of choice for aqueous preparations is moist heat sterilization (in an autoclave) and, therefore, it should be used whenever possible [10].

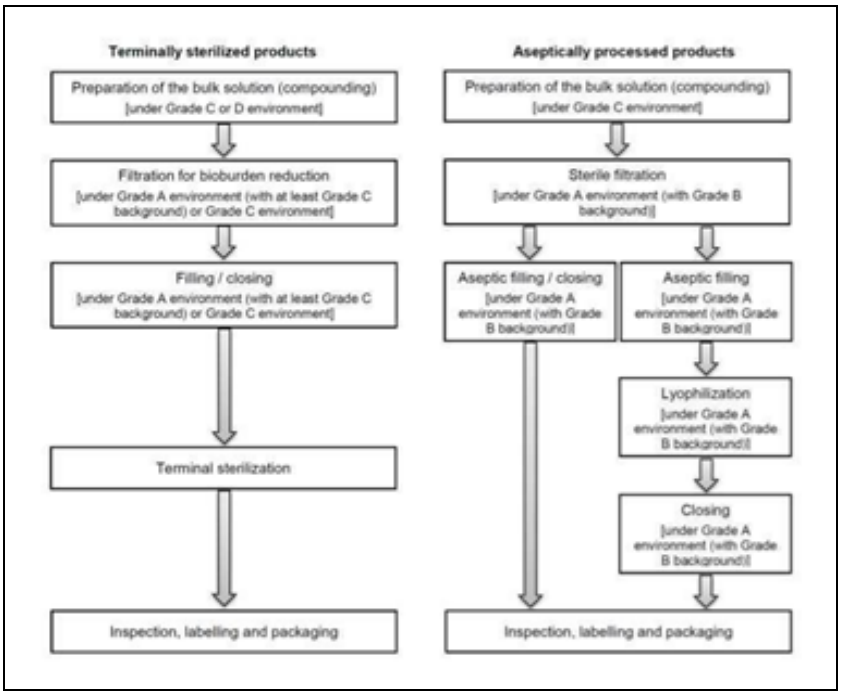

FIG. 1: SUMMARIZES THE STEPS INVOLVED IN THE MANUFACTURE OF TERMINALLY STERILIZED PRODUCTS AND ASEPTICALLY PROCESSED PRODUCTS

1.2 Aseptic Processing: In aseptic processing, the product and the container closure system are previously subjected to sterilization methods separately. Since the product is not sterilized in its final container, it is required that the filling and closing processes occur under aseptic conditions and following aseptic technique [5]. Usually, different sterilization methods are applied to the individual components of the final product. Glass containers are subjected to dry heat sterilization (in a depyrogenation tunnel), rubber closures are subjected to moist heat sterilization (in an autoclave) or purchased irradiated (pre-sterilized), and liquid dosage forms are subjected to sterilizing filtration (through a sterilizing-grade filter). Each one of these manufacturing steps should be properly validated and controlled. Any manipulation of the sterilized components poses the risk of contamination and, therefore, appropriate controls should be in place in order to avoid obtaining a non-sterile product [6].

2. Freeze Drying Process: The freeze-drying process is comprised of three main stages: freezing, primary drying, and secondary drying. An equilibration step is often included prior to beginning the freezing process to assure all liquids are at the same temperature. This involves keeping a formulation batch usually at room temperature for a short period before initializing chilling. This is to a lesser extent a measure to minimize batch variation although the control of ice nucleation

(control of ice crystal formation and growth) or annealing (thermal hold treatment) in batches will be required to minimize inter and intra batch variation [5, 6, 7]. The underpinning principle of freeze-drying surrounds an understanding of the phase diagram of water. A liquid formulation solidifies during freezing, which must be complete before a vacuum is pulled to prevent frothing. A frozen product is then supplied with heat energy (the latent heat of sublimation) at the primary drying stage using a vacuum pressure below the triple point of water [8].

FIG. 2: AN INDUSTRIAL SHELF LYOPHILIZER

The freeze-drying process is comprised of three main stages: freezing, primary drying, and secondary drying.

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

- Pretreatment / 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.

In addition to providing an extended shelf-life, successful freeze-drying should yield a product that has a short reconstitution time with acceptable potency levels. The process should be repeatable with well-defined temperature, pressure, and time parameters for each step. The visual and functional characteristics of the dried product are also important for many applications. When detailing the lyophilization process, it is common to break it down into its component stages: freezing, primary drying, and secondary drying [7, 8, 9].

2.1 Freezing Phase: The freezing stage involves freezing the product in containers until the solution changes from liquid to a solid-state. The solvent, generally water, forms ice crystals with the protein and any other components squeezed into the interstitial space between the crystals. The rate at which the shelf temperature is lowered is critical in determining the crystal size. Rapid cooling rates typically result in small crystals, whereas slow cooling rates result in large crystal formation. There is a tradeoff to consider as larger crystals can impose higher mechanical stresses on the protein structure while the high surface area of small crystals increases the size of the ice-water interface, and such interfaces are known to damage proteins [10, 11]. From an engineering perspective, larger crystals mean larger pore sizes forming during the primary drying phase. This means the rate of flow of vapor from the ice front through the dried layer can proceed at a faster pace with some reports of an increase of up to 3.5 times the initial primary drying rate.

The challenge during freezing is to ensure the product freezes. As obvious as this may sound, it cannot be taken for granted as ice nucleation is a random process that cannot be engineered easily [12, 13]. As mentioned previously, the frozen product is a mixture of crystalline ice and amorphous/glassy regions containing the protein in the interstitial spaces. In order to ensure all material is solid, one has to bring the temperature down below the glass transition temperature, otherwise known as the Tg'. Even at this temperature, ice nucleation rather than supercooled liquid is not a certainty and the purer the solution, and the more perfect the surface of the container, the lower the chances of nucleation. While attempts have been made to force the solution to nucleate through mechanically shaking the containers in some way, no viable method exists today on an industrial scale [14]. This means that in the case of biopharmaceuticals where the product is lyophilized in vials, ampoules or

syringes, each individual container will freeze at a different time and one will have heterogeneous nucleation between the containers Furthermore; ice crystal growth continues during freezing, so containers that freeze early will have larger crystals compared to those that freeze later. A solution to the problem of heterogeneity is through the use of annealing.

During annealing, the shelf temperature is increased to about 10-20 °C above the Tg' after freezing and held for a period of time [15, 16]. This allows the crystals to grow. As this process is controllable, one can improve the quality by design of the systems by using an annealing step that results in a more defined crystal size in all vessels. The Tg' of the formulation is a crucial characteristic when designing a lyophilization cycle. In fact, in the past, many cycles were designed purely on the knowledge of the Tg'. There are several analytical methods for determining the Tg, and these are discussed under section 1.6 Formulation characterization.

Eutectic / Collapse Temperature: This critical temperature determines the maximum temperature that the product can withstand during primary drying without it melting or collapsing. Crystalline products have a well-defined "eutectic" freezing/ melting point that is its collapse temperature [16].

Glass-Liquid Transition or Glass Transition: It is the gradual and reversible transition in amorphous materials (or in amorphous regions within semicrystalline materials) from a hard and relatively brittle "glassy" state into a viscous or rubbery state as the temperature is increased. An amorphous solid that exhibits a glass transition is called a glass. The reverse transition, achieved by supercooling a viscous liquid into the glass state, is called verifications [17]. The glass-transition temperature Tg of a material characterizes the range of temperatures over which this glass transition occurs. It is always lower than the melting temperature, Tm, of the crystalline state of the material, if one exists [17, 18].

Hard plastics like polystyrene and poly (methyl methacrylate) are used well below their glass transition temperatures, *i.e.* when they are in their glassy state. Their Tg values are well above room temperature, both at around 100 °C (212 °F). Rubber elastomers like polyisoprene and poly-isobutylene are used above their Tg, that is, in the rubbery state, where they are soft and flexible [18].

2.2 Primary Drying Stage: During this phase, the pressure in the chamber is lowered to create a pressure difference between the vapor pressure directly above the ice front and the chamber. The process of sublimation of ice at low temperature and low pressure can easily be visualized using the phase diagram. At subzero temperatures below 6.1 mill bars, the solid ice will sublime to vapor. The sublimation process requires energy, the source of which is the heated shelf. It is essential throughout the process that the temperature of the contents of the container does not exceed the Tg' throughout otherwise, melt back can occur [17, 18, 19]. However, by carefully adjusting the shelf temperature, one can provide maximum thermal energy while maintaining a product temperature below its Tg'.

Overall this process can be described as a heat and mass transfer process. The mass transfer is driven by the difference in pressures between the vapor pressure and the chamber pressure. However, the vapor pressure is dependent on the product temperature, which will drop as the ice sublimes unless sufficient heat transfer from the shelf to the product occurs to replace the loss of energy due to enthalpy of sublimation. The rate of vapor flow from the drying chamber to the condenser is dependent on the respective vapor pressures in each chamber. However, as the condenser is generally kept at a much lower temperature than the drying chamber, the water vapor condenses back to ice on the coils of the condenser resulting in far lower vapor pressure in the condenser than the drying chamber [19].

Although, in theory, by changing the temperature in the condenser, one could alter the vapor pressure in the condenser, the pressure in the condenser is likely to remain the same as the chamber pressure due to the vacuum pump to which the condenser is attached.

In the event the operator raises the condenser temperature, thereby raising the vapor pressure in the condenser, this would result in vapor flow into the vacuum pump.

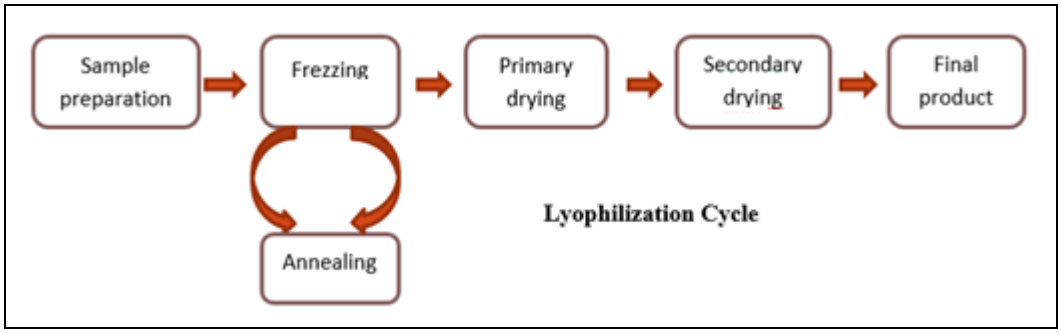

FIG. 3: LYOPHILIZATION CYCLE

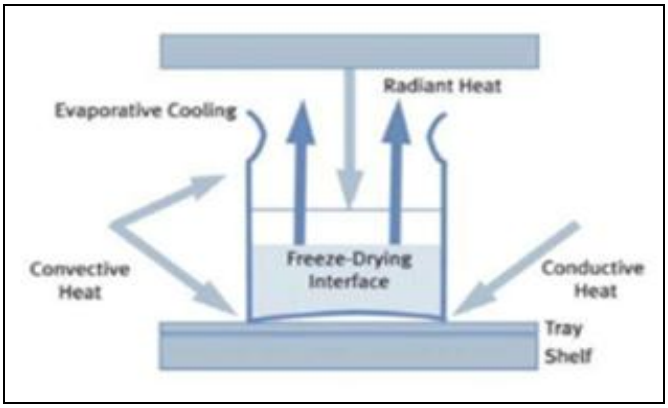

FIG. 4: HEAT TRANSFER IN A SHELF LYOPHILIZER

As such, the condenser temperature is not seen to be a driving factor during freeze-drying. Because the driving force behind primary drying is a pressure difference, it is logical to conclude that the greater the number of ice particles at the ice/air interface, the greater the number of particles are subject to the pressure difference, and therefore the greater the rate of primary drying [20]. From an engineering perspective, this means that one should aim to have a low aspect ratio between fill depth and surface area. It is better to have a large diameter vial filled to a depth of 4mm than a small diameter vial filled to a depth of 20 mm. It has been shown that there are three factors that resist mass transfer from container to condenser: a) the dried product layer that forms on top of the ice front; b) the container stopper if present; and c) the chamber resistance. The chamber resistance is generally the result of the interconnecting tube between the product chamber and the condenser. While at aggressive drying rates, the chamber-condenser pathway can become an issue to the point where the vapor is traveling at mach 1. Pikal *et al*., found that the dominant resistance accounting for nearly 90% of resistance was due to the dried – product layer. In light of this, it is easy to understand why large ice crystals resulting in large pore sizes are important to increase the rate of primary drying.

Heat transfer generally takes place through three mechanisms: conduction, convection, and radiation [20, 21].

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 degrees Celsius [18-20]. 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 the removal of water during lyophilization.

At atmospheric pressure (approx. 1,000 mbar) water can have three physical states

- Solid
- Liquid
- Gaseous

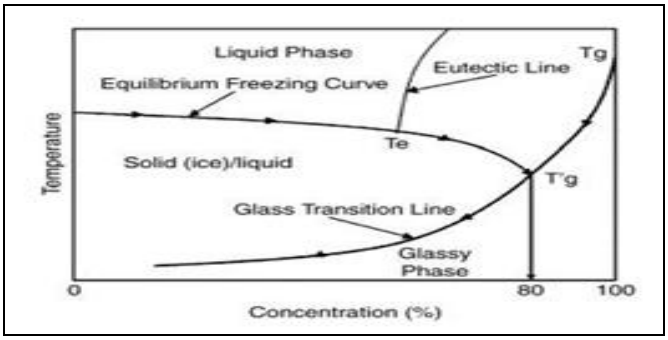

FIG. 5: PHASE DIAGRAM OF WATER SHOWING TRIPLE POINT OF WATER

2.3 Determination of the End of Primary Drying: Several analytical methods are available for determining that primary drying is complete.

The most basic method is to monitor the product temperature with a thermocouple probe. The measured product temperature will be colder than the shelf temperature setpoint during active primary drying because the heat from the shelf is being used for the sublimation phase change. When the sublimation of ice crystals is complete, the product temperature will increase and approach the shelf temperature. When the product temperature equals the shelf temperature, it can be inferred that primary drying is complete. Note: the specific vial that contains the thermocouple wire will typically dry faster than the other vials on the shelf because the wire will conduct more heat into that specific vial. Similarly, if bulk drying, the area around the thermocouple wire will dry more quickly than other areas in the product tray. It is important to allow a modest amount of additional drying time (30 min to 2 h depending on the product characteristics) after the product thermocouple temperature increases to ensure that all of the ice in the entire batch of the product has been completely sublimated [23].

Because the product will dry from the top down, the tip of the thermocouple should always be placed at the very bottom and center of the container. It is OK if the thermocouple touches the bottom of the container. If drying in vials, it is good practice to insert the thermocouple in a vial located in the middle of the shelf. Radiant heating effects will cause vials/products on the perimeter of the shelf to dry more quickly.

2.4 Secondary Drying Stage: As stated above, this step involves the desorption of the water molecules from the cake structure. In extreme cases, this can also involve the removal of bound water from the protein superstructure. This is done by raising the temperature of the product giving the ice molecules energy to sublime and diffuse through the cake structure. It is important to note that following primary drying, the Tg of the product is now above zero, so the shelf temperature can sometimes be raised as high as 50 °C without melting the product [24]. While the low pressure must be maintained, there is little to gain from extremely low pressures as the temperature is the determining factor for the rate of secondary drying. Although the Tg of the product is generally above zero after primary drying, the presence of up to about 25% moisture means the Tg can be around room temperature.

As the whole purpose of lyophilization is to produce a material that is stable for long term storage at ambient conditions, this is unacceptable as the product is likely to melt or collapse during storage at room temperature. Secondary drying actually starts during the primary phase, but at elevated temperatures (typically in the 30 ºC to 50 ºC range), desorption proceeds much more quickly. Secondary drying rates are dependent on the product temperature [25]. System vacuum may be continued at the same level used during primary drying; lower vacuum levels will not improve secondary drying times [25, 26].

Amorphous products may require that the temperature increase from primary to secondary drying be controlled at a slow ramp rate to avoid collapse. Secondary drying is continued until the product has acceptable moisture content for long term storage. Depending on the application, the moisture content in fully dried products is typically between 0.5% and 3%. In most cases, more dry the product, the longer its shelf life will be. However, certain complex biological products may actually become too dry for optimum storage results, and the secondary drying process should be controlled accordingly [24].

3. Quality by Design: Pharmaceutical formulations and the manufacturing processes associated with them are quite complex because they involve a large number of manufacturing variables (stirring speed, temperature, *etc*.) and formulation variables (excipients, chemicals, *etc*.). The relationship and interaction between these variables play an important role in deciding the quality attributes (QAs) of a pharmaceutical product. In order to study the multi-factorial relationship between the variables, knowledge of statistical tools such as the statistical design of experiments (DoE), response surface methodology (RSM), optimization and multivariate data analysis is important [35]. This knowledge will assist with the screening of variables and optimizing them so that the desired quality attributes are achieved.

The published literature has highlighted the use of DoE, optimization, and multivariate data analysis in applications related to pharmaceutical product and process development. Pharmaceutical quality by design (QbD) is a step-by-step approach to the

development of formulations that starts with defining objectives and has an emphasis on understanding the process variables and process control [22]. Implementation of aQbD system in the pharmaceutical formulation development in this process involves investigating all the factors that may have an impact on the quality of the final product. The QbD approach defines the potential critical quality attributes (CQAs) of the finished product by identifying critical processing parameters (CPPs). This approach applies various principles and tools at different stages so that a better understanding of the product and processes is obtained. It includes a selection of CPPs, CQAs, and quality risk assessment (QRA) tools such as risk filtering, fishbone diagrams, and failure mode and effect analysis (FMEA).

These principles and tools help identify an initial list of potential variables/factors that may affect the QAs of the final product. Descriptions of QbD can be found in ICH Q8 (2006), ICH Q9 (2005), and ICH Q9 (2008). These have been provided with the aim of maintaining product quality. For establishing or defining the design space, there is a need to perform DoE for single- or multiple-unit operation, and in the case of complex processes, several DoEs are required [25]. The robustness of the design space is important in the case of pharmaceutical formulations as there is variation within different lots of excipients frequently. In such situations, the established design space should have sufficient robustness to minimize the impact of these variations on the quality of the final product.

4. Critical Variables Parameter of Lyophilization Cycle: A critical parameter is defined as follows: A process control variable that: when operating beyond its acceptance range, has a major effect on Product safety or efficacy, or is likely to operate beyond a narrow range and have an impact on process consistency.

Following are steps of lyophilization and critical parameters of it:

- Freezing
- Freezing temperature and time
- Annealing
- Primary drying
- RAMP
- Target product temperature
- Shelf temperature
- Primary drying endpoint
- Chamber pressure
- Secondary drying
- Heating rate

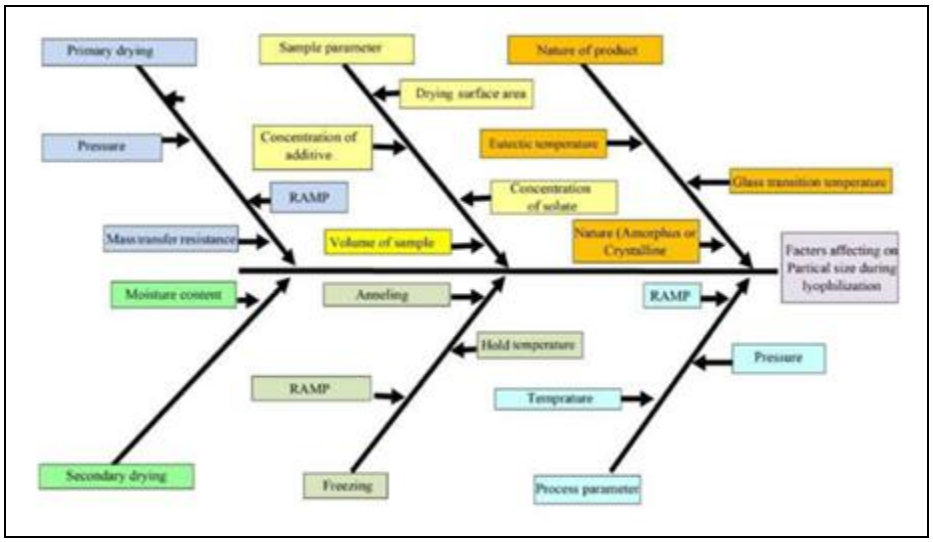

FIG. 6: ISHIKAWA DIAGRAM FOR LYOPHILIZATION

The critical factors affect the particle sizes of the product are explained by the Ishikawa diagram, which shows the root causes of the critical factors in that are the effect of the process variables sample variables, instrumental variables, or capability of the instrument. The Ishikawa diagram explains the root causes, which will be helpful in controlling the variables. According to the QbD approach, the identification of root causes of variables plays an important role in controlling product quality [22]. The

design of the experiment is a powerful tool for identifying the critical process parameter to optimize the respective condition. Critical factors in the lyophilization are optimized using DoE relationships to predict ideal formulations. However, with the increasing complexity of active moiety manufactured for therapeutics, the ability to classify them is limited.

More importantly, the intelligent freeze dryer, which utilizes steady-state models and the pressure rise test to determine cycle parameters, does not show where the edges of failure are. Indeed, the recommended cycle could even be on the edge of failure. In terms of QbD, this is not ideal. This thesis aims to develop a systematic step - by step approach which the engineer can use to develop and optimize a formulation for lyophilization. Such a platform technology would allow the user to optimize formulation development easily and gain sufficient understanding to allow process validation as well as an optimum formulation. Furthermore, the use of DoE will be used to develop an empirical specific process model to allow the user to optimize the lyophilization cycle. The resultant model will provide a detailed map of the design space showing the edges of failure. Such a method allows for a quality by design approach to cycle development, which, until further steps are made in PAT, is vital in lyophilization development.

5. Methods:

5.1 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.

5.2 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.

5.3 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 a stopper 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.

5.4 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 maybe the same thickness as the 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 **Fig. 6**. 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 airtight containers. Bulk drying is generally reserved for stable products that are not highly sensitive to oxygen or moisture [26].

6. Characterization and Evaluation of Lyophilized Formulation:

6.1 Determination of Reconstitution Time: For calculating reconstitution time, the contents of a lyophilized vial should be reconstituted, using aseptic technique, to 50 mg/mL as follows:

1. To minimize foaming, AVOID vigorous agitation or shaking of the vial during or after reconstitution.

2. Remove the polypropylene flip-off cap from the lyophilized vial to expose the central portion of the rubber stopper.

3. Wipe the top of the rubber stopper with an alcohol swab or other antiseptic solution and allowed it to dry. After cleaning, do not touch the rubber stopper or allow it to touch any other surface.

4. Slowly transfer 10 mL of 0.9% sodium chloride injection through the center of the rubber stopper into the lyophilized vial, pointing the transfer needle toward the wall of the vial. It is recommended that a beveled sterile transfer needle that is 21 gauge or smaller in diameter, or a needleless device is used, pointing the transfer needle toward the wall of the vial.

5. Ensure that all of the lyophilized powder is wetted by gently rotating the vial. 1. Allow the wetted product to stand undisturbed for 10 min.

Gently rotate or swirl the vial contents for a few minutes, as needed, to obtain a completely reconstituted solution.

6.2 Freeze-drying Microscopy: Temperature controlled microscopy (also known as hot stage microscopy) is a tool used in the pharmaceutical industry to characterize polymorphic forms of API compounds. Freeze-drying microscopy (FDM) is a variant of temperature controlled microscopy in which the sealed temperature-controlled stage is connected to a vacuum pump in order to generate a low-pressure sample environment. Commercial systems available today are sophisticated and use liquid nitrogen to generate the low temperatures and generally include a Pirani vacuum gauge in order to measure and control the vacuum level in conjunction with a PC, which also controls the temperature and pressure and crucially microscopic images acquired during the experiment. FDM allows the freeze-drying scientist to observe the gross structural changes associated with collapse. With programmable systems, the sample is loaded into the FDM stage; then the experiment follows a predetermined temperature profile, e.g., freezing, then reduction in pressure followed by a cool heat cycle around the temperature region of interest. In order to simplify the identification of the collapse temperature, images can be processed into movies or viewed by plotting the temperature profile against time and then viewing the images associated with the temperature range of interest. A representative drop of the sample solution is frozen on a coverslip, which is placed on the heating/cooling element. A vacuum is applied, and the ice sublimation/diffusion begins when the temperature of the element is increased slowly. The first virtual sign of texture change denotes the collapse temperature.

The glass transition is defined as time or frequency-dependent physical change in the state of a material from an amorphous solid to a rubbery/viscose state. The glass transition is observed when the experiment and molecular relaxation time scales cross. In the DSC method of Tg and Tg' determination, the temperatures are sensitive to the

heating/cooling rates used in the test. The enthalpies and their temperature range vary, as demonstrated by Taylor and York's studies of trehalose dihydrate and sucrose, where by DSC was less sensitive, in detecting the lost water from the enthalpy of dehydration at a slower scanning rate. A faster scanning rate increases the sensitivity to detect weak transitions, and a lower heating rate improves resolution e.g., the heating rate influences the Tg' measured for 10% Dextran solution. The Tg' was -10 ℃ at 20 °/min heating rate and -14 °C when measured at 2 °C/min. Slow cooling rates are thought to increase the extent of structural relaxation and the intensity of enthalpy relaxation compared to faster cooling rates.

The typical primary drying temperatures depend on the Tg' or the collapse temperature Tc of a combination of excipients and concentrations used in the formulations. The Tg ' of typical excipients used in the freeze-drying range from -8 ℃ *e.g.*, gelatin to -62 ℃ for glycine whereas sucrose is -32 ℃, trehalose at -34 ℃ and Mannitol at -35 °C (Costantino and Pikal 2004). The Tg' of lysozyme is -67 °C and for BSA is -81 ℃, both measured by DSC. The Tg' of a formulation can be increased by increasing the weight fraction of excipients with higher Tg' or by including additional excipients that have a higher Tg. Theoretically, the Tg of individual components can be calculated using the Gordon Taylor equation from the Tg /Tg' of the mixture; however, practically, this information can be directly measured by either DSC or freeze-drying microscopy. The temperature of the samples during primary drying is maintained below the Tg/Tg' of the formulation to prevent collapse; therefore, most freezing drying work systematically uses the temperature range -30 ℃ to -50 ℃ for drying, depending on the formulation.

6.3 Determination of Collapse Temperature: Determination of collapse temperature was done using Impedance analysis. Electrical impedance analysis is a relatively new method and has been demonstrated to detect events that other methods appear to miss, some of which may be relevant to freeze-drying, including mobility changes in regions of the frozen matrix that could alert the freeze-drying scientist to the possibility of micro collapse or micro melting occurring in a formulation. Impedance analysis was carried out for the formulations to below - 150.0 °C according to standard procedure. The impedance of the formulation was measured at 5-sec intervals during cooling and warming. The data was exported directly to Microsoft Excel for graph plotting, and interpretation of the warming profile of the analysis carried out to determine the temperatures of the significant events, which could be attributed to increases in ionic mobility within the formulations.

6.4 Powder X-ray diffraction (P-XRD): Experimentally, if we know the wavelength of the X-ray λ and measuring θ we can know the atom plane spacing d. By knowing d, the crystal structure and phase can be identified. Another application of XRD is to estimate the particle size by using Scherrer's formula: t = 0.9 / cos Where is the half-maximum width of the peak in radians, λ is the wavelength of the X-ray and θ is the corresponding constructive interference angle. Philips expert pro MPD diffractometer (PAN analytical Inc. Germany) an automated diffractometer equipped with Bragg-Brentano geometry with a resolution of 0.001 A0 was used to perform powder X-Ray diffraction study. About 10 mg of powdered sample was sprinkled over vacuum grease coated glass slide at room temperature to form a sample layer of approximately 0.5 mm thickness. The samples were targeted using, Cu Kα radiation (λ = 0.15418 nm) as target filter having a voltage/current of 40 KV/30 mA and scanned at a speed of 40/min, radiation diffraction angle (2θ) ranged from 5° to 70°, *i.e.,* 3≤ 2θ ≤ 400. Step width was 0.040C, and step counting time was 0.5 seconds, and the acquisition time was 1 h.

6.5 NMR Spectroscopy: NMR has become the dominant method of analysis for organic compounds because, in many cases, it provides a way to determine an entire structure using one set of analytical tests. It is also increasingly used in inorganic chemistry and biochemistry, where it also provides a lot of valuable structural information. Nuclear Magnetic Resonance is a property of the nucleus of an atom, concerned with what is known as nuclear spin (I).

This is equivalent to the nucleus acting like a miniature bar magnet. Although isotopes can have a variety of values for I (including zero), the most useful for spectroscopy are those nuclei which have

I = ½. This includes hydrogen 1 (1H), carbon 13, fluorine 19, and phosphorus 31, so that some of the commonest elements in organic chemistry can be analyzed using NMR. When a nucleus with I = 1/2 is placed in a magnetic field, it can either align itself with the field (lower energy) or against it (higher energy). If radio waves are applied, nuclei in the lower energy state can absorb the energy and jump to the higher energy state. We can observe either the absorption of energy or the subsequent release of energy as the nucleus "relaxes" back to the lower energy state. Traditionally this was done by scanning slowly through a range of radio wave frequencies (this is called a continuous wave, CW). However, this has largely been replaced by the faster Fourier Transform (FT) method, where one big, broad pulse of radio waves is used to excite all nuclei, then the results are analyzed by a computer.

The NMR spectra were recorded on a Bruker Advance II 400 MHz NMR spectrometer equipped with a 5 mm1H cryoprobe head with a z-gradient (BrukerBio Spin, GmBH, Germany) in CDCl3 at 25 °C. The data were acquired and processed using the Topspin 3.1 software (Bruker). The pulse sequences from the Bruker pulse sequence library were used for 1H NMR. A line broadening factor of 0.3 Hz was applied, and baseline correction was performed prior to the integration of the 1H NMR signals. Sample quantities of the lyophilized sample (typically 50 mg), was used for recording the NMR spectrum.

6.6 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, mano-metrically, 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.

7. Desired Characteristics of Freeze-Dried Products:

- Intact cake
- Sufficient strength
- Uniform color
- Sufficiently dry
- Sufficiently porous
- Sterile
- Free of pyrogens
- Free of particulates
- Chemically stable

8. Applications:

8.1 Pharmaceutical and Biotechnology: Pharmaceutical companies often use freeze-drying to increase the shelf life of products, such as vaccines and another injectable. 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.

8.2 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.

8.3 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 bio-separations, freeze-drying can also be used 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.

8.4 Nutraceuticals: In the case of Nutraceuticals, the process of freeze-drying used for stabilization as well as increasing the shelf life of products. Liquid Nutraceuticals are converted to powder form, which helps in preserving them for a longer time. Nutraceuticals that can be preserved by freeze-drying include aloe Vera, seaweeds, *etc*.

8.5 Starters and Cultures (Edible Cultures): In the case of regular drying methods, the resultant product loses its quality. This might bring about adverse changes in the properties of the products. Products preserved using this technique include probiotics, buttermilk, *etc*.

8.6 Research: Botanical samples are preserved by freeze-drying to be used for research purposes. Laboratory samples which can be preserved by freeze-drying include API, pathological samples, microbiological cultures, viruses, bacteria, anti-bodies, *etc*.

8.7 Document Recovery: The process of freeze-drying can be used for recovery & saving of documentation facing damage through fire, floods, *etc*. FD using a vacuum can be used to restore books damaged by water as well as paper containing water-soluble inks.

8.8 Floral: The moisture content present in flowers is eliminated through vacuum extraction, followed by freezing at low temp. This prevents floral shrinkage and maintains the quality of flowers and structure.

8.9 Taxidermy: FD is not an alternative to taxidermy but can be considered as an asset to taxidermy. Animals processing a large quantity of lipids contain need to processed accordingly in order to achieve optimal quality of FD.

8.10 Pet food: Removal of moisture is necessary for long term preservation of pet food. FD helps in preservation as well as maintains product quality. Pet foods which are subjected to FD can be regarded as close to naturally occurring diet.

CONCLUSION: The lyophilization technique proved to be an advantage for the 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. In the freeze-dried solid-state, chemical or physical degradation reactions are inhibited or sufficiently decelerated, resulting in 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 the freeze-drying process as well as various parameters is essential for successful designing of a freeze-drying cycle which makes the process more efficient and less complicated so that product gets dried in shorter time retaining its properties so that operating costs and other inputs can be minimized and in return, it will maximize the capitals.

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REFERENCES:

1. Wang DQ, Hey JM and Nail SL: Effect of collapse on the stability of freeze-dried recombinant factor VIII and alpha-amylase. J Pharm Sci 2004; 93: 1253-63.
2. Overcashier DE, Patapoff TW and Hsu CC: Lyophilization of protein formulations in vials: investigation of the relationship between resistance to vapor flow during primary drying and small-scale product collapse. J Pharm Sci 1999; 88: 688-95.
3. Etzl EE, Winter G and Engert J: Toward intradermal vaccination: preparation of powder formulations by collapse freeze-drying. Pharm Dev Technol 2014; 19: 213-22.
4. Pikal MJ and Shah S: The collapse temperature in freeze-drying: dependence on measurement methodology and rate of water removal from the glassy phase. Int J Pharm 1990; 62: 165-86.
5. Depaz RA, Pansare S and Patel SM: Freeze-drying above the glass transition temperature in amorphous protein formulations while maintaining product quality and

improving process efficiency. J Pharm Sci 2016; 105: 40-49.
6. Awotwe-Otoo D, Agarabi C and Read EK: Impact of controlled ice nucleation on process performance and quality attributes of a lyophilized monoclonal antibody. Int J Pharm 2013; 450(1-2): 70-78.
7. Izutsu K, Yoshioka S and Kojima S: Physical stability and protein stability of freeze-dried cakes during storage at elevated temperatures. Pharm Res 1994; 11: 995-99.
8. Patel SM: Lyophilized drug product cake appearance: what is acceptable? Journal of Pharmaceutical Sciences 2017; 106: 1706-21.
9. Lionberger RA, Lee SL, Lee L, Raw A and Yu LX: Quality by Design: Concepts for ANDAs. The AAPS Journal 2008; 10: 2.
10. Little T: Using a Systematic Approach to Select Critical Process Parameters - Harmonized regulations call for a risk-based and systematic approach to evaluating and selecting CPPs. Bio Pharm International 2012; 25: 7.
11. Mourya VK, Choudhari Y and Padame MK: Quality by design: impact of product variables and their interaction on the particle size in lyophilization of sodium fluoride. Soft Nano science Letters 2016; 6: 1-10.
12. Pandey AP, Karande KP, Sonawane RO and Deshmukh PK: Applying Quality by Design (QbD) concept for fabrication of chitosan coated nanoliposomes. J Liposome Res 2014; 24: 37-52.
13. Sawant K, Pandey A and Patel S: Aripiprazole loaded poly (caprolactone) nanoparticles: Optimization and *in-vivo* pharmacokinetics. Mater Sci Eng C 2016; 66: 230-43.
14. Liu J, Viverette T, Virgin M, Anderson M and Paresh D: A study of the impact of freezing on the lyophilization of a concentrated formulation with a high fill depth. Pharm Dev Technol 2005; 10(2): 261-72.
15. Passot S, Tréléa IC, Marin M, Galan M, Morris GJ and Fonseca F: Effect of controlled ice nucleation on primary drying stage and protein recovery in vials cooled in a modified freeze-dryer. J Biomech Eng 2009; 131(7): 074511.
16. Alina P, Maria M and Lucia R: Sylvester Bianca, A quality by design approach for the development of lyophilized liposomes with simvastatin. Saudi Pharmaceutical Journal 2017; 25(7): 981-92.
17. Reddy B: Improved tablet production by modified granulation techniques-a review. Int Journal of Research in Pharmacy and Life Sciences. 2014; 2: 224-35.
18. Andrea A and Roberto P: Application of the Quality by Design Approach to the Freezing Step of Freeze-Drying: Building the Design Space. Journal of Pharmaceutical Sciences 2018; 107(6): 1586-96.
19. Ravnik J, Sitar A, Avanzo M, Cegnar M and Ramsak M: Lyophilization model of Mannitol water solution in a laboratory scale lyophilizer. Journal of drug Delivery Science & Technology 2018; 45: 28-38.
20. Moussa M, Sinha K, Hirth M, Shang S and Jameel F: Predictive models of lyophilization process for development, scale-up/tech transfer and manufacturing. European Journal of Pharmaceutics & Biopharmaceutics. 2018; 128: 363-78.
21. Cyrus A, Elizabeth C, Scott L, Khan M and Shah R: Quality by design: Impact of formulation variables and their interactions on quality attributes of lyophilized monoclonal antibody. International Journal of Pharmaceutics 2012; 438(2): 167-75.
22. Tiwari S and Verma P: Spherical crystallization. a novel drug delivery system. Int Journal of Pharmacy & Life Sciences 2011; 2(9).
23. Christine W, Barthelemy D and Michael A: Quality by Design Approaches to Formulation Robustness-An Antibody Case Study 2016; 105(5): 1667-75.
24. Ryo O and Chikamasa Y: Effects of temperature ramp rate during the primary drying process on the properties of amorphous-based lyophilized cake. Journal of Drug Delivery Science and Technology 2017; 39: 131-39.

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