

## INDEPENDENT PROTOCOL DEVELOPMENT OF LYOPHILIZATION FOR DIFFERENT COMPOUNDS

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This article is available online at [www.ssjournals.com](http://www.ssjournals.com)

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### ABSTRACT

A multidimensional space that encompasses combinations of machine design and processing variables that provides assurance of suitable product performance. This review article mainly focuses on design space in the concern of developing and scaling up all the process variables with reference of one particular material for the development of individual product protocol. The design space should be developed as an envelope for every product specifying its sublimation rate, shelf temperature, and chamber pressure, one boundary of the design space is established by failure of the formulation under aggressive cycle conditions. Other boundaries of the design space are determined by equipment performance including refrigeration capacity, condenser capability, heating capacity or limitations of the dynamics of water vapor flow within the system. The characterization of this design space assures a thorough understanding of both the product and the process. It minimizes the probability of unpleasant surprises in the technology transfer process. Hence we can develop a collection of protocols regarding the individual product lyophilization in a very similar manner like I.P., B.P., U.S.P. etc. This will result in restriction from process variation and thus different troubles can be resolved.

**Keywords: Multidimensional, scaling, Refrigeration, Lyophilization**

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### 1. INTRODUCTION

Wide range of the products such as foodstuffs, pharmaceuticals, biotechnology products, vaccines, diagnostics and biological materials can be dehydrated by the technique of sublimation and the process is known as Lyophilization (Freeze-drying) and this can be carried out on various scale i.e. from bench top through pilot-scale to a full-scale and the process supports various advantages over the conventional drying and many other traditional methods of drying<sup>1</sup>. The quality of the product dehydrated by sublimation is much more superior than the product dehydrated by traditional methods.

### 2. ITINERARY OF ACCOMPLISHMENT

The process of Lyophilization is mainly divided into three steps<sup>1,2,3</sup>. First the product is frozen at adequately low temperature to reach a vitreous state where pure water gets crystallized and in its interstitial region the amorphous content remains stable. Freezing as crystals and sublimation involves two steps ice nucleation followed by ice crystals growth. Once the liquid gets solidified with the accurate temperature then crystal formation occur and results in to the amorphous

solid. In second stage the product is sublimed at the temperature below the glass transition or collapse temperature and hence the crystallized water is sublimed. This overall phenomenon is an endothermic process which is controlled by chamber pressure and shelf temperature and various other factors such as heat transfer in container, container design, stopper design, shelf space design, freezing behavior and freed structure of the compound etc are all important factor for the sublimation rate of the freed crystals. The precaution should be taken during the whole cycle so that collapse can be controlled, Dumitru Mnerie<sup>1</sup> in 2008 explained that the end of this stage the maximum moisture of the content are removed and the product is dried up to 80-90% and thus only the homogeneous mixture of the compound is obtained. The number of parameters influencing this process i.e. why this process of freeze-drying is so complex. Hence after primary drying 10-20% remains in the homogenous mixture which is removed off by the application of the third step which is known as desorption and this step is also known as secondary drying. In this step the process of drying is done at the higher

temperatures i.e. in positive temperatures so that the adsorbed water from the interstitial phase can be removed off. Maintenance of the correct temperature should be regulated during whole process. Indeed if the collapse of the temperature will occur the structural integrity of the cake will get destroyed and will result in the melting. Hence, during this phase the chances of the temperature collapse will be higher and to overcome this problem the shelf design should be best to restrict the overheating. However once the area of stability is reached the product is kept in positive temperature for several hours for the complete removal of moisture. At this phase diffusion helps in removal of moisture and as the diffusion kinetics is slower is slow this results in much consumption of time i.e. approximately one third of the total cycle and even though just removing 10-20% of the moisture. The maximum temperature for secondary drying for every product is defined according to its function and it is usually below +40°C.

This is the topic to discuss that there is need for the optimization of every cycle which is to be undertaken with the highest allowed product temperature and sufficient safety margins to skip any variations thus the product will be maintained within proven acceptable limits. Earlier freeze-drying cycle was not optimized and the process was trial and approach basis till the satisfactory result was not obtained. Nowadays process evaluation and validation approach is used but then also there is lack of information regarding its authenticity. Hence, Lyophilization process should be initially evaluated as according to particular compound with reference to their characteristics (i.e. physical or chemical). The critical parameters such as shelf temperature, solvent humidity etc are established and then proven acceptance range is set (i.e. shelf temperature  $\pm 1^\circ\text{C}$  or  $\pm 5^\circ\text{C}$  or more) as according to compounds composition. Finally the validated unit should be an S.I. unit which can be accepted globally. Then any one of the standard publication should provide this information across the world as the standards of the pharmaceutical preparations has been described worldwide in the standard publications like I.P., U.S.P. and B.P. etc.

### 3. GENERAL FORMS OF A DESIGN SPACE FOR FREEZE DRYING

A simplified sketch of graph from a paper published by Chang and Fischer<sup>3</sup> in 1995 includes a graph that described the simplified

process of the freeze-drying and the safe zone from collapse temperature by which the degradation of the material could be controlled and that can be used for the process of optimization and validation for particular compounds.

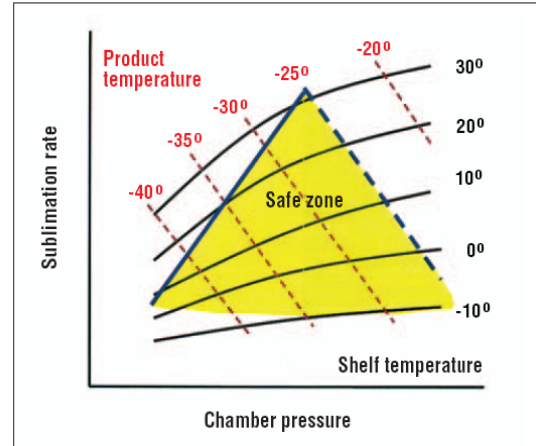


Figure 1 (Chang and fisher, 1995)

**Figure1:** General layout of a proposed design space for freeze-drying, showing general relationships among sublimation rate, system pressure, shelf temperature, and product temperature (in degrees Celsius), with sublimation rate shown on the y-axis and chamber pressure shown on the x-axis, illustrating the functional relationships among sublimation rate, product temperature, and the two independently controlled variables in the process: shelf heat-transfer fluid inlet temperature and chamber pressure.

### 4. Chamber pressure has a complex effect on product temperature and sublimation rate, as follows:

1. The heat transfer rate gets increased due to the higher chamber pressure which supports the sublimation rate by increasing the thermal conductance of the gas in the narrow gap between the heating plate of the shelf and the bottom of the vial. Eventually results in to the increase of the water vapor pressure at ice interface and helps in driving the moisture from the product into the chamber thus helps in the sublimation of the product. This occurs because the improved heat transfer provided by higher pressure outweighs the negative effect on the sublimation rate of decreasing the driving force for flow of water vapor from the

product to the chamber. Therefore for the most efficient processing it is desirable to operate at the highest possible shelf temperature and the lowest chamber pressure that still maintains the target product temperature during primary drying.

2. The decreased driving force of water vapor from ice interface is due to the higher chamber pressure. The driving force is defined as the difference between the pressure at the ice interface within the product and the chamber pressure ( $P_i - P_c$ ).

These all the facts are suitable for lyophilization<sup>3</sup> when all the product temperature remains the same but this is impossible that all the products will have the same vapor pressure temperature. So, requirement for independent protocol development for different compounds with reference to the international units is their which can be accepted globally.

## 5. MEASUREMENT OF SUBLIMATION RATE

Sublimation rates are being measured gravimetrically till date<sup>2</sup>. A representative number of vials are weighed before beginning the cycle and the cycle is terminated before the end of primary drying. The average sublimation rate is calculated by re-weighing the pre-weighed vials after partial drying and by knowing the time interval during which drying took place. Of course, this method is destructive and weighing the vials can be tedious but the process information is generally worth the loss of material and the work involved. There is a new process analytical technology in freeze-drying; however that provides instantaneous and nondestructive measurement of sublimation rate. A paper by Gieseler H, et al<sup>4</sup>, 2007 describes that Tunable diode laser absorption spectroscopy is a technique in which sensing hardware is placed in the connecting duct between a chamber and a condenser. TDLAS is not applicable to freeze-dryers designed with an internal condenser. A near-infrared beam is directed at an angle to the axis of the duct and the Doppler shift of the water absorption band is measured by comparison with a sealed reference cell containing water vapor at a known partial pressure. The frequency shift between the two absorption maxima is proportional to the velocity of water vapor in the duct. By measuring the concentration of water vapor by traditional absorption spectroscopy and

by knowing the cross sectional area of the duct the instantaneous mass flow rate was determined.

## 6. LIMITS FOR SPACE DESIGN

The approach to identify freeze-drying cycle for any given product has to be developed specifically through trial and error method and by taking set of parameters (shelf temperature, chamber pressure and time duration) with reference to international units for a single product could be optimized and in same manner the list of protocols for number of products can be made which produce a Qualitatively acceptable product. During the validation of the process the variation of  $\pm 1$  to  $\pm 5$  for temperatures, pressures and times are tolerable from the standards to make improvement during the processing. The technical person handling this design approach should have a superior understanding of process conditions to produce an acceptable product.

## 7. IDENTIFICATION OF INDIVIDUAL PRODUCT-IMPOSED BOUNDARY FROM THE DESIGN SPACE

The upper product temperature limit during primary drying should be determined during characterization of formulations intended for freeze-drying using low temperature thermal analysis, freeze-dry microscopy or both. Suppose that characterization of the formulation shows an upper product temperature limit during primary drying at  $-25$  °C. This upper temperature limit is shown by the broken blue line in Figure 1 and it represents the boundary of the design space imposed by the characteristics of the formulation. For a failure mode involving ejection of solids from vials the product-imposed boundary on the design space would be a horizontal line corresponding to the sublimation rate above which significant solids ejection takes place.

## 8. IDENTIFY EQUIPMENT-IMPOSED BOUNDARIES ON THE DESIGN SPACE

When there is demand from the market for the development of any freeze-drying cycle either from pharmaceutical or any other industry, usually any biotech company develops the protocol on laboratory scale with the help of their development scientists and provides the data to the concern firm but this results in the variation from the results obtained from the pilot scale and the production scale freeze-drying equipment. This is because of the limitations of performance of laboratory scale to pilot scale and production

scale. Thus, there is requirement for independent protocol development on every scale for every product and the dependence for the technique on third party should not be there.

The necessity of knowing the capability of equipment at the site where a product will be manufactured during initial cycle development<sup>2</sup> is there. The various types of the limitations are explained in their review paper, taking one as an example: the condenser has a limit as to the flow rate of water vapor that can be condensed while keeping the surface temperature of the condenser adequately low. This condition may occur because of limitations in refrigeration capacity at the condenser because of limited surface area of the condenser or perhaps restrictions in water vapor flow around the condenser make some surfaces relatively inaccessible for condensation. In freeze-drying choked flow is characterized by loss of pressure control in the chamber. As the equipment limitations described above, the limitation imposed by choked flow depends upon the pressure in the system, where the mass flow rate choke point is directly proportional to pressure which is imposed by choked flow that is the general shape shown by the solid blue line in Figure 1. In this example the cycle is bounded by the upper product temperature isotherm on the right and by the line representing the choke point on the left. Any process conditions in the design space would be acceptable. It is most desirable to operate near the apex of this space because the apex represents the most efficient process conditions. This example also explains that the requirement of the individual protocol for every compound every compound has its own physico-chemical characteristics.

## 9. CONCLUSION

Freeze-drying is not only a process to be followed or done which is a well defined critical technique to be handled by the trained professionals who have the great understanding of the subject. To generate a design space similar to one it is necessary to have a thorough understanding of both the characteristics of the formulation and the capability of equipment. This is the major intent of the Quality by Design paradigm. A paper published by Pikal et al.<sup>4</sup>, in 1983 describes about the thickness of the vial (containers) and viscosity of the different compound with their own partial pressure will require different self temperatures and work of Marco et al.<sup>5</sup>, 1995 explains the 12% variation in the results when the parameters are not

optimized. Again in 1995 March Wei-youh<sup>6</sup> with James Mcshane and Joseph Wong found that optimization and monitoring was important for reduction in the experimental trials not the major alterations in the freeze-drier. In 2004 S.C. Tsiontides et al.<sup>7</sup>, explained equivalent drying rates should be base for scale-up of freeze-drying but in industrial process or scaling-up process shelf temperature and chamber pressure set-points may not be adequate since different product have their own characteristics irrespective of size thus yielding different rates of heat transfer to the product and in 2005 Wei-youh, Hardwick and Akers in 2005 concluded in their paper that the time required for a cycle will be approximately 91% when compared with industrial lyophilizer with the lab scale. Hear this article also emphasizes that all the above journals discussed have the same thought regarding the integrated process development and this article is just stretching its thought regarding the specificity of the different compound for the development of the process as protocol and which can be used commonly for the production at any scale. As all of the above points of discussion explains clearly that the process of the lyophilization should be an integrated process for its development as a protocol and it should also be very specific for each product as different product have their own identity regarding physio-chemical behavior. So it is important to view the product and process development for freeze-dried formulation as an integrated process rather than as a collection of independent activities for every product. The development scientist must be aware of the type of equipment to which the product will be transferred in the next stage of development. The scientist must also understand the capability of the equipment and must take it into account as cycle conditions are developed. This approach becomes particularly important for formulations that will withstand aggressive cycle conditions.

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