The Impact of the Freezing Stage in Lyophilization: Effects of the Ice Nucleation Temperature on Process Design and Product Quality

Michael J. Pikal, Ph.D., Shailaja Rambhatla, & Roee Ramot

School of Pharmacy, University of ConnecticutAuthor Company

Lyophilization is the most common method for manufacturing solid protein pharmaceuticals (1) and is central to the preservation of materials which must be dried very thoroughly (< 1% moisture) in order to ensure stability and require a gentle, sterile process for doing so. However, the multitude of variables inherent in a large batch of individual vials in a complex drying chamber configuration makes process control difficult at best; and a thorough understanding of the process and the materials science of different formulations is necessary to avoid product damage.

A solution's lyophilization occurs in three steps: first, the solution is cycled through a freezing step, in which most of the water separates into ice crystals throughout a matrix of glassy and/or crystalline solute. This step is of paramount importance (2) in determining the details of the remainder of the cycle, and it is here where two main problems come into play: First, the concentrations of all dissolved materials will increase dramatically as water freezes into ice, potentially imparting massive pH changes and/or significant increases in the rate of second order degradation processes which could degrade the product. Note that this also means most of the desiccation actually occurs at freezing, as the solute is dramatically concentrated (3). Second, the temperature at which the solution will form ice, Tn, is stochastic, as well as dependent on a number of process and formulation variables and thus induces heterogeneity in a vial batch (2), causing process control problems in the primary drying stage.

The primary drying stage involves sublimation of ice under vacuum and normally an increase in shelf temperature to provide energy for sublimation. However, if the temperature increases to the eutectic temperature (or collapse temperature, Tg', for solutes which form amorphous solids rather than crystals), gross defects (collapse) occur in the product cake, making it generally unsuitable for pharmaceutical use (3). Most of the lyophilization cycle is spent on primary drying, the duration of which is usually days, and its rate is evaluated from the equation below:

$$dm/dt = (P_o - P_c) / (R_p + R_s) \quad (1)$$

where dm/dt is the rate of mass transfer for the water vapor, Po is the equilibrium vapor pressure of ice at the temperature of the frozen mass, P_c is the chamber pressure, R_p is the resistance of the dried product layer to the transfer of vapor, and R_s is the resistance of the stopper. Since the vapor pressure of the ice varies exponentially with the temperature, product temperature

control is in primary drying is critical, and the main process variables for control of product temperature during primary drying are the shelf temperature and chamber pressure. A constant product temperature requires a balance between the heat transfer rate to the product and removal of heat by sublimation, which in turn is coupled to the mass transfer rate of water vapor. While the former depends largely on the design of the freeze dryer, the type of vials used, and the chamber pressure, the latter depends to a great extent on the product resistance which in turn, is a reflection of how the initial solution was frozen. The small dimensions of the pores in the solute matrix previously occupied by ice crystals means that the greatest resistance to the flow of water vapor comes from the product.

The remaining unfrozen water requires a secondary drying step, or a desorption step, for its removal. The amount of sorbed water is usually about 15-20 percent w/w whereas the final lyophilized product will usually have less than one percent water. Secondary drying temperatures are usually much higher than those of primary drying because the rate of water desorption from the solid is extremely slow at primary drying temperatures. Apoint should be made here regarding the effect of ice crystal size obtained from the freezing step: larger ice crystals result in larger pores left by their absence during primary drying and, consequently, decreased product resistance to vapor flow and decreased primary drying time, but increased secondary drying time due to a lack of surface area (1). Normally, the decrease in primary drying time dominates.

Importance of the Freezing Step

The importance of the freezing step is almost obvious since it is the first process step, and because the characteristics of the frozen matrix (or "cake", after water has been removed) determine how the rest of the MANUFACTURING American Pharmaceutical Review 2 The Impact of the Freezing Stage in Lyophilization: Effects of the Ice Nucleation Temperature on Process Design and Product Quality Michael J. Pikal, Ph.D., Shailaja Rambhatla, and Roee Ramot School of Pharmacy, University of Connecticut process will run. The freezing of a solution starts with nucleation, the spontaneous aggregation of water molecules to form a template to which other molecules can attach and ultimately form a crystal. As the temperature drops below the equilibrium freezing point, the probability of nucleation increases, because of a thermodynamic driving force, and ultimately decreases due to kinetic limitations imposed by high viscosity. The nucleation observed in pharmaceutical solutions is largely heterogeneous nucleation, meaning the event was initiated by nucleation sites either in the solution or on the surface of the container in which the solution was placed, as opposed to homogenous nucleation, where clusters of water molecules spontaneously form to generate nuclei (4).

One important goal of the freezing step is to produce a *uniform* product batch, which is difficult because of the stochastic nature of nucleation. That is, the degree of super-cooling, defined as the difference between the equilibrium freezing point and the temperature at which ice crystals first form, is both a statistical or random event as well as one that depends on the solution properties and process conditions. The degree of super-cooling is important because it determines the number of nuclei at any time, and thus determines the number of ice crystals formed (3). More ice crystals from the same amount of water means smaller crystals, which means smaller pore size and thus longer primary drying time (2, 3, 5). The reduction in drying rate with increasing degree of super-cooling is a significant effect. The Searles work (2) shows about a 3% increase in drying time for a 1°C decrease in ice nucleation temperature (Fig. 1), and current (unpublished) studies in our laboratories show an effect of the same magnitude. The "freezing rate" or simply, the temperature change of the heat sink, is also a factor that can affect the degree of super-cooling. In general, the freezing process has to be chosen so as to produce a moderate and uniform super-cooling and also relatively fast ice growth to minimize residence time of a labile drug molecule in a reactive environment. While it is difficult to achieve both the desired features since the only controllable factor in freezing is the cooling rate of the heat sink, the aim is to achieve uniformity within a vial and between vials of the same batch.

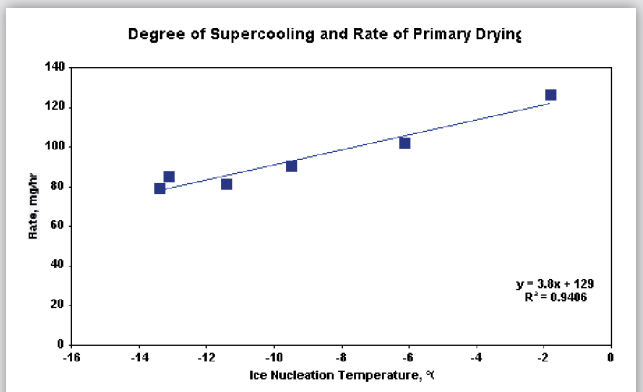

Figure 1. The Effect of Ice Nucleation Temperature on Rate of Primary Drying. (Data Taken From Ref. 2, Table 2.)

Scale-Up Issues

Most laboratory experiments occur on a scale much smaller than that of a pharmaceutical production plant and in an environment that contains far more air-borne "ice-nucleating" particles than a typical Class 100 production environment. The scale issue of greater variation in shelf surface temperature in a large freeze dryer will cause heterogeneities in ice nucleation temperature beyond what is observed in the laboratory. Thus, a freezing procedure employed for a laboratory scale for achieving a desired level of uniformity may not transfer exactly to a manufacturing scale. Further, and most important, the cleaner air in the production environment means fewer heterogeneous nucleation sites in the production solution. This creates a much lower probability of nucleation at a given temperature, effecting higher degrees of

super-cooling. Higher degrees of super-cooling means higher product resistance, and the freeze drying cycle developed in the laboratory will produce slightly higher product temperature (≈1°C or more) and a longer primary drying process (≈10% or more) in manufacturing. The extension in primary drying time is usually the more serious problem, particularly if fixed time cycles are used. It is thus important, to be able to control the nucleation temperature in order to control product resistance, and drying times.

Annealing

In the interest of eliminating inter-vial heterogeneity, Searles et al (5) suggested incorporating an annealing step during the freezing stage of the process. Maintaining the samples below the equilibrium freezing temperature but above their Tg' for a sufficient amount of time leads to Ostwald ripening, a growth of larger crystals at the expense of smaller ones, thereby leading to a larger and more uniform crystal size. By comparing scanning electron microscope (SEM) images of annealed versus un-annealed samples, Searles et al were able to show that annealing could eliminate inter vial heterogeneity in primary drying and that annealing times as low as even 30 minutes resulted in significant reduction of primary drying rate. However, the detrimental effects of annealing cannot be ignored. Of course, the annealing step does take time, but it is potential instability that constitutes the more significant problem. Crystallization of buffer components resulting in pH shifts, incompatibility between polymers leading to phase separation, and longer residence time in a reactive environment due to annealing are some of the issues that, especially for proteins, can become causes for instability of formulations. Thus, while the idea of annealing does impart a possible solution to the issue of heterogeneity, ability to control the nucleation temperature would still be preferable.

Temperature-Controlled Nucleation

The cooling rate of the shelf during the freezing step has an effect on the probable nucleation temperature of the liquid, as discussed earlier: A fast cooling rate normally gives greater super-cooling, inducing a lower nucleation temperature and therefore smaller ice crystals, increasing primary drying time (1).

The concept of temperature-controlled nucleation has been explored by Searles et. al.(2) in an attempt to precisely fix the nucleation temperature in order to study its relation to primary drying rate and the nucleating agents used. Common ice nucleating agents such as silver iodide, *Pseudomonias syringae*, and air-borne particulates, along with methods such as scoring the vials to provide a crystallization surface were explored, and it was found that the use of ice nucleating bacterium produced the lowest degree of supercooling and the highest primary drying rate, They were able to conclude from this study that the ice nucleation temperature is the primary determinant of inter-vial drying heterogeneity. However, the idea of adding nucleating agents to control the nucleation temperature is not one of practical utility for a pharmaceutical product.

At the International Symposium on Biological Products Freeze-Drying and Formulation (6), Rowe suggested an ice-fog technique for nucleating samples at a particular temperature. The procedure involves cooling the sample to a particular temperature in a partial vacuum. Next, a flow of cold nitrogen is introduced into the chamber. MANUFACTURING American Pharmaceutical Review 3 FIGURE 1. THE EFFECT OF ICE NUCLEATION TEMPERATURE ON RATE OF PRIMARY DRYING. (DATA TAKEN FROM REFERENCE 2, TABLE 2.) The nitrogen gas, being at a very low temperature as a result of circulation through coils immersed in liquid nitrogen, creates an ice fog as it enters the chamber resulting in the nucleation of the solution at the desired temperature. Because of its use of ambient air to create nucleation, this could be a promising method for temperature-controlled nucleation, and current studies in our laboratories support this conclusion. However, this methodology has not yet been developed fully, still requiring refinement in the laboratory.

Evaluation of Freezing Process by Characterization of the Dry Product

Samples taken from the dried cakes produced in a freeze drying run prove valuable in assessing the performance of the freezing method used. The main concern here is morphology and "structure" of the cake, such as the size and shape of the crystals formed during freezing and the size and morphology of the resulting pores. Two main factors - the degree of super-cooling and the composition of the solution - affect the so-called ice crystal morphology. MacKenzie (7) proposed distinct composition/temperature curves along which one could expect a certain crystal type to form. For instance, hexagonal crystals seem to form at a higher temperature, while dendritic crystals and finally spherulitic crystals form at much colder temperatures (7). The resulting crystal structure does impact primary drying time (8). Depending on the mass transfer characteristics of the pores left in relief from the ice crystal structure, a pre-selected nucleation temperature achieved uniformly by the techniques described above could give manufacturers better control over the product.

To examine cake morphology, techniques such as scanning electron microscopy can be used. Photomicrographs of freeze dried products produced with different ice nucleating agents clearly show the differences in pore structure, reflecting the difference in ice crystal morphology (4). It is also important to realize that since temperature is rarely uniform across the material, the cake structure may vary with depth into the cake. Comparing SEM micrographs of the bottom and top of a lyophilized cake from Searles, one observes smaller, spherulitic crystals near the bottom, indicating a lower nucleation temperature and thus uneven cooling in the vial (4).

For a quantitative measurement of surface area, the most common and practical method is the "Brunauer Emmett Teller specific surface area", or BET SSA, method. Used by chemical and material engineers extensively, this method has not been applied in earnest to lyophilized cakes, but recent studies in our laboratories (unpublished) suggest BET SSA may prove useful in checking freezing step efficiency. Measurement of lower SSA can be used to validate the claim that higher nucleation temperatures increase pore size, decreasing surface area. From experiments that establish a correlation between Specific Surface Area and product resistance, obtained from Manometric Temperature Measurement (9), a useful estimate of the inter-vial variation in product resistance can be obtained. With the aid of simple heat and mass transfer theory (11), one can then estimate temperature and drying time variations.

Conclusions

The freezing method used can have a significant effect on ice morphology, affecting both the resistance to vapor flow during primary drying and also quality of the final product when collapse is a problem (10). It is vital to control nucleation temperature during freezing in order to optimize and properly scale-up the freeze drying process. Control and characterization of the degree of super-cooling can provide a solution to what is perhaps the biggest freeze drying scale-up problem.

This article has reviewed current views and ongoing research pertaining to the importance of the freezing step during lyophilization and its impact on the freeze drying process. It is clear that additional studies are required to properly assess the quantitative impact of variation of nucleation temperature. It would also be useful to develop scale-up algorithms to allow "correction" for this effect, so as to maintain similarity among product temperature profiles, in both the lab and in manufacturing freeze drying operations.

References

1. *W. Wang, "Lyophilization and development of solid protein pharmaceuticals", International Journal of Pharmaceutics, 203, 1-60(2000)*
2. *J. A. Searles, J.F. Carpenter, T. W. Randolph, "The ice nucleation temperature determines the primary drying rate of lyophilization for samples frozen on a temperaturecontrolled shelf", Journal of Pharmaceutical Sciences, 90, 860-871, (2001).*
3. *M. Pikal, Freeze Drying, in Encyclopedia of Pharmaceutical Technology, Vol. 6, J. Swarbrick and J. Boylan, Editors, Marcel Dekker, 2001.*
4. *T. A. Jennings, "Supercooling", Insight, 5 (5), (2002)*
5. *J. A. Searles, J.F. Carpenter, T. W. Randolph, "Annealing to optimize the primary drying rate, reduce freezing-induced drying rate heterogeneity, and determine T(g)' in pharmaceutical lyophilization", Journal of Pharmaceutical Sciences, 90, 872-877, (2001).*
6. *T. D. Rowe, " A technique for the nucleation of ice", International Symposium on Biological Product Freeze- Drying and Formulation, 1990.*
7. *A. P. MacKenzie, "The physico-chemical basis for the freeze-drying process" Development in Biological Standardization, 36, 51-67 (1977)*
8. *M. Kochs, C. Korber, B. Nunner, I. Heschel, " The influence of the freezing process on vapor transport during sublimation in vacuum-freeze-drying", 34(9), 2395-2408, (1991).*
9. *N. Milton, M.J. Pikal, M.L. Roy, S.L. Nail, " Evaluation of Manometric Temperature Measurement as a method of monitoring product temperature during lyophilization" PDA Journal of Pharmaceutical Science and Technology, 5, 7-16, (1997).*
10. *T. A. Patapoff, D. E. Overcashier, " The Importance of freezing on lyophilization cycle development", Biopharm, 3, 16-21, (2002).*
11. *Pikal, M.J., "Use of Laboratory Data in Freeze Drying Process Design: Heat and Mass Transfer Coefficients and the Computer Simulation of Freeze Drying," J. Parenteral Sci., and Tech., 39, 115-138 (1985).*

***Dr. Pikal** is currently Professor of Pharmaceutics at the University of Connecticut. Prior to September 1996, he was with Lilly Research Laboratories for more than 24 years. His research has focused on the science and technology of freeze drying.*

***Ms. Rambhatla** is a Ph.D. candidate in Pharmaceutics at UConn, and Mr. Ramot is an undergraduate in chemical engineering at UConn.*