Review Article

A Review on Rational Design to Optimize Stable Lyophilized Parenteral Products

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ABSTRACT

The most common form of sterile parenteral powder is freeze dried or lyophilized powder. Design of lyophilization process is often approached with a trial and error experimental plan, which can be minimized with scientific approach and existing literature to improve product quality and less freeze drying cycle runs. Formulation and process development of lyophilized parenteral products need a systematical understanding of the physical chemistry of freezing and freeze drying, material science and related mechanisms of heat and mass transfer. This review presents an outline on freeze drying of injectable pharmaceutical products. It will provide description of various freeze dried critical process parameters and their effect on the formulation for developing robust freeze dried cycle to assure quality of product.

Keywords: Freeze dried parenterals, QbD, primary and secondary drying, freeze drying cycle.

INTRODUCTION

The most important markets of freeze-dried products are pharmaceutical industry, biotechnology as well as food industry. In Pharmaceutical industry lyophilized products account for largest volume in parenterals including new anti-infective products, biologicals, *in vitro* diagnostics and also for very fast dissolving oral solid dosage forms.[1] Freeze-drying(FD)has become a successful commercial process in the recent years because it allows greater storage stability for otherwise labile biomolecules, provides a convenient storage and shipping format and following reconstitution rapidly delivers the product in its original formulation.

Lyophilization (FD) is the process of solvent removal (usually water) from pharmaceutical product. Solvent would froze first and then removed by sublimation in a vacuum environment. The process consists of three separate, unique, and interdependent processes; freezing, primary drying (sublimation of ice at high vacuum & at low temp), and secondary drying (desorption water at low vacuum & at a temperature above freezing point).[2-7]

It is a GMP integrated fundamental process in the biopharmaceutical industry. It is estimated that over 50 % of biopharmaceuticals products are lyophilized in order for these unstable agents to be applied as therapeutic agents. Lyophilized products are elegant with very low moisture content and amendable to being carried out in an aseptic environment.[1,8] It is more compatible with sterile operations than dry powder filling since a solution can be sterile-filtered immediately before filling vials. Fill weight control is more precise for liquid than for powder filling and the absence of powder at the filling step minimizes problems with particulate contamination in a clean aseptic environment.[1,9] This way lyophilization process increases degree of confidence in product sterility, preserving chemical, biological potency, & homogeneity. It shows greater speed and completeness of rehydration in comparison with dry powder fills for products like injectable penicillin.[10,11]

The main component of parenteral formulation is water. Since most common instability mechanism of parenterals is hydrolysis.[8] Therefore, lyophilization widely used for protection of sensitive molecules from degradation (thermo labile& water sensitive) and improves storage life along with improved marketing of the end product. It also resolves a practical problem for certain drug delivery system, for example, the packaging of constituents that cannot be mixed in the liquid state but which are solidified in successive stages and then freeze dried. Freeze-drying technique takes place at low temperature which could be minimized the chemical decomposition of the product. Reconstitution of freeze-dried product will be fast and complete, which is promoted by its very high surface area. Therefore lyophilization process is advantageous in many ways such as ease of processing a liquid, which simplifies aseptic handling, to enhance stability of a dry powder, removal of water without excessive heating of the product and gives rapid and easy dissolution of reconstituted product.

Difficulties for optimization of freeze drying cycle

It is well recognized that freeze drying is complex technology associated with the manufacture and control of a lyophilized pharmaceutical dosage form.[2] The operating conditions required to obtain a product with the desired characteristics are found mostly by trial and error: this is a consequence of the impossibility of directly measuring and thus of controlling in-line parameters of interest mainly the product temperature and the residual water content.[12]

Although the highest quality dried product can be obtained by freeze drying, the process is multi-stage, relatively slow and most expensive both in the capital investment (cost and complexity of equipment) and operating expense such as increased handling time, processing time and need for sterile diluent upon reconstitution, etc.[1] These drying times could take a lot of additional time (even days) to freeze-dry a certain product of interest if the process is not well-designed.[12,13,14]

Optimization of the freeze drying cycle for a given formulation requires a balanced understanding of the fundamental science of freeze-drying, formulation characteristics, equipment capabilities and practical risks associated with process parameters. Ultimately, the optimized drying cycle should be efficient and robust without introducing significantly great manufacturing risks or compromising the pharmaceutical quality of the product.[3] Some of the important aspects of these operations include: the formulation of solutions; filling of vials and validation of the filling operation; sterilization and engineering aspects of the lyophilizer; scale-up and validation of the lyophilization cycle; and testing of the end product.[2] This is also one of the targets of the Guidance for Industry Process Analytical Technology (PAT) issued by US Food and Drugs Administration in 2004. In fact, it encourages formulators to use the latest scientific advances in pharmaceutical manufacturing and technology so that quality is no longer tested into products but it should built-in.[12]

There are several publications and reviews on the freeze drying of protein formulations and on the engineering aspects of freeze drying process.[10, 11, 15, 16, 17,18]The primary goal of research on freeze drying is to improve process economics by reducing processing time.[10] A brief review article on freeze drying of pharmaceutical products was published by Sadikoglu et al. involving excipients in appropriate quantities and effects of glass transition temperature (Tg) in the formulation period and freezing, primary, secondary drying stages, optimal control and remote monitoring from viewpoint of mathematical modeling.[19] Review by Qian and Zhang based on the discussion of the theory of freezing, basics of freeze drying and the dependence of pore size, pore volume and pore morphology on variables such as freeze temperature, solution concentration, nature of solvent and solute.[20] Some processing parameters, significance of thermal transitions, control of ice nucleation and crystallization during the freezing step, along with guidelines for optimization of primary as well as secondary drying were given by Tang andPikal.[4, 5, 13, 21] Sadikoglu and Liapis developed a model to describe the primary and the secondary drying stages of freeze-drying on trays for skim milk freeze drying.[22] And further used this model to develop a control strategy to minimize the drying time by using the heat input and the drying chamber pressure as the control variables inferring maximum interface (melting) and surface (scorch) temperatures as constraints.

However till date a comprehensive analysis of the freeze drying process parameters, processing problems associated with parenterals formulations and feasibility characteristics for freeze- dried formulations does not exist in the literature. To have an up-to-date perspective of the process and more importantly its application in formulating parenterals, this article gives the recent investigations and achievements in this exciting area. This review provides an outline shows impact of parenteral formulation variables on different stages of the freeze-drying process and offers guidance for selection of critical process parameters during freeze-drying cycle development, optimization and scale-up. This will also address some of the problems associated with the manufacture and control of a lyophilized dosage form.

PRINCIPLE AND DESCRIPTION OF FD PROCESS

FD is based on the principle of sublimation of ice, without entering the liquid phase. The concentration gradient of water vapor between the drying front and condenser is the driving force for removal of water during lyophilization. With the help of phase diagram showing in Figure 1 the principle of sublimation can be explained. The phase diagram of water shows that two phases coexist along a line under the given conditions of temperature and pressure while at the triple point (0.0075°C at 0.61KPa or 610 Nm^{-2}; 0.01°C at 0.00603 atm.) all three phases coexist. Lyophilization is performed at temperature and pressure conditions below the triple point, to enable sublimation of ice.[23]The process is usually carried out with aqueous systems, although in recent years freeze drying of solutions with special solvents have become increasingly important.

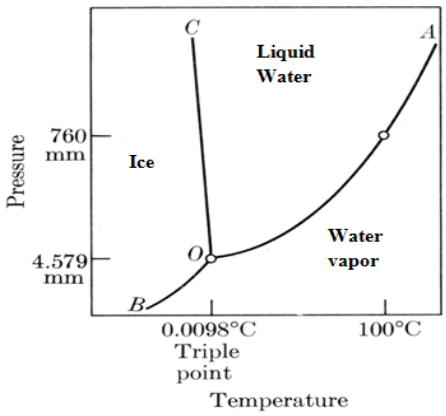

Figure 1: Phase diagram of water

The product is first frozen to a low enough temperature to allow complete solidification of the content of each vial. To help crystallize the bulking agent an annealing step is sometimes included whereby the shelf temperature is raised to near or above the formulation's Tg in the frozen state. Then the chamber is evacuated until the pressure is less than the vapor pressure of ice at

the temperature of the product. The difference between the vapor pressure of ice and the chamber pressure provides the driving force for sublimation. By maintaining the chamber under vacuum, the chamber pressure is constantly maintained below the saturated vapor pressure of ice and sublimation continues. In order for sublimation to occur, energy must be supplied to balance the latent heat, ΔH of ice sublimation.[10] Primary drying is completed after removal of frozen bulk water. But there is still some bound unfrozen water remaining in the product that can be removed by desorption at higher temperatures experienced during secondary drying. And product temperature plays an active role in all three phases.

Basic components for a freeze dry system for production of pharmaceutical dosage forms including chamber containing shelves, system for pumping, heating, cooling the fluid, vacuum pumping system, condenser for trapping water vapor and refrigeration system for cooling the condenser. Detailed instrumental description of lyophilization process could found in other literature.[3]The freeze-drying equipment also has provisions for defrosting the condenser, clean-in-place (CIP) and steam-in-place (SIP) of the freeze dryer and computer interface to input, monitor, and control the cycle parameters via a Programmable Logic Control (PLC).[3] The process is conventionally divided into three stages are freezing, primary and secondary drying.

1. Freezing

This is the first most crucial step because the overall performance of freeze drying process significantly depends on freezing. Based on the physical and chemical properties of material, the freezing protocol can be optimized to produce high product quality and short drying time.[24] The freezing temperature, time, freezing rate and super cooling degree are all important factors influencing the overall drying time and product quality and are described in Table1. The characteristics of the frozen matrix strongly affect drying rates at primary and secondary stages.[25, 26]

The nucleation temperature is important factor which controls the crystal growth. The balance between crystal growth and ice nucleation temperature determines the number, shapes and sizes of ice crystals. 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. If the solute separates out in crystalline form, it is known as the eutectic temperature (Teu). In contrast, if an amorphous form is produced, the temperature is referred to as the glass transition temperature (Tg). This critical process temperature is the collapse temperature for amorphous substance or eutectic melt for the crystalline substance.[7, 10, 27] Determination of this critical temperature is important for development of an optimized lyophilization cycle. This freezing temperature chosen should be below the Tg of the formulation as well as sufficiently low to initiate nucleation of the bulking agent.[3]

Table 1: Processing Parameters of freeze drying

Parameters involved in Freezing	Input	Output
Speed of Freezing	Fast or very fast	• Small and numerous ice crystals • Crystals • At pilot level easy to achieve • Unrealistic for Production due to lots of the problem e.g. filling, loading time, etc.
	Slow freezing	• Large and less numerous
Freezing Temperature	The shelf temperature for freezing should be set below Tg or Teu	• Tg for amorphous state • Teu if it is in the crystalline state
Freezing time	Depends on fill volume	• Larger fill volume takes longer time to fully freeze
End of freezing	Formulation in Liquid state	• Formulation in Solid state

a. Annealing or thermal cycling

Annealing is an additional step recently involved in freeze drying process which will help to accelerate primary drying. This process involves holding the product at a temperature above the final freezing temperature for a defined period to crystallize the potentially crystalline components during the freezing stage.[26, 28] This temperature should be between the ice melting temperature and the Tg of the freeze concentrate.[10, 13, 26] This step shows dramatic effects on particle size distribution of ice crystals.[29] Another purpose of annealing is to completely crystallize bulking agents or co solvents in the formulation during the freezing stage. Completing the crystallization of these excipients during the freezing process may be beneficial as the storage stability of the product can be compromised if residual amorphous or hydrated forms crystallize during

storage.[30, 31]This also helps to minimize the vial to vial variations and obtain homogeneous batch.[7, 10]

Temperature and time are two important parameters need to be justified for annealing. The annealing temperature could be chosen on the basis of the devitrification temperature, which is determined by Differential Scanning Calorimetry (DSC). The selected temperature should be about 10°devitrification temperature since higher annealing temperatures normally produce a greater degree of crystallinity and require less time to completely achieve the percent crystallinity that is feasible at that temperature.[3]Annealing could predispose the formulation to greater instability during storage by drug degradation or by modifying of structures of certain proteins.[10, 13]Therefore this modification step may or may not always beneficial for every parenteral product.

2. Primary drying

This is the longest and energy consuming step in the lyophilization process. Because of this, optimization of primary drying has a large impact on process economics. Two key parameters can be controlled during this steps are shelf temperature and chamber pressure to dry the product as fast as possible by retaining the integrity of cake. Other parameters are not controllable but they significantly affect the on primary drying process e.g. container, formulation, fill volume, stopper, total product load, condenser capacity, condenser temperature, location of condenser, intra and inter shelf temperature variability.[3]

In this step the partial pressure of the vapor surrounding the product must be lower than the pressure of the vapor from the ice at the same temperature.[3] The product is first frozen at a low temp from (-40°C to -10°C)to reach a vitreous state. Then it undergoes sublimation under vacuum maintain the product temperature below its Tg or collapse temperature. That specific temperature is determined by the stabilizers chosen and is measured with specific tools e.g. Cryomicroscope. The product elegance is directly depends on this temperature. Therefore, primary drying temperature should be kept as high as possible but below the critical process temperature to avoid a loss of cake structure.[32, 33, 34] Very high drying temperature can leads to meltback/collapse phenomenon in case of crystalline or amorphous substance respectively.[23] The energy supplied in the form of heat must remain lower than the product's eutectic temperature. To avoid vial to vial inconsistencies in heat transfer, chamber pressures in the range of 100–200 mTorr are preferred.[36, 37]Therefore the information of the sublimation rates and product temperatures associated with multiple combinations of shelf temperature and chamber pressure as well as the knowledge of commercial equipment capabilities are critical for the selection of appropriate process parameters.[3]

Barresi and et al have studied the effect of operating temperature on the process and product characteristics (apparent density, residual moisture and rehydrability) and the drying rate.[12] They have found that optimal results for process duration and product quality can be achieved when the primary drying is carried out as closer as possible to the maximum temperature allowed by the product.[3] The mathematical and schematic representation of interplay between shelf temperature, chamber pressure, product temperature and sublimation rate has given by Chang andTang.[3, 13] The completion of primary drying is directly related to the ice sublimation rate and can be monitored in a number of methods at pilot scale as well as at production scale and these details are described by Chang and Patro.[3]

3. Secondary drying

Secondary drying phase involves additional drying time for desorption of residual moisture (10 to 35%)by diffusion from the solid matrix for optimal stability.[10,38]Since this slower diffusion kinetics, secondary drying almost takes 1/3rd of total process length. It is initiated by increasing the shelf temperature usually to above room value and further reducing the chamber pressure.[11]

Residual moisture in the product is generally dependent on four factors which are product matrix (both in frozen and sublimation mode), vacuum in the drying chamber, duration of secondary drying and maximum temperature allowed for the product drying. The governing relation of moisture in porous media during the secondary drying is the adsorption-desorption equilibrium. It depends on temperature and moisture content.[35, 39] Glassy products are limited in their drying rate at this stage due to the slow molecular diffusion within the dried cake.[40] But for the crystalline products it may be shortened because higher drying temperature can be applied without the risk of damaging the product.[7, 13] Approaches for the optimization of the transition from primary to secondary drying are described by Change and it is related to heating rate and shelf temperature. The final moisture level of freeze dried product critically depends on the final product temperature and duration of secondary drying. The acceptable level of residual moisture content has to be predetermined during formulation development based on stability data.[3]

HEAT TRANSFER DURING FD

Heat is the essential component required to remove water in the form of vapor from the frozen product. It should be carefully controlled to avoid overheating of the product. Heat input depends on the temperature of the product, duration of primary, secondary drying phases and chamber pressure. Freeze dried product dynamics and temperature are affected by heat transfer in vial, as well as bulk. In primary drying stage the sublimation occurred at specified product temperature which results from a balance between heat transfer rate to the product

and the drying rate or mass transfer rate of water vapor.[4, 13] It is an important rate limiting process. Heat transfer by conduction takes place through a series of resistances-the bottom of the vial, the frozen layer of product, the metal tray (if used) and the vapor phase caused by lack of good thermal contact between the vial and the shelf.[9]

The heat conduction and the phase change are two main contributions to achieve effective thermal conductivity. At high temperature, the phase change is dominant while heat conduction is significant at low temperature. As the moisture level decreases, the heat transfer would change from heat conduction to phase transitions. This is critical for the collapse temperature. The temperature of a freeze drying process must be close to collapse temperature to run an effective operation also it cannot exceed this temperature due to the process requirement and product quality concern. [10]

Few studies revealed on the techniques to calculate the heat transfer parameters or to estimate their values by means of experiments and also heat flux between the heating shelf and container is the result of several mechanisms that depend on dryer, container geometry, as well as on pressure and temperature of the surrounding gas.[10] The heat transfer for vial freeze-drying at a steady state was presented by Pikal.[26] Recently, the use of mathematical modeling, coupled with few experiments to determine model parameters has been proposed to get the design space quickly.[11]Because of the high heat input required for sublimation (670 cal/g), transfer of heat from the heated shelf to the sublimation front is often the rate-limiting step in the coupled heat and mass transfer process.[9]

MASS TRANSFER DURING FD

For freeze drying process the total resistance to mass transfer is the sum of several resistances in series e.g. partially dried layer, the vial or container, a partially inserted stopper and the pathway from the chamber to the condenser.[7, 11] The maximum resistance occurs across the dried product layer where water molecules have to pass the pores and channels which were formed during the freezing step to reach the condenser and that causes the greatest pressure drop.[7]

The diffusion of water vapor in the partially dried layer is one of the major factors affecting the mass transfer rate and which is closely related to the pore size. Large ice crystals will be helpful for the movement of water vapor. The pressure difference is essentially the driving force for the transport of water vapor. The smallest chamber pressure gives the highest ice sublimation rate.[13] In their report of the effect of chamber pressure on heat and mass transfer, Livesey and Rowe noted that the rate-limiting factor in freeze drying changed as drying process proceeds.[37] Initially, the process is limited by heat transfer when the dried layer is thin and an unrealistic heat flux is required to push the sublimation rate to its maximum. After a certain thickness of the dried layer has been developed, the process becomes controlled by mass transfer since as they claimed the required heat flux is easily maintained for the decreasing sublimation rate. [11]

Mass transfer in freeze-drying refers to the transfer of water vapor from the sublimation front through open channels in the partially dried layer created by prior sublimation of ice through the head space of the vial, past the lyostopper and through the chamber to the condenser. Briefly the rate-limiting step in mass transfer is transfer of water vapor through the partially dried matrix of solids. Mass transfer of the unfrozen water through a glassy phase during secondary drying occurs more slowly than bulk flow of water vapor by direct sublimation since no open channels are present in the glassy phase. The high resistance of the solid material to the mass transfer creates secondary drying the most time-consuming phase of the freeze-drying cycle for amorphous solutes containing a large percentage of unfrozen water. According to studies reported by Pikal, shelf temperature is the most critical process variable, affecting the rate of secondary drying and final moisture level.[13]Chamber pressure had no measurable influence on secondary drying kinetics.[9]

COMMONLY USED EXCIPIENTS FOR FD PARENTERALS FORMULATIONS

In the freeze-dried formulations, excipients are mainly included to improve the functional properties and stability of the lyophilized product. Excipients added to the lyophilized cake should have regulatory acceptance as they are intended for parenteral administration. Various reviews are available listing excipients used in lyophilized formulations.[1, 10, 23, 28]Baheti et al focused specifically on the issues related to excipient selection in lyophilized formulations of small molecules.[23]Polwellet al. have given compendium of excipients used for parenteral formulations with reference to individual products.[43] A review by Neema et al., listed the excipients and frequency of their usage in marketed injectable formulations.[41] The list of parenteral formulations of small molecules marketed in the United States is compiled by Strickley.[42, 43] The selection of these excipients would depend on the formulation. Commonly used excipients in freeze-dried formulation are bulking, solubilizing, buffering, antimicrobial agents, collapse temperature modifiers and tonicity modifiers. The main purpose of the bulking agent is to provide a dried matrix in which active pharmaceutical ingredient can dispersed and it offers support to prevent cake collapse even if the amorphous phase is being dried above its collapse temperature. Many drugs are present in a too small dose (less than 2 %) to form a well-defined freeze-dried cake and must be formulated with a bulking agent.[13, 44, 45] The nature of lyophilized cake also depends on the ratio of drug and bulking agent showing an increased crystallization with an increase in amount of bulking agent.[10, 23, 40] Buffering agent is required to avoid degradation of drug during processing, storage and

reconstitution which could be caused due to changes in pH level. Selection of a suitable buffer and its concentration is important for sensitive molecules. For example aspartame lyophilizes in the presence of 0.1M phosphate buffer shifts the half-life of the material to 98 days while in unbuffered it was found 921 days. And further increase in the buffer concentration causes a reduction of half-life to 77 days.[23] For the selection of buffering agent the pH of maximum stability of drug should be known and maintained. The detailed description of use of buffering agent and various case studies has described by Baheti.

Collapse temperature modifiers are used only for amorphous materials which require the primary drying temperature to be kept below the collapse temperature of the formulation. These excipients have high individual collapse temperatures and generally not used. Commonly used collapse temperature modifiers are dextran, gelatin and hydroxyl ethyl starch, etc.[46] Antimicrobial agents are added to multi-dose formulations to prevent microbial growth during its shelf life. The examples of commonly used antimicrobial agents are benzyl alcohol, ethyl paraben, methyl paraben, phenol and m-cresol. These agents are often typically included in the diluent for reconstitution.[47]

Isotonicity with human plasma should be basic need of parenteral formulations. Some formulation requires tonicity adjusting agent to form isotonic formulation. According to formulation development, the tonicity modifiers may be added during lyophilization or in the reconstitution medium. The well-known tonicity modifiers are dextrose, mannitol, glycerol and sodium chloride.[48] Different solubilizing agents such as surfactants, co-solvents and complexing agents can also be used in parenteral formulation. Surfactants used at very low levels to aid reconstitution if the drug does not show good wetting behavior. Co-solvents are added due to their high vapor pressure to facilitate faster removal from the product during drying process and thus speeding up the lyophilization process. Co-solvents like organic solvents are sometimes used to increase the primary drying rate by increasing the sublimation rates, improve product stability, decrease reconstitution time by improving drug wettability or solubility and also enhance the sterility assurance of the sample solution.[49] The most commonly reported solvent is tertiary butyl alcohol.[50]

ESSENTIAL PARAMETERS REQUIRED TO DESIGN ROBUST FD CYCLE FOR PARENTERAL FORMULATION

An understanding of the effect of formulation on freeze-drying behavior is very important to the formulation scientist. For FD product development must focused parameters are exact formulation conditions, essential excipients in optimal quantities, thermo-physical properties of formulation to obtain stability, biological activity, safety and quality of a FD product. Subtle variations in the composition of formulations e.g. changes in the ionic strength or pH, may have a significant effect on the physical chemistry of the freezing and freeze-drying processes.[9] This section aims extensive study of feasibility parameters and constraints for lyophilization process optimization. Preformulation parameters of active ingredient such as stability (thermal, pH, photo stability), pH solubility profile, excipient, container closure system compatibility and dose units have to be studied. The correlation of freeze drying processing conditions on the final product has described in Table 2. The development of a suitable formulation and a freeze-dry cycle requires knowledge of some basic properties such as Tg/Tc, temperature effect on solubility, thermal properties of the frozen solution, residual moisture content, degree of super-cooling and other related properties.

Table 2: Freeze drying Processing conditions impact on final product

Freeze drying Processing conditions	Significant Parameters
Freezing conditions	Freezing rate, shelf temperature during freezing, duration of the freezing step and possible annealing steps
Primary and secondary drying conditions	Shelf temperature, pressure, ramp from freezing step to primary drying step and from primary to secondary drying
Stoppering conditions	Vacuum, type of gas used during stoppering
Factors affecting stability of freeze dried parenterals	Storage temperature, moisture content, Tg, Formulation, types & conc. of formulation& excipients, reconstitution medium, crystallization of amorphous excipients

The concept of glass transition temperature (Tg) applies to amorphous system and corresponds to a change in the viscosity of solution from a viscous liquid to a glass or an essentially solid solution of solute in water. It represents the maximum allowable product temperature during the primary drying.[10] Above Tg the solute flows after the supporting ice structure is removed resulting in collapse of the product. The Tc and the Tg are closely related and the collapse temperature is normally a few degrees centigrade higher than Tg. In one study on collapse, Pikal and Shah found that collapse occurred at a temperature a few degrees higher than Tg.[51] Wang suggested that Tc is closely related to Tg although the decrease in viscosity at Tg is not sufficient to cause the structural collapse.[10] Other researchers assumed that they are equally important.[24, 52] The collapse temperature depends both on the residual water content and on the nature of the solute. It appears to be determined in large part by the molecular weight and by the structural groups of the solute molecules.[12] Tc is one of critical parameter influenced by excipient properties and stability of drug.[13] Also slower reconstitution is generally experienced with

the collapsed product due to the loss of porosity and resultant reduction in powder surface area.

A eutectic is an intimate physical mixture of two or more crystalline solids that melts as single pure compound. When eutectic crystallization is initiated, the temperature of the product increases to the eutectic temperature (Te). After eutectic crystallization is completed at the point Te, no more liquid is present and no further changes in microstructure of frozen system take place with further reduction in temperature. Below the eutectic temperature, the product temperature decrease more rapidly toward the shelf temperature. However, eutectic behavior is only observed when the solute crystallizes. In the most cases, the solute does not readily crystallize during freezing. The term eutectic temperature is often misused in reference to freeze-drying. However many solutes do not crystallize during the freezing process, but instead form a glassy mixture with unfrozen water.[9] In contrast to collapse, meltback is due to the eutectic melting of crystalline agents in the frozen formulation. To prevent meltback, the product temperature must be kept below the eutectic melting temperature (Te) of the crystalline component(s) of the formulation during primary drying.

When the temperature of the glassy maximally freeze concentrated solution increases, an endothermic glass transition first occurs. Further increase in temperature above Tg may lead to an exothermic event, corresponding to the recrystallization of a component such as mannitol.[53] This temperature is termed devitrification temperature. Devitrification is a process by which a metastable glass forms a stable crystalline phase on heating above Tg.[54, 55, 56, 57]

An important objective is the determination of the drying rate-limiting factors.[10] Heat transfer and mass transfer are the two most likely rate-controlling factors. They depend on operating parameters such as temperature and pressure. The requirements for an effective process also include low partial pressure, a short distance between the product and the condenser surface and sufficient energy transfer to the product. The drying time is proportional to the layer thickness.[12]

For the total solids concentration greater than 10% (w/w) freeze drying might be difficult to achieve. Also if the solutions are too diluted, then it will lead to the formation of highly porous freeze dried cake with insufficient support and non-elegant appearance. For less porous lyophilized cake reconstitution time would be prolonged. An additionally it would be more difficult to remove the absorbed water from the smaller surface area.[13]

In addition, during developing lyophilization cycle at lab scale or at pilot scale the equipment capability at commercial scale should take into consideration. For large-scale development runs it is necessary to match product resistance with that of small-scale runs.[3] Usually all development trials are taken at small scale below 5000 units and after clinical consistency higher scale trial can be done to get target value of developed cycle. Statistical analysis is done on the result to underline any deviation compared to early development results. The transferring for large-scale production requires people with good knowledge of clinical and industrial freeze-dryers along with set up for process evaluation & validation and specific sampling procedure is developed to assure homogeneity.

QbD Approach for Lyophilization Process

Optimized freeze-drying process based on well established scientific principles, knowledge of the critical properties of the formulation and how to apply this information to process design to obtain a consistent, stable, and esthetically acceptable product. QbD (quality by design) approach in the developing a freeze dried product would assure the quality requirements of final product. These qualities including preserve original chemical or biological potency after reconstitution, acceptable cake appearance, moisture level, rapid and complete dissolution. The robust freeze drying cycle can be obtained using design space. The input and output parameters of freeze drying process are interdependent as shown Figure 2. The goal is to obtain the same range of output using wide range of input parameters. The design of experiments (DOE) approach is perfectly suitable for freeze drying process since there are only a few well-characterized input parameters for this closed system process.

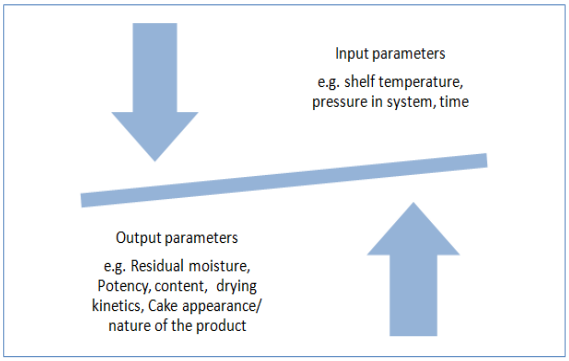

Figure 2: Input and output parameters of freeze drying

Cooling rate is important to minimize the surface area of ice by growing large ice crystals. But it is not practical to manipulate the cooling rate (usually less than 2°C/min).[13] The product temperature depends on the properties of formulation, shelf temperature, chamber pressure and container-closure system. The product temperature should always be several degrees below Tc in order to obtain a dry product with acceptable appearance. Optimized freeze-drying process runs with the product temperature as high as possible which gives the target product temperature close to Tc.[4] The chamber pressure of freeze dryer should be low to improve the rate of ice sublimation and shown impact both on heat and mass transfer. The optimum chamber pressure is a

compromise between high sublimation rate and homogenous heat transfer. The most time-consuming part of freeze-drying is the determination of the adequate shelf temperature for primary drying. The product temperature during primary drying is lower than shelf temperature and changes with chamber pressure, shelf temperature and heat transfer coefficient of the container. In secondary drying it's usually better to run a high shelf temperature for a short time which will decrease the water desorption rate dramatically when compared to low temperature for long period.[13]

The design space for the primary drying portion was developed by Koganti et al., on the basis of very few experimental runs of freeze drying and using mathematical models.[58] Critical process parameters (CPP) form primary drying (shelf temperature, chamber pressure & duration for primary drying) were used as set points and input parameters (heat transfer coefficient & mass transfer resistance) were obtained from separate experimental runs. And then two lyophilization runs were conducted to verify the model predictions which added the confidence in the design space. On this basis they establish the functional dependence of product temperature during drying and sublimation end point on shelf temperature and chamber pressure. And they successfully establish design space for primary drying portion of freeze-drying process.[58]

At pilot scale lyophilization cycle characterization needs to occur with broader tolerances while at production scale with reduced tolerances around all critical parameters. This information provides ranges for critical parameters over which the product is demonstrated to be stable. The lyophilized product needs to be tested for all critical quality attributes via stability-indicating assays along with biophysical characterization and also to test any potential conformational changes. These efforts should be thorough enough to ensure success invalidation efforts in terms of inter and intra batch consistency for all critical quality attributes.[3]

Process Analytical Technology for FD

Process analytical technology (PAT) will help to improve process control and deliver more consistent product quality and achieve higher operational efficiency in freeze-drying process. There are various tools available for freeze drying process development. Critical temperatures (Tc/Tg/Te) can be determined by microscopy, Lyostat™, and DSC. While crystalline-amorphous transitions can be confirmed by DSC and X-ray Diffraction.[10, 52]

The end point of primary drying can be detected by several different methods. The end point is indicated when the product temperature approaches the shelf temperature and this can achieve from product temperature data.[3, 13] It can be also detected by monitoring dew point sensors which can detect the vapor composition change or the relative humidity in the freeze drying chamber and show a sharp dew point decrease at the end of primary drying due to the vapor compositions in the chamber changing from almost 100% water vapor to essentially 100% nitrogen.

Previous developers had only two online tools to monitor the process. First was an invasive R probe placed in a vial and another tool was a view port through which a product can be seen during drying. Recent lyophilizers are equipped with microbalance and cold plasma system. Microbalances are easy to use and follow the loss of weight of product during whole cycle. Cold plasma system can measure water vapor flux inside freeze drying chamber during development phase of the stabilizers.

Scale-up for the lyophilization cycle is very difficult and requires knowledge of the many variables that may have an effect on product. The list of the variables would include vial filling issues, validation of filling operations, freezing rate and temperature ramping rate. The lyophilizer should also have the necessary instrumentation to control and record the key process parameters. These include shelf temperature, product temperature, condenser temperature, chamber and condenser pressure.[2]

Analytical Techniques for FD Formulation Characterization

The complex physical changes in frozen formulations are characterized by number of analytical methods. Details of the freeze-dried product characterization are not part of the scope of this document. The characterization may validate the applied conditions of the process and the optimized formulation. Physico-chemical characterization of freeze dried product includes various parameters to assure quality and consistency of the product. These evaluation parameters are such as reconstitution time, measurement of particle size (if applied), thermal analysis, drug content, dose uniformity, powder surface analysis, study of water sorption, determination of residual moisture, microscopic and macroscopic aspect of freeze-dried product, stability and sterility testing. These methods include differential thermal analysis, DSC, electrical resistance, thermo-mechanical analysis and freeze-drying microscopy. Moisture content of lyophilized formulations can be determined by loss-on-drying, Karl Fischer titration, thermal gravimetric analysis (TGA), gas chromatography (GC), or near IR.[3]According to FDA sterile water for injection should be used to reconstitute products instead of bacteriostatic water for injection. Stability study is very significant for selection of the final freeze dried product and should be conducted according to specified guidelines.[2, 10]

Selection of containers and stoppers

The selection of compatible containers and stoppers are paramount important for long term stability of lyophilized formulation. Both the vial type and stopper type impact the lyophilization process. The vial type influences the heat transfer properties while the stopper type influences

those of mass transfer. Therefore, it is highly recommended to use the same type of vials and stoppers for full-scale practice runs and commercial purposes.[3] Usually Type I borosilicate glass (treated or untreated) is used due to its strong chemical resistance and low level of leachable. Processes related to stopper-induced which can lead to increase in moisture content of lyophilized solid protein formulations during storage are described by Wang.[11] There are various types of stoppers available. Among them halo butyl stoppers, bromobutyl stoppers have been shown to be more resistant than chlorobutyl stoppers to moisture absorption during storage and steam sterilization.[59]Therefore, stoppers should be carefully chosen based on their compatibility with the lyophilized formulation, formulation pH, excipients and sterilization, moisture, vapor transfer property and resealability.[10]

CONCLUSION

Lyophilization is the most common process for formulating solid parenteral pharmaceuticals. However optimized lyophilization cycle required various tedious parameters to control. Therefore, efforts should be made to design a cycle, which is robust, efficient and has minimal adverse effects on parenteral stability. To design such a cycle, parenteral formulations need thorough characterization and all critical temperatures need to be determined. Even successful lyophilization scale-up is critical to meet the clinical and market demand for the drug in a timely manner. Due to equipment differences between small and large-scale freeze dryers some practical considerations and challenges need to understand by formulation scientist. And this will help to develop a robust freeze drying cycle.

REFERENCES

1. Nema S, Ludwig J, Pharmaceutical dosage forms: Parenteral medications, Informa Healthcare, 3,2, 2010.
2. FDA guideline Lyophilization of parenterals, Guide to inspections of lyophilization of parenterals.
3. Chang BS, Patro SY, Freeze-drying process development for protein pharmaceuticals, Lyophilization of Biopharmaceuticals, American Association of Pharmaceutical Scientists, 2004, 113-138.
4. Pikal MJ, Freeze-drying of Proteins. Part I: Process Design,BioPharm, 3, 1990, 18-27.
5. Pikal MJ, Freeze-drying of Proteins. Part II: Formulation Selection,BioPharm, 3, 1990, 26-30.
6. Goldblith SA, Rothmayr WW,Freeze-drying and advanced food technology, Academic Press, New York, 1975.
7. Avis KE,Lieberman HA, Lachman L,Pharmaceutical Dosage Forms: ParenteralMedications, 2, Marcel Dekker, New York, 1993.
8. Gibson M, Pharmaceutical preformulation & formulation: A practical guide from candidate drug selection to commercial dosage form, 2, Drugs & the pharmaceutical sciences, 1999, 333.
9. Banker G, Rhodes C, Modern Pharmaceutics, 4, Informa healthcare, 2009, 392-402.
10. Wang W,Lyophilization and development of solid protein Pharmaceuticals, International Journal of Pharmaceutics, 203, 2000, 1–60.
11. Wang W, Chen M, Chen G, Issues in freeze drying of aqueous solutions, Chinese Journal of Chemical Engineering, 20, 3, 2012, 551—559.
12. Barresi AA,Ghio S, Fissore D, Pisano R, Freeze-drying of pharmaceutical excipients close to collapse temperature: influence of the process conditions on process time and product quality, Drying Technology, 27, 6, 2009, 805-816.
13. Tang X, Pikal MJ, Design of freeze-drying processes for pharmaceuticals: practical advice, Pharmaceutical Research, 21, 2004, 191-200.
14. Sadikoglu H, Ozdemir M, Seker M, Freeze-drying of pharmaceutical products: Research and Development needs,Drying technology, 24,7, 2006, 849-861.
15. Aurelie H, Julien A, Severine V, Sublimation kinetics during freeze-drying of pharmaceutical protein formulation, Drying Technology, 25, 2007, 753-758.
16. Schersch K, BetzO, Garidel P, Systematic investigation of the effect of lyophilizate collapse on pharmaceutically relevant proteins I: Stability after freeze-drying,Journal of Pharmaceutical Sciences, 99,5, 2010, 2256-2278.
17. Kasper JC, Friess W, The freezing step in lyophilization: Physicochemical fundamentals, freezing methods and consequences on process performance and quality attributes of biopharmaceuticals, European Journal of Pharmaceutics and Biopharmaceutics, 78, 2011, 248-263.
18. Mayeresse AY, Cupere VD, Veillon R, Considerations for transferring a bulk freeze-drying process from a glass container to a tray, Pharmaceutical Engineering, 2009, 1-8.
19. Sadikoglu H, Liapis AI, Mathematical modeling of the primary and secondary drying stages of bulk solution freeze-drying in trays: parameter estimation and model discrimination by comparison of theoretical results with experimental data, Drying Technology, 15, 1997, 791-810.
20. Qian L, Zhang HF, Controlled freezing and freeze drying: A versatile route for porous and icro-/nano-structured materials, Journal of Chemical Technology & Biotechnology, 86, 2011, 172-184.
21. SwarbrickJ, Boylan J,Encyclopedia of Pharmaceutical Technology, Marcel Dekker, New York, 2002, 1299-1326.
22. Sadikoglu H, Liapis AI, Crosser OK, Optimal control of the primary and secondary drying stages of bulk solution freeze drying in trays, Drying Technology, 16, 1998, 399-431.
23. Baheti A, Kumar L, Bansalb AK, Excipients used in lyophilization of small molecules,Journal Excipients and Food Chem,1, 2010, 41-54.
24. Searles JA, Carpenter JF, Randolph TW, The ice nucleation temperature determines the primary drying rate of lyophilization for samples frozen on a temperature-controlled shelf, Journal of Pharmaceutical Sciences, 90, 2001, 860-871.
25. Tang XC, Nail SL, Pikal MJ, Mass transfer in freeze drying: measurement of dry layer resistance by a non-steady state method,AAPS Annual Meeting, New Orleans, Louisiana, 1999.
26. Yoshioka S, Aso Y, Kojima S, The effect of excipients on the molecular mobility of lyophilized formulations, as measured by glass transition temperature and NMR relaxation-based critical mobility temperature, Pharm Res, 16, 1999, 135-140.
27. Wassim A, Ghania D, Serge S, Hatem F, Freeze-drying of nanoparticles: Formulation, process and storage considerations, Advanced Drug Delivery Reviews, 58,2006, 1688–1713.
28. Nemati F, Cavé GN, Couvreur P, Lyophilization of substances with low water permeability by a modification of crystallized structures during freezing, Assoc. Pharm. Galenique Ind., Chatenay Malabry, 3, 1992, 487–493.

29. Yu L, Milton N, Groleau EG, Mishra DS, Vansickle RE, Existence of a Mannitol Hydrate during freeze-drying and practical implications, Journal of Pharmaceutical Sciences,88, 1999, 196-198.
30. Johnson RE, Kirchhoff CF, Gaud HT,Mannitol-Sucrose Mixtures: Versatile formulations for protein lyophilization, Journal of Pharmaceutical Sciences, 91,2002, 914-922.
31. Greiff D, Development of cycles for lyophilization, Dev. Biol. Stand., 74, 1992, 85-92.
32. Carpenter JF, Pikal MJ, Chang BS, Randolph TW, Rational design of stable lyophilized protein formulations: some practical advice, Pharm Res, 14, 1997, 969-975.
33. Craig DQM, Royall PG, Kett VL,Hopton ML, The relevance of the amorphous state to pharmaceutical dosage forms: glassy drugs and freeze dried systems, Int J Pharm, 179, 1999, 179-207.
34. Nail SL, The Effect of Chamber Pressure on Heat Transfer in the Freeze-drying of Parenteral Solutions,J Parent Drug Assoc, 34,1980, 358-368.
35. Livesey RG, Rowe TW, A Discussion of the effect of chamber pressure on heat and mass transfer in freeze-drying, Journal of Parenteral Science Technology, 41, 1987, 169-171.
36. Carpenter JF,Prestrelski SJ, Arakawa T, Separation of freezing-and drying-induced denaturation of lyophilized proteins using stress-specific stabilization. I. Enzyme activity and calorimetric studies,Arch. Biochem. Biophys, 303, 1993, 456-464.
37. Chen GH, Wang W, MujumdarAS, Theoretical study of microwave heating patterns on batch fluidized bed drying of porous material, Chem. Eng. Sci., 56, 2001, 6823-6835.
38. Wang W, Chen GH, Heat and mass transfer in dielectric-material-assisted microwave freeze-drying of skim milk with hygroscopic effect, Chem. Eng. Sci., 60, 2005, 6542-6550.
39. Wang W, Pikal MJ, Shah S, The collapse temperature in freeze drying: dependence on measurement methodology and rate of water removal from the glassy phase, International Journal of Pharmaceutics, 2, 1990, 165-186.
40. Powell MF, Ngugen T, Baloian L, Compendium of excipients for parenteral formulations, PDA, Journal Pharmaceutical Science Technology, 52 1998, 238-311.
41. Neema S, Washkuhn RJ, Brendel RJ, Excipients and their use in injectable products, PDA Journal Pharmaceutical Science Technology, 51, 1997, 166-171.
42. Strickley RG, Parenteral formulations of small molecules therapeutics marketed in the United States Part I, PDA Journal Pharmaceutical Science Technology, 53, 1999, 324-349.
43. Strickley RG, Parenteral formulations of small molecules therapeutics marketed in the United States Part II, PDA Journal Pharmaceutical Science Technology, 54, 2000, 69-96.
44. Jennings TA, Effect of formulation on lyophilization, part 1, IVD Technology Magazine, 1997.
45. Sugimoto I, Ishihara T, Habata H, Nakagawa H, Stability of lyophilized sodium prasterone sulfate, Journal Pharmaceutical Science Technology, 35, 1981, 88-92.
46. Swarbrick J, Boylan JC, Encyclopedia of Pharmaceutical Technology, Marcel Dekker Inc., New York, 6, 1992, 275-303.
47. McNally EJ, Hastedt JE, Protein formulation and delivery, Informa Healthcare, New York, 175, 2008, 197-217.
48. Remington,The Science and Practice of Pharmacy, Mack Publishing Co., PA,2000, 246-262.
49. Teagarden DL, Baker DS, Practical aspects of lyophilization using non-aqueous co-solvent systems, Eur J Pharm Sci, 15, 2002, 115-133.
50. Jennings TA, Lyophilization: Introduction and Basic Principles, Informa healthcare, New York, 2008.
51. Pikal MJ, Shah S,The collapse temperature in freeze drying: dependence on measurement methodology and rate of water removal from the glassy phase, International Journal of Pharmaceutics, 2, 1990, 165-186.
52. Franks F, Improved freeze-drying: an analysis of the basic scientific principles, Process Biochemistry, 24, 1989, 3-7.
53. Hatley RHM, Franks F, Application of DSC in the development of improved freeze-drying process for labile biologicals, Journal of Thermal Analysis, 37, 1997, 1905-1914.
54. Meredith P, Donald AM, Payne RS, Freeze-drying: In situ observations using cryo-environmental scanning electron microscopy and Differential Scanning Calorimetry, J. Pharm. Sci., 85, 1996, 631-637.
55. Chang BS, Randall CS, Use of subambient thermal analysis to optimize protein lyophilization, Cryobiology, 29, 1992, 632–656.
56. Philips RD, Finley JW, Protein Quality and the Effects of Processing, Marcel Dekker, New York, 1989, 9-124.
57. Rey L, May JC, Freeze-Drying: Lyophilization of Pharmaceutical and Biological Products, Marcel Dekker, New York, 96, 1999, 1–30.
58. KogantiVR, Evgenyi Y, Shalaev MR, Thomas B, Youssef DN, Hiebert FA, Investigation of design space for freeze-drying: Use of modeling for primary drying segment of a freeze-drying cycle, AAPS PharmSciTech., 12, 2011, 854–861.
59. Corveleyn WS, Smedt DS, Remon JP, Moisture absorption and desorption of different rubber lyophilization closures, Int. J. Pharm., 159, 1997, 57–65.

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