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Introduction

In the early days of lyophilization cycle design, it was not uncommon for development scientists to run upwards of 15 to 20 pilot runs to develop a cycle that worked well for their product in commercial manufacturing. In many of these cases, the main criteria for determining if the cycle was acceptable was based on the physical appearance of the dried product (solid, intact cake), the reconstitution time, the residual moisture content, and the stability of the active ingredient (immediately after freeze-drying and after long term storage). While these attributes are still important today and must still be achieved from a lyophilization cycle, modern lyophilization cycle design must also take into account the time that it takes to dry these products. When talking about cycle optimization, the question that begs to be asked is, why worry about how long it takes to freeze-dry a product if one is achieving product that consistently looks good and meets all of the release specifications? The answer to this question is that it doesn't matter at all for a company that is only producing several batches of product a year (for example a product with orphan drug status). In these cases it would likely take more time and effort to optimize the cycle than the time savings that could be saved by doing such. However, for those drug products that are produced regularly on a large scale, cycle optimization is critical for two distinct reasons. The first reason simply comes down to the manufacturing costs. It is well understood that commercial freeze-drying is the most expensive unit operation as part of a complete manufacturing process. This cost increases dramatically the longer the product spends in the freeze-dryer.

The second reason lyophilization cycle optimization is important in commercial manufacturing is throughput. It is not uncommon for companies that manufacture drug products that are produced on a massive scale do not have enough capacity to meet the market demand for the product. In these cases, the manufacturer must either simply undersupply the market, or look to outside commercial manufacturing companies to make up the difference. By reducing lyophilization cycle time through empirical cycle optimization studies, it is possible in many cases, to meet the market demand for high volume drug products while keeping the entire manufacturing process in-house.

Building a Firm Foundation

Just like building a house, building a quality lyophilization cycle must occur on a strong, well built foundation if a robust, stable product is going to be achieved. In the case of a freeze-dried product, the formulation is the foundation. The development scientist may be able to design an optimal lyophilization cycle for a poor formulation; however eventually, product quality and or product performance during the freeze-drying process are going to be compromised unless a strong formulation is developed prior to starting cycle design.

Prior to beginning the design of a fully optimized cycle, the development scientist should conduct a thorough data review. This will include data from the bulk drug substance (development and stability studies), data from pre-formulation studies, data from the formulation studies, data from any stability studies, Clinical Trials results, etc. Listed below in Figure 1 is a partial list of the information that should be acquired prior to beginning optimized lyophilization cycle design studies for a small molecule:

- Stability Indicating Analytical Method
- pH Stability Profile
- pH Solubility Profile
- Thermal Stability Profile
- Oxidative Stability Profile
- Light Stability Profile
- Route of Administration
- Dose
- Route of Degradation
- All Excipients and Concentrations
- Container Closure System
- Stability Data of Bulk Drug
- Existence of Polymorphs
- Crystalline/Amorphous/Metastable Character
- Fill Volume and Administration Volume

Figure 1

Understanding each of the points in Figure 1 can help the development scientist design an optimized cycle that will produce a stable product. For example if the thermal stability data for the bulk drug substance shows that the active ingredient has increased degradation rates when temperatures exceed 45°C, the cycle should be designed so that the product never exceeds 45°C during the secondary drying phase. By taking the time to conduct a thorough data review, the development scientist will save time and effort in the cycle design phase, and will help to ensure that the newly developed cycle will produce a quality product.

The development scientist must also have a thorough understanding of the thermal characteristics of the formulation. A combination of techniques including differential scanning calorimetry (DSC) and freeze-dry microscopy are two techniques that are well suited to provide a complete thermal picture of the formulation. Both of these techniques are discussed in detail in other papers within this series.

Additionally, the development scientist should talk to the engineers and equipment operators who thoroughly understand the commercial freeze-dryer where the cycle will be used. This is done to make sure that the newly designed cycle will transfer flawlessly from the development-scale, pilot freeze-dryer to the larger commercial unit. The development scientist needs to understand the limits of the larger equipment and design the cycle accordingly.

Finally, sample preparation is very important in cycle design, and the development scientist must try and duplicate the formulation and filling as closely as what will be conducted at the commercial level. This includes following the same formulation steps under similar conditions, sterile filtration of the product into a Class A bench, using the same fill volume, using the same vial and stopper combination, having the vials and stoppers cleaned and sterilized using production equipment (this can be duplicated in the lab if production cleaning and sterilizing equipment is not available), filling the vials under Class A air, etc.

Equilibration and Freezing

The initial temperature of the lyophilizer shelves prior to loading the vials can play a vital role in the stability of the product. If pre-chilled shelves are necessary, the initial shelf temperature for loading is typically 5°C. In rare cases, temperatures below 0°C have been used for extremely temperature sensitive drug products; however, at temperatures below freezing, frost and or ice will build up on the shelves as the door is opened and closed during the loading process. This can present a problem, especially for robotic loading systems, as vials have a tendency to “chatter” along the shelf and tip over during loading. The development scientist should talk to equipment engineers and operator to find out if shelf loading below freezing has caused problems in the past.

Once the shelves in the development dryer have reached set point, the vials may be loaded. Vial arrangement in cycle design is critical and cannot be overlooked during development. Figure 2 below shows a typical vial arrangement that has been used successfully in the past.

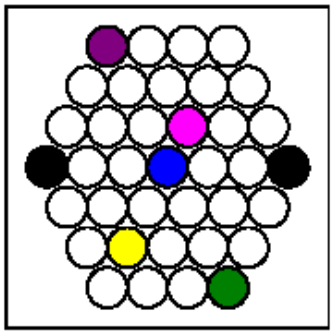

Figure 2

The vials are placed in this arrangement in order to account for the thermal barrier that occurs during freeze-drying, which results in a temperature gradient across the samples. Part of good cycle design includes understanding the temperature gradient that occurs and taking steps to account for it. Additionally, it is imperative that the vials remain in this configuration during the entire cycle. Development scale freeze-dryers tend to vibrate during processing, so steps must be taken to ensure that the vials don't drift apart. Personal experience has shown that the simple act of taping a thin strip of paper around the vials works exceptionally well in keeping the vials together. Using a large rubber band to keep the vials in position will work as well.

As shown in Figure 2, there are several circles (representing vials) that are colored, and represent vials that contain thermocouples. The goal here is to probe different locations across the cluster in order to determine the temperature gradient that will occur across the samples. The center vials represent the coldest spots while the outer vials represent the warmer spots during primary drying. The white circles represent product vials without thermocouples. Note that these vials could contain a placebo if the bulk drug substance if prohibitively expensive (mannitol at the same total solids content works well as a placebo).

Once the vials are loaded, they will be held until all of the thermocouple vials reach equilibrium. Note that the goal here is not to have each thermocouple reach shelf temperature (edge vials will almost always be slightly warmer than interior vials), but to simply come to equilibrium. Once equilibrium has been achieved, cooling to freezing temperatures may begin. Figure 3 below represents a typical profile for the initial temperature equilibration step and the freezing step. (Note that the colors representing the thermocouples in Figure 3 do not correspond to the colors shown in Figure 2.)

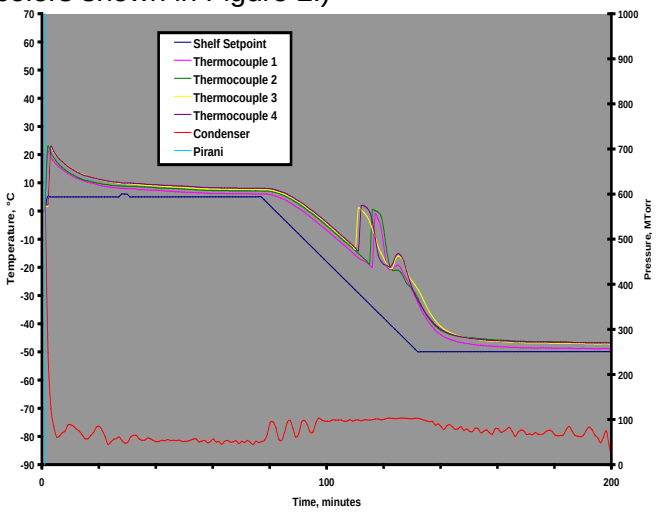

Figure 3

As shown in Figure 3, the shelf was pre-chilled to 5°C prior to sample loading and the vials were allowed to sit undisturbed for approximately 1.5 hours. Note that this is longer than what is needed; however, in these early studies, it is common to hold these samples longer than necessary to obtain additional data points, with the understanding that a reduction in the hold time can be made in future runs.

Also included in Figure 3 are shelf cooling and equilibration at freezing set point. For this particular run, shelf temperature set point was -45°C. As with the equilibration at 5°C, the equilibration at -45°C is extended in this initial run to accumulate additional data points.

The rate of shelf cooling chosen is based on several factors. The first issue being the limitation of the larger dryer where the new cycle will eventually be transferred. Do not design a cycle with faster rates than what can be achieved on the larger equipment. The second issue is based on

the product itself and how freezing rate translates into product stability. It is well understood that the freezing rate can affect product stability as the stresses associated with freezing can damage some sensitive molecules[1]. While it is out of the scope of this paper to go into detail on freezing induced damage, it is important for the development scientist to investigate the potential for freezing induced damage and super-cooling, and adjust shelf cooling rates accordingly.

The final shelf temperature set point for freezing should be based largely on the thermal characterization studies mentioned above. For example, if the thermal studies indicate that a Tg' occurs at -25°C, the product temperature (not necessarily the shelf temperature) should be taken below -25°C, or part of the product (interstitial space) will still be a fluid when primary drying starts. Unless a particular product has an unusually low critical temperature (Tg', Te, Tc), a shelf temperature of -45°C will ensure that the entire product is completely frozen prior to beginning primary drying.

Annealing

If an annealing step is needed, the shelves are warmed at the quickest heating rate until the product reaches the correct temperature determined from the thermal characterization studies. The holding time at annealing temperatures can generally be gauged by what was observed in the DSC annealing studies. If the results show that the metastable glassy phase is easily crystallized once annealing temperatures are reached, the development scientist can feel confident that all of the product vials are completely annealed after several hours of holding time (generally 2 to 3 hours). If the thermal results indicate difficulty in metastable glass crystallization, additional studies to investigate longer hold times will be required. Regardless, tests should be conducted after the fact to ensure proper crystallization of the metastable glass. High temperature DSC and x-ray powder diffraction (XRPD) are well suited to confirm proper and complete crystallization.

Once the product has been held at annealing temperature for the proper amount of time, the shelves are cooled back to the initial freezing temperature using the quickest possible rate. Proper time must be allowed for equilibrium to be reached again.

Primary Drying

Once freezing (and annealing if necessary) are complete, the vacuum pump is engaged, ice sublimation begins, and the cycle moves into the primary drying phase. Primary drying will continue until all of the pure ice, surrounding the interstitial space, is removed.

The two parameters that need to be determined in this phase of development are the proper shelf temperature and the proper vacuum level necessary to keep product temperature at the correct value determined from the thermal characterization studies. For example, if a thermal characterization study revealed that a Tg' occurs for a particular product at -10°C, the development scientist needs to ensure that every vial in the dryer must stay below -10°C during the entire primary drying phase or risk collapse.

It is well understood that product temperature during primary drying will rise over the course of drying due to increased resistance from the growing dried layer. As such, the development scientist must account for this and set the initial product temperature lower than the critical product temperature. As a rule of thumb, setting the initial product temperature 5°C to 7°C below the critical product temperature at the start of primary drying will ensure that the critical product temperature is not exceeded during the course of primary drying. For the example described above, the product temperature at the start of primary drying should be somewhere between -17°C to -15°C.

Once initial product temperature has been determined, the appropriate vacuum level must be determined. This is done by considering the vapor pressure of ice as a function of temperature.

Column 1 and 2 of Figure 4 show how ice vapor pressure is extremely affected (logarithmical) by temperature. This table highlights the need to be drying as closely as possible to the product's critical temperature, as the sublimation rate is maximized and the samples dry faster. If primary drying was conducted well below the critical temperature, it would increase the drying time exponentially.

Ice Temp, °C	Pi of Ice (µm)	25% of Pi (µm)
-45	54	14
-40	96	24
-35	168	42
-30	286	72
-25	476	119
-20	776	194
-15	1241	310
-10	1950	488
-5	3013	753

Figure 4

Primary drying (ice sublimation) will only continue if the vapor pressure of ice does not reach equilibrium in the product chamber. This is done by keeping a pressure gradient between the ice at the sublimation front in the vials and the surface of the condenser. Reducing the chamber pressure below the vapor pressure of ice will ensure the water vapor will flow from the product to the condenser. According to published literature reports, keeping the chamber pressure between 10 to 30% of the vapor pressure of ice will be sufficient to drive sublimation sufficiently[2]. Column 3 in Figure 4 shows the 25% value of the vapor pressure of ice as a function of temperature. These are the values of pressure that would need to be maintained in the chamber to keep sublimation going during primary drying. For the example described above, which assumes a product temperature of -15°C at the start of primary drying, the vacuum set point should be approximately 300 µm. Once the vacuum level achieves set point, according to the capacitance monometer vacuum gauge, the shelf temperature is then incrementally increased. The shelf temperature is increased until the product temperature (according to the warmest thermocouple), reads -15°C for this particular example. As observed in Figure 5, a stepwise increase in shelf temperature is used until the correct product temperature is achieved.

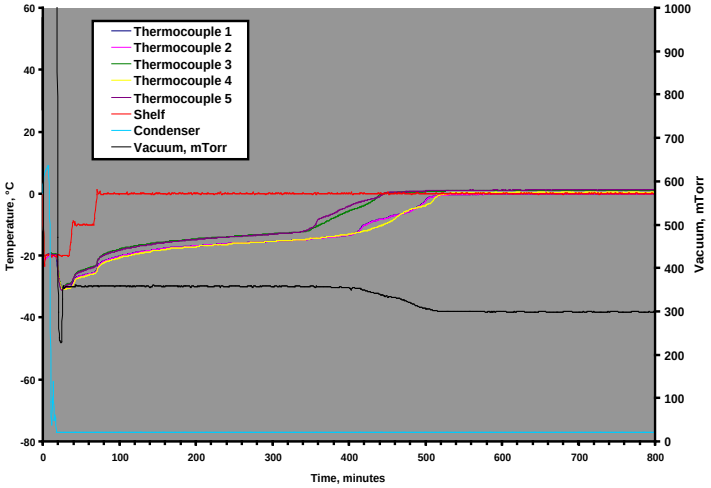

Figure 5

In this particular example, a shelf temperature of 0°C and a chamber pressure of 300 mTorr will result in a product temperature of approximately -15°C at the start of primary drying. Note that the vacuum gauge (Pirani gauge) is reading higher than set point. The Pirani gauge is thrown off set point by water vapor; however, this can be used to an advantage as described below.

At this point, the samples must be re-prepared and the dryer reloaded in the same arrangement as described above. The equilibration and freezing protocols determined previously will be run and then the vacuum level and shelf temperature will be set to 300 µm and 0°C, respectively. These conditions will be held and the data monitored. Specifically, the development scientist should be looking at the thermocouple and the Pirani data. As primary drying completes, the sublimation front passes the tip of the thermocouple (a rise in product temperature will be observed), and the

Pirani gauge will reach set point. Figure 6 shows a cycle printout that highlights the thermocouple and Pirani gauge data indicating the completion of primary drying.

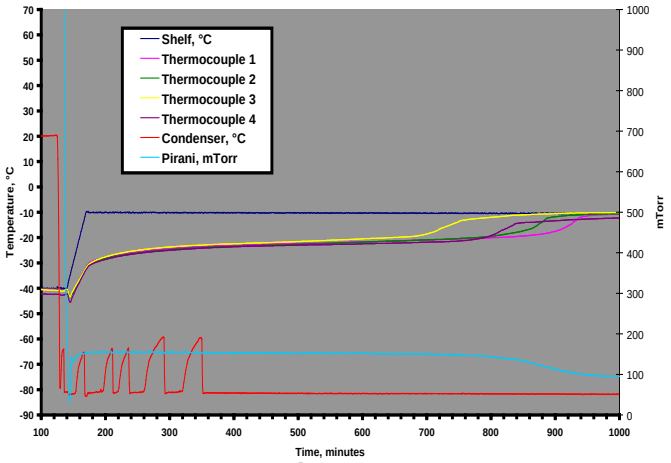

Figure 6

Also of interest in Figure 6 are the different times that the thermocouple vials are completing primary drying. The reason for this time difference is due to the location of the vial within the cluster. The center vials are more shielded and will dry slower while the edge vials see a warmer environment and will dry faster. This phenomenon is termed the edge effect, and highlights the importance of keeping the vials together tightly during cycle development and probing various locations with thermocouples.

Secondary Drying

The function of secondary drying is to drive off unfrozen water that is either loosely associated with crystal surfaces or buried within a glassy phase. As in primary drying, the thermal characterization data proves invaluable in secondary drying cycle development. If the thermal data shows that the system is entirely crystalline, it is known that the samples are already 99% percent dry at the end of primary drying since all of the water was pushed out into the ice channels when the interstitial space formed the crystalline lattice. As such, ramp rates are as fast as allowed by the production equipment. In the case of amorphous or partially amorphous systems, unfrozen water is embedded inside the glassy phase, and has to diffuse to the surface before it can be vaporized and removed from the product. Care must be taken as warming too quickly may exceed the Tg of the solid and result in collapse.

The secondary drying product temperature should be based on the thermal stability data obtained from the initial data review. If the thermal stability of the active ingredient does not exceed 25°C, it would be wise to only take the product to -10°C or -15°C during secondary drying to prevent degradation.

The length of the secondary drying phase will depend on the desired residual moisture content. Typical manufacturers strive to reduce the residual moisture to less than 1%; however certain biologically based formulations require slightly higher water contents for adequate stability. Stability studies will need to be done to confirm this. The most efficient means to determine secondary drying time is with the use of a sample thief. This device allows samples to be removed from the freeze-dryer and tested for moisture content without interrupting the cycle. A typical testing routine would include removing and testing a sample at the completion of primary drying, after the shelf temperature reaches set point, and after 1, 2, 4, and 6 hours (longer times may be necessary depending on the moisture content). In addition to conducting residual moisture testing, a purity test should be conducted to ensure that secondary drying is not causing degradation to the product.

Confirmation Run

At the end of cycle design, a confirmation run should be conducted to ensure that the new cycle produces acceptable product and runs flawlessly. A full dryer (or at least one full shelf) should be processed using placebo in most of the vials if necessary. The active vials should be 100% inspected for any physical defects (collapse, meltback, shrinkage, etc), and a statistically acceptable amount should be tested to ensure they meet all of the release specifications.

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References

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2. S Rambhatla and MJ Pikal, "Heat and Mass Transfer Issues in Freeze-Drying Process Development", Lyophilization of Biopharmaceuticals. American Association of Pharmaceutical Scientists Press, 2004.

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