Application of Thermal Analysis in Lyophilization Development and Optimization

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Lyophilization (or freeze-drying) is a process that removes the water from a product in order to impart higher stability, broader temperature tolerance, and longer shelf-life to pharmaceutical formulations that are unstable in aqueous solution. Lyophilization works by freezing the material and then reducing the surrounding pressure and adding enough heat to allow the frozen water in the material to sublime directly from the solid phase to the gas phase. While the demand for lyophilization is growing due to the introduction of new products, specifically biologics such as monoclonal antibodies (Mabs) and recombinant proteins, there are also several disadvantages to lyophilization. The principle disadvantages of lyophilization are: high capital cost of equipment (about three times more than traditional sterile solution or sterile dry powder fill technology); high energy costs (two to three times more than conventional methods); and long process time (typically a minimum of 24-hours per drying cycle). However, it should be pointed out that for many products, including the vast majority of high-value proteins, peptides, and vaccines, lyophilization is the only way to deliver stable, biologically active products with a commercially viable shelf-life. With the growing number of drugs in development requiring lyophilization, finding methods to shorten lyophilization development and ensuring optimization of lyophilization cycles can significantly impact the time and cost to develop and produce lyophilized product. Thermal analysis is a technique used to accelerate lyophilization development and optimization and through which lyophilization becomes a logical, time-sensitive, and cost-effective option for pharmaceutical and biotech companies.

Thermal analysis is used to study the properties of materials as a function of temperature. With respect to lyophilization, thermal analysis can be utilized to determine the critical thermal properties of a substance including glass transition (Tg'), eutectic melt, and collapse temperatures. Knowledge of these parameters is vital for the development of robust and efficient lyophilization cycles that consistently produce pharmaceutically elegant lyophilized cakes. Two primary methods of thermal analysis testing are Freeze-Dry Microscopy (FDM) and Differential Scanning Calorimetry (DSC). FDM and DSC use micro-quantities of product to evaluate the physical properties of a formulation under a variety of thermal conditions. FDM is a technique that is performed using highly specialized microscopy equipment. A miniature-scale lyophilization cycle is performed on the microscope stage; amorphous and/or crystalline phases of frozen formulations can be identified as well as temperatures at which the various phases of the lyophilization cycle occur, including the critical collapse temperature of the formulation. DSC is a technique that measures heat flow as a function of temperature applied to a sample across a thermal profile (either a heating or a cooling curve). The endothermic or exothermic profiles that result correlate to critical glass transition and eutectic melt temperatures of the product. The observed Tg' is the highest allowable product temperature during primary drying that will result in a lyophilized cake without experiencing collapse. Lowering the primary drying temperature results in a less
efficient lyophilization cycle and may increase the overall cycle length. In combination, these techniques provide information upon which to base the initial lyophilization pilot studies or optimization of an existing lyophilization cycle. Several case studies are presented below.

**Case Study 1: Thermal Analysis During the Preclinical Phase for a Contract Manufacturing Company and their Client**

Our client was collaborating with another firm in early development of their new preclinical product’s lyophilized process. They needed to ascertain the critical design parameters to conduct lyophilization cycle development for engineering and then clinical batches. As is often the case during preclinical periods, they were under significant time, monetary and drug substance supply constraint. As part of the thermal analysis, we needed to determine the critical thermal properties of the formulation, including glass transition or eutectic melting temperature, and/or collapse temperature by differential scanning calorimetry (DSC) and freeze dry microscopy (FDM). Two thermal events were identified in the DSC thermograms - a glass transition at -43.3°C and the onset of the eutectic melt at -3.34°C. FDM confirmed that the glass transition at -43.3°C is the limiting thermal event for lyophilization cycle development.

In less than 1 week, BioConvergence scientists developed the thermal analysis profile required to set the initial primary drying conditions (temperature and pressure) for the new product formulation based on the most critical temperature. Ultimately, our client conserved their scarce drug substance, reduced time spent in lyophilization process development, and maintained their aggressive timeline for initial clinical supply.

**Case Study 2: Thermal Analysis During the Clinical Phase for a Small Biotechnology Company**

Our client’s new lyophilized drug product successfully completed early clinical testing and their production date for Phase 3 clinical supplies was set. However, Phase 2 study results established that their product required five times the dose given at one third the frequency used for Phase 2 studies. To provide clinical supplies, they needed a new presentation capable of delivering
the higher dose. But there was a potential glitch - they had conflicting development history data and theories regarding the formation of crystalline mannitol during lyophilization and its role in solubility, reconstitution and product stability. Early studies indicated a relationship between lyophilized plug dimensions relative to vial dimensions and crystalline mannitol formation. However, the product formulation, lyophilization cycle, and vial presentation had remained the same since preclinical development and cGMP clinical production had delivered acceptable product quality, reconstitution, and stability results. Therefore, no additional development studies had been performed to prove or disprove the theory regarding crystalline mannitol formation. To develop a new larger dose presentation, they needed to understand the intersection of these key product design parameters for their current formulation and presentation.

The first step was to conduct studies on the current product to determine the presence and level of crystalline mannitol. The client provided 6 vials from each of 9 product lots currently undergoing ICH stability. BioConvergence development scientists used 2 vials each to perform thermal analysis by differential scanning calorimetry (DSC), freeze dry microscopy (FDM), moisture analysis by Karl Fischer (KF), and reconstitution studies. Due to the presence of mannitol and known issues with drug substance crystallization, attention focused on identifying evidence of increased crystallization.

Thermal analysis studies, analyzing both solids and solutions, produced no observations of significant endothermic events near 160°C during DSC analysis (i.e. typical melt range for mannitol of 164-169°C). Thermal analysis by DSC provides a viable method for detecting crystalline mannitol melt in the final product even at concentrations as low as 1%. No significant crystalline mannitol formation was found in existing product lots.

DSC endotherms identified 2 significant thermal events with a glass transition temperature of -24.4°C and ice melt at -4°C, and FDM results of the collapse temperature onset at -24.4°C/111mTorr and full collapse at -20.3°C/111mTorr. Engineering and clinical product batches were produced using multiple drug substance lots, at various scales, in different lyophilizers and by several contract manufacturers. All the batches yielded similar thermal, moisture, and reconstitution profiles. Thermal analysis by DSC and FDM results provided the necessary design data for primary drying temperature and vacuum settings for the new larger product presentation.
In less than 2 weeks, BioConvergence development scientists reviewed and assessed historical lyophilization development data and production records, developed DSC methodology to quantify and definitively address crystalline mannitol formation theory, and developed the thermal analysis profile required to set the initial primary drying conditions for the new larger presentation. The ultimate result was that our client was able to proceed to the next step in lyophilization development on schedule.

Case Study 3: Thermal Analysis During the Commercial Phase for a Large Global Life Sciences Company

Our client’s lyophilized protein diagnostic product had been successfully launched and sales exceeded expectations. Therefore, they were facing the decision whether to expand their lyophilization capacity (and by how much) to meet demand. However, they suspected their formulation and lyophilization cycle had not been optimized due a low collapse temperature of -48°C and lower than expected yields during commercial production. They sought a collaborative partner who could assess their current lyophilization cycle and recommend meaningful (rather than small incremental) changes. They wanted to minimize formulation changes, reduce cycle length, increase yields and improve quality. Because the partner would be suggesting changes to a top selling product, they realized choosing a reputable partner was key to making their case for improvement to internal stakeholders.

As an initial step of what was to become a much larger lyophilization cycle development project, thermal analysis by differential scanning calorimetry (DSC) and freeze dry microscopy (FDM) were performed on the current formulation to determine the critical thermal temperatures and pressures. These data were then compared to the existing lyophilization cycle parameters.

DSC thermograms produced using two methods failed to identify critical temperatures – neither glass transition nor eutectic melt. Working collaboratively with the client, it was theorized that the unusually low protein concentration of <10 mg/mL might make it difficult to detect a low-energy thermal event, such as a glass transition, using DSC. FDM results were unique as well due to the low protein concentration and the resulting freeze-dried product was lacking in structure and substance and exhibited a very thin and spongy nature. However, the onset of collapse was found to be -48°C/37mTorr and complete collapse occurred at -40.5°C/37mTorr. Other noteworthy findings were: A change in the morphology of the freeze-dried layer was observed in addition to the presence of bubbles at the sublimation front. The phenomena indicate the melt of at least one component of the solution and speculation that the physical changes were indicative of a glass transition not detectable by DSC. At full collapse, there was no longer any observable structure to the freeze-dried layer. A large temperature range between the onset of and full collapse is unusual. It is possible the lack of significant structure and/or the
presence of multiple proteins in the formulation may have contributed to a complex collapse process.

Thermal analysis results indicated that a lyophilization cycle: Should ensure the product is frozen to at least -60°C to make certain the ice and interstitial space are solid. That product temperature at the start of primary drying should be approximately -53°C. That a chamber pressure of less than 10 mTorr should be maintained to ensure pressure differential between the vapor pressure of ice at this temperature and the vapor pressure at the condenser.

The initial client lyophilization cycle, which consumed 60+ hours in use, was not optimized. Their dilute formulated solution, low fill volume, and low collapse temperatures of the product resulted in critical temperatures that are not readily achieved by commercial lyophilizers. This resulted in low freezing and primary drying temperatures, and vapor pressures that resulted in a low sublimation rate. These are the drivers for the very long and difficult to control freeze dry cycle and low product yield. BioConvergence suggested the client change their formulation by adding a bulking agent that would yield a discernable cake structure during freeze-drying and increase the collapse temperature to a level achieved by most commercial lyophilizers.

The development scientists worked collaboratively to incorporate the client’s significant knowledge and limitations of the multi-protein product and BioConvergence’s significant knowledge regarding lyophilized product formulation and process development. Ultimately, BioConvergence performed additional development and created a product with improved quality and stability with a lyophilization cycle of less than 30 hours (or put another way, a 50% reduction). The client is performing engineering runs in their commercial lyophilizers and assuming success, will transition to the new product formulation and associated lyophilization process. When fully implemented, our client will save more than $100,000 per batch in processing costs and effectively double the output produced by each lyophilizer.

Thermal analysis has been demonstrated to be a useful tool for determining the critical thermal properties of a drug product and establishing preliminary parameters for lyophilization. FDM and DSC are relatively inexpensive to run and require only small
amounts of formulated material; yet they provide valuable data for determining initial or optimized lyophilization cycle parameters. It is expected that over the next couple of years, 40% of New Molecular Entities are expected to require lyophilization.\textsuperscript{1} With tight budgets, costs for adding lyophilization capacity, and a competitive market, thermal analysis testing can provide great benefits for pharmaceutical and biotech companies, with relatively minimal risk in terms of cost, time, and materials.

\textsuperscript{1} “Streamlining Lyophilization Process from the Start,” Pharma Manufacturing.com.