Myth Busting: Misconceptions in Lyophilization

Dr Andrew Bright Ph. D,
Biopharma Group, UK

Freeze-drying (lyophilization) is a means of stabilizing a perishable product and extending its shelf life and is frequently used in the pharmaceutical industry for drugs, vaccines, antibodies and other biological material. The freeze-drying process has several steps, each requiring careful optimization to maintain a product's quality and function after drying. With each product, optimization conditions may differ, and these may also change when scaling up to product manufacturing. Several assumptions based on previous knowledge or experience are often made but as with many techniques that have become well-developed over time, there remains a number of misconceptions that persist in this process.

Recently, Dr. Andrew Bright, Ph.D., Senior Scientist at Biopharma Group, UK presented a two-part series of webinars questioning ten assumptions that are made when developing the freeze-drying process for pharmaceutical products and how these may be counteracted. This tech note summarizes both webinars and includes a selection of questions from the Q&A sessions.

1. 'Less complex formulations are easier and cheaper to freeze-dry'

Using less formulation ingredients in a pharmaceutical product is thought to reduce time and cost of freeze-drying. Some excipients and stabilizers, such as cyclodextrins, can be more expensive than the active ingredient itself so omitting these can substantially reduce manufacturing costs. Simpler formulations can also increase the drying rate in the lyophilization process due to reduced impedance to sublimation from lower density of the dry layer and potentially lead to more rapid reconstitution.

However, a simple formulation is not always optimal as the active substance may need additional ingredients, such as cryo-/lyo- protectants or buffers to remain stable during the freeze-drying process. Additionally, these protectants and other ingredients can act as thermal stabilizers, increasing the critical temperature of the product in the frozen and dried state allowing for increased temperature to be utilized while freeze-drying, reducing the time required.

Some small molecules, may have different polymorphs or crystallization issues that are hard to control by freeze-drying alone so may need excipients to inhibit the crystallization process completely or influence the polymorph form. In some cases, the formation of a cohesive cake may not occur in the absence of excipients e.g. when the product is at a low concentration, and therefore bulking agents maybe required.

2. 'There is little difference between using the glass transition temperature (T_g') or the collapse temperature (T_c) of a product to measure the critical formulation temperature'

Although the T_g' and T_c of a product can occur at the same temperature, they are not measuring the same parameters – the T_c values have been reported to be many times higher than the corresponding T_g' values for some formulations.

T_g' is the temperature at which an amorphous frozen system changes from a brittle to a flexible state, where as the T_c is based on the temperature at which the viscosity of the product decreases to a point at which it can no longer support itself and loses structure (Figure 1). For this reason, it is standard practice to determine the

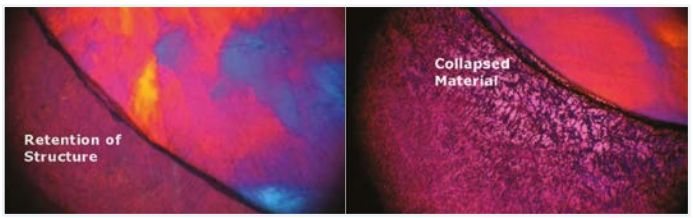

Figure 1: *Observation of collapse using FDM*

T_c value at the onset of collapse by Freeze-Drying Microscopy (FDM, LyoStat). Depending on how the T_g' is determined and who is evaluating it, T_g' is usually based on the effect of the phase transition rather than the transition itself, creating considerable variability in results. The most common method to ascertain T_g' is Differential Scanning Calorimetry (DSC) which relies on detecting the change in heat capacity accompanying the glass transition. Other methods examine changes in mechanical properties

(Dynamic Mechanical Analysis and Thermal Mechanical Analysis) or softening events (Atomic Force Microscopy). Biopharma Group has developed their own instrument (Lyotherm) that can measure T_g' by combining Differential Thermal Analysis (DTA) and impedance analysis to identify electrical and thermal changes within a sample. This enables a more complete picture of the thermal and physical characteristics.

Freeze-drying most products successfully requires that the product temperature is maintained at least below its T_c, or in some cases where the product maybe highly unstable below its T_g', as increasing the molecular mobility can potentially increase the rate of degradation, eventually leading to loss of cake structure.

3. 'An iterative development process is not necessary for cycle development'

An iterative process involves data gathered from one cycle to refine the subsequent one. This step wise approach helps identify issues early and prioritize what to focus on in subsequent cycles which will reduce the risk of product failure. For example, if the product passes all testing but drying is not efficient, it might be worth increasing the product temperature in primary drying next time. Although part of a Quality by Design approach, this iterative concept does not use the Design of Experiment (DoE) idea which would have completed many runs before obtaining data on drying efficiency.

Using Process Analytical Tools (PAT) and SMART™ technology can increase the information and data about product at each cycle. This can help increase the understanding of the process and enable robustness to be built into the developmental stages for better scaling up of the process.

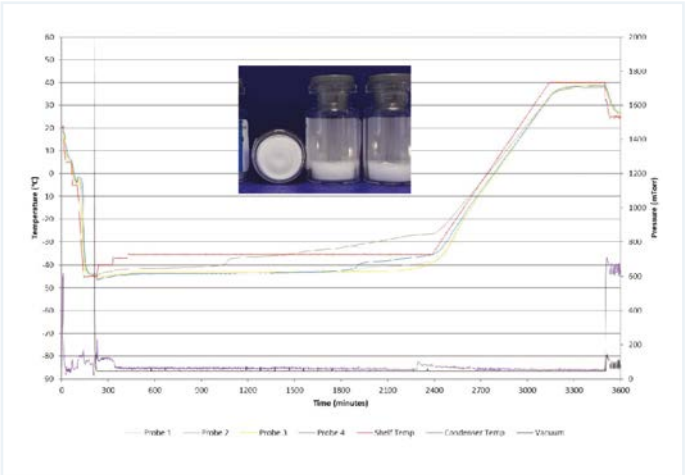

Figure 2: *Case Study: SMART software with low T_c*

In a case study of a product with mixed amorphous and crystalline components and surfactant, a conventional lyophilization cycle lasted 120 hours with primary drying of 90 hours. This often gave variable product quality, appearance and moisture content. With the use of SMART software, the cycle was analyzed to optimize shelf temperature and pressure. In the following cycle the primary drying time was reduced to 37 hours (67% shorter) and the total cycle time to 60 hours (59% shorter) (Figure 2).

4. 'Everyone uses +20°C and low pressure for secondary drying so these must be the best conditions'

Typically, secondary drying is performed at +20°C with a lower pressure than in primary drying, however studies have demonstrated that the rate of secondary drying is more influenced by temperature than pressure. Some proteins cannot withstand temperatures of +20°C; they will aggregate at these conditions and therefore may need a lower temperature to maintain their stability. In addition, low pressure may not be required as higher pressures during secondary drying can create more gas collisions and better heat transfer in a product.

A more scientific approach is needed to determine the optimal temperature and pressure to be applied during secondary drying which establishes the relationship between moisture levels after primary drying (and during secondary drying) and the dry state T_g. This can be done experimentally or calculated mathematically.

5. 'The lower the temperature of the condenser surface, the better'

It is thought that colder condenser temperatures equate to faster freeze-drying by removing the water from the product faster. However, it has been demonstrated that it is the difference in vapor pressure between product and condenser that increases the speed of the drying process, not the condenser temperature alone. As the condenser temperature is reduced, the vapor pressure also reduces so that the difference in temperature is not significant enough to drive the process.

It is also worth noting that unnecessarily cold condensers will increase the cost and complexity of equipment and the running costs.

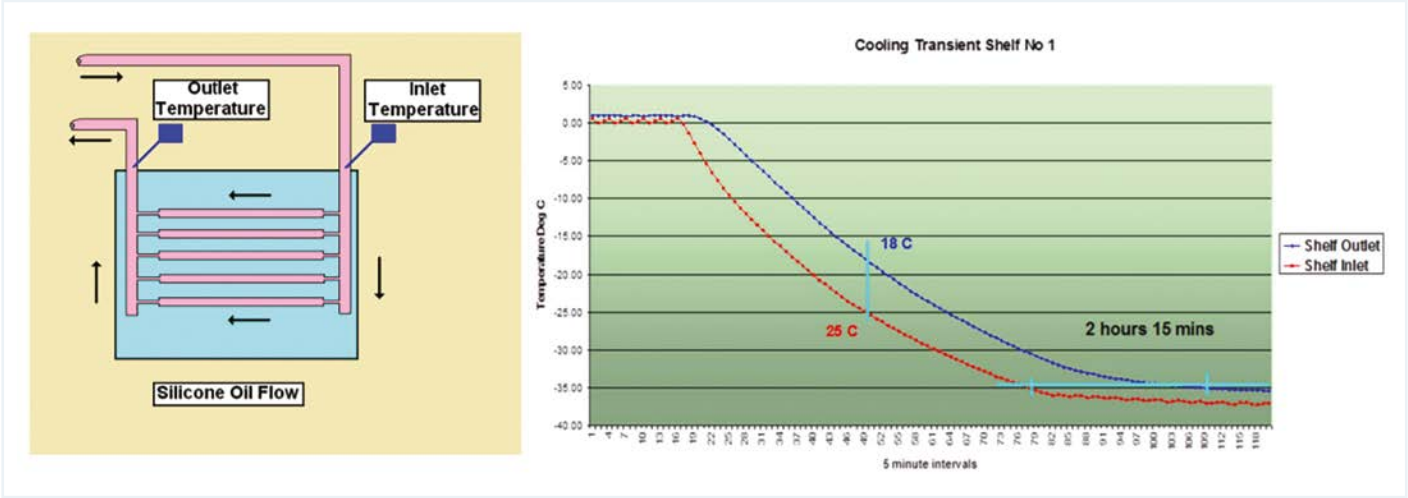

Figure 3: *Differences in temperature between shelf inlet and outlet*

6. 'The shelf temperature is a measure of the surface of the shelf during the freeze-drying process'

The shelf temperature of a freeze dryer is not a direct measurement of the temperature at the surface of the shelf. The actual measurement is based on the temperature of the inlet fluid. When comparing this to the outlet temperature, there is often a big difference of several degrees (Figure 3). The temperature of the shelf itself will therefore differ across the surface during the drying cycle although each shelf in the dryer should behave similarly to each other.

7. 'Monitoring product temperature using conventional probes will represent product temperature in all vials'

Conventional probes placed in a vial during freeze-drying may affect the product temperature so that the recorded measurement may not represent all other vials in the same batch. There are several factors that may affect these differing results. Placing a probe in different positions within a vial may alter measurements from within the vial. A probe placed at the center and bottom of a vial will optimize the length of time data can be obtained suggesting this position should be used in all experiments. The presence of the probe itself can increase the size of pores in the ice and reduce cake resistance, increasing the sublimation rate and produce a greater cooling effect. This could result in the recording of lower temperatures than other vials in the same batch. The presence of the probe can also form a nucleation site where ice crystals will form changing the crystal structure and therefore differing from the surrounding vials.

These issues associated with conventional probes can be alleviated using non-invasive instrument such as LyoFLux, Tunable Diode Laser Absorption Spectroscopy (TDLAS) from SP that monitors vapor flow and use of this data to calculate the batch average product temperature. Another option is Manometric Temperature Measurement (MTM) which forms part of the SMART software mentioned earlier. The MTM calculates the mean dry front temperature from a rapid pressure rise test and various known product batch parameters.

8. 'Shelf and product temperatures optimized for small scale freeze drying can be used when scaling up the product manufacturing process'

It seems obvious that the shelf temperatures optimized in small-scale studies should be scalable for larger product manufacturing but there are several effects that should be considered. The performance of the freeze dryer itself could affect the scalability of the conditions, for example some freeze dryers may struggle with lower temperatures due to the capabilities of the condenser or the differential temperatures between inlet and outlet may differ more widely between freeze dryers. Different load sizes and volumes may also change the optimal parameters that are required during the drying process.

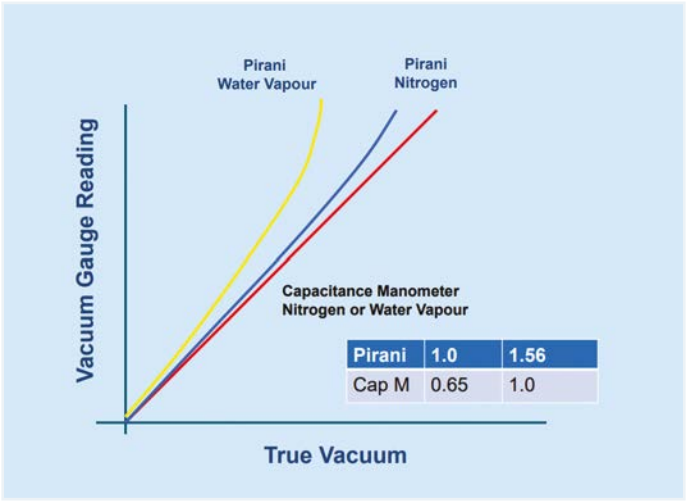

Figure 4a: *Pirani v Capacitance Manometer*

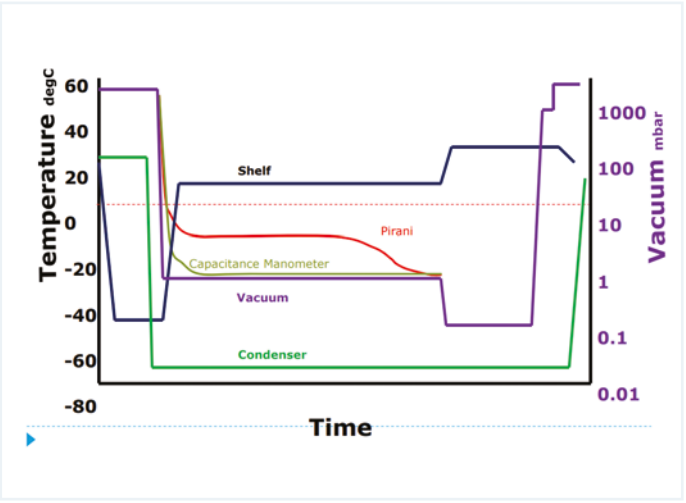

Figure 4b: *Pirani v Capacitance Manometer*

Product temperature is also not necessarily a truly scalable parameter. Temperature is simply the net difference between heat in and heat out and these parameters can be affected by impurities in sample causing more nucleation points and ultimately leading to changes in drying times and sublimation rates. This could be an issue if you are scaling up from R&D where there may be more impurities in a sample than in the subsequent stages of development.

9. 'Any measurement of pressure can be used when scaling up product development'

Pressure is dependent on the position and type of pressure gauge. A capacitance manometer (e.g. MKS) provides a direct measurement of pressure inside the freeze dryer. It is tolerant to small changes and unaffected by different gases. This contrasts with the thermocouple gauge (e.g. Pirani) which will deviate from capacitance and is affected by water vapor (Figure 4a and 4b). The end point of the primary drying stage can be determined by observing when the capacitance and thermocouple gauge measurements meet, i.e. in the absence of any remaining water vapor that causes deviation in the thermocouple gauge measurement.

10. 'Repeatability is the same as robustness'

Demonstrating repeatable results several times does not necessarily mean the process is reproducible enough to cope with any changes in any single input parameter or robust enough to cope with unintended internal changes, e.g. temperature excursions or external factors.

A robust freeze-drying process will be able to produce a quality product even when there are small deviations in critical parameters. Determining optimal design space parameters into cycle development will benefit the robustness of the set up and maintain batch consistency.

Conclusion

Successful freeze-drying requires a balance of several factors which can be complex to manage and time consuming to optimize. Over the years, more experience has been gained and advanced technology has enabled more detailed analysis to be captured. This has led to the questioning of several assumptions, some of which have been mentioned in this webinar series and many have also been discussed in a dedicated booklet 'Misconceptions in Freeze-Drying' written by Biopharma Group.

To view the full webinar and download the slides, please go to the archived webinars on our website ...
https://www.spscientific.com/Webinars/Archives/

Q&A Session

1. **How to determine if it is micro-collapse or product characteristics, and does micro-collapse have any impact during long term stability?**

   Micro-collapse usually occurs in freeze-dried products which contain both a crystalline phase and an amorphous phase, and is due to the amorphous phase undergoing collapse where the crystalline phase retains its structure. The freeze-dried material which has undergone micro-collapse when observed by the naked eye (macroscopic) maybe appear to be elegant but will have evidence of collapse at the microscopic scale. If micro-collapse is observed, it would be recommended to reduce the temperature for primary drying, or reformulation to mitigate the defect.

   Micro-collapse can be observed by the use of Freeze-Drying Microscopy (FDM, LyoStat). It can have an impact on long term stability as the amorphous phase has exceeded its collapse temperature and therefore has increased molecular mobility. This in turn can increase the rate of degradation during the freeze drying process, and can have an impact on the long term stability of the product.

2. **What would be your conclusion if the Pirani and CM Gauge reading don't quite meet (small difference observed)?**

   If trying to use the Pirani and CM gauge reading to determine the end point of primary drying, the point at which ice sublimation is complete, it is best to observe the Pirani and CM gauge over time. Either the Pirani and CM gauge meet as this will indicate little, or no water is being released by sublimation. In some cases, the reading will not meet and this can be due to the a calibration error, or a small amount of water vapor is being released from the product by desorption during primary drying conditions which will be registered by the Pirani. In this scenario, it would be best to observe the Pirani and CM gauge and once the Pirani has started to decrease, or undergone a step change to determine when the two readings are running in parallel for a period of time.

3. **When using an annealing process, how do you determine the optimum time/temperature to ensure it is complete?**

   Typical annealing temperatures are usually 10°C above the T_g', and should be held there until the product temperature matches the shelf temperature, and then held for a further two hours. Typical hold times are between 3 to 5 hours but for larger volumes the hold time required should be increased and determine experimentally.