Lyophilization, or freeze-drying, is a crucial process technology for many parenteral drugs, and an essential part of extending product shelf life. Although lyo is fairly straightforward at the lab level, at the facility level it can be costly, complex, and labor-intensive. With a flood of protein-based therapeutics and other injectable products in the drug development pipeline—it is expected that 40% of approved new molecular entities (NME’s) in the next five years will require lyophilization—there is a real need for leaner, more efficient freeze-drying methods [1].

One thing manufacturers must do is to gain better control over their freeze-drying process conditions, starting with formulation activities. Even in formulation, lyophilization is typically done on large machines, which only adds to the time and cost of lyophilization throughout development and commercialization. To minimize cost and streamline this process, therefore, many manufacturers are finding it in their best interest to optimize their lyophilization cycles by looking at pre-lyophilization methods such as freeze-dry microscopy.

Freeze-dry microscopy focuses on the direct examination of freezing and freeze-drying via a special microscope and thermal stage. It allows formulators to determine how their products will react in varying thermal conditions using small samples, instead of wasting large quantities of products by freezing them at less than optimal temperatures. This article will briefly describe how the technique is performed, the essential equipment required, and finally will show how one company has successfully used it.

**Traditional Lyo**

Freeze-drying typically has three stages: freezing, primary freezing, and secondary freezing. During freezing, material is cooled below its eutectic point, the lowest temperature at which the solid and liquid phases of the material can coexist. Then, the product goes through primary freezing, the main step in the lyophilization process, which involves removing water from the frozen product. This is primarily done via sublimation. During this phase, temperature is critical—if too much heat is added,
the material's structure could be altered and spoiled. Finally, any unfrozen water molecules are removed during secondary drying and the product is sealed.

Freeze-dry microscopy, as part of a complete thermal characterization study, is critical in developing a scientifically based, optimized lyophilization cycle. Understanding the thermal properties of a particular formulation prior to starting pilot studies in a develop-scale freeze-dryer offers several advantages over the trial and error approach that has traditionally been used for cycle design. The first piece of vital information obtained is the identification of amorphous, metastable, and or crystalline phases in the frozen system. This information is important in that these different phases solidify and freeze-dry very differently, and certain steps in the freeze-drying cycle must be carefully designed during development in order to prevent the physical loss of structure.

The next piece of vital information obtained from thermal characterization is the identification of the various critical temperatures associated with the different phases. As formulations get more complex and specialized, so do the associated critical temperatures. When studying the frozen system, the critical temperatures that are routinely encountered are the glass transition temperature (Tg) and the eutectic melting temperature (Te). These temperatures, for the most part, identify the maximum product temperature that can be achieved during primary drying without risking structural loss of the product. Freeze-dry microscopy is especially well suited for these studies since the user is able to visually observe the physical loss of the structure as a function of temperature.

Finally, by understanding the thermal properties of the product, the development scientist is more prepared to identify, diagnose, and correct a problematic formulation and or cycle. Product or process failures routinely result in physical damage to the physical structure of the cake (meltback, collapse, shrinkage, cracking, lensing, etc). By understanding the physical characteristics of the product, through thermal characterization, and on the type of structural damage, the development scientist is in a better position to diagnose and correct the problem.

**Critical Temperature Regulation**

Every formulation has a definitive critical temperature, a point after which the formulation experiences processing defects and may be unusable. For this reason, knowing the critical temperature of a formulation before lyophilization takes place is essential. By using a microscope and thermal stage, researchers can determine optimal lyophilization conditions using less time and product.

There are three kinds of critical temperatures: eutectic temperature (Te),
Figure 1. Tc viewed via a polarized light microscope with a thermal stage. The collapse that occurs at -36.0°C is readily visualized.

The glass-transition temperature is typically followed by the collapse temperature (Tc) as the sample is warmed. Tc is best measured by freeze-dry microscopy. Most formulations exist in an amorphous state, and the critical temperature for freeze-drying will be their collapse temperature.

This is the temperature at which the formulated product weakens to the point of not being able to support its own structure, leading to incomplete drying, inadequate stability in re-constitution and poor product appearance. Tc is best viewed via a polarized light microscope and thermal stage (Figure 1).

Setting Up a Freeze-Dry Microscopy Lab

Necessary components for the standard freeze-dry microscopy lab include a polarized-light microscope, a liquid-nitrogen-cooled thermal stage, a vacuum pump and an imaging system. The optimal polarized-light microscope system includes a strong light source, preferably 12V/100W, a Bertrand lens, proper working distance objectives, and polarizer/analyzer. Polarized-light microscopy allows researchers to visualize critical temperature. It also allows them to determine if their sample is crystalline or partially crystalline based on the birefringence of anisotropic crystals within the frozen matrix. Figure 2 shows the dendritic ice morphology under crossed polars.

Equally important is the thermal stage (Figure 3). An ideal thermal stage possesses these key capabilities:
• Temperature range of -196°C to 125°C
• Temperature stability less than 0.1°C
• Temperature accuracy of 0.01°C
• X-Y sample manipulation functionality
• Vacuum tight sample chamber to 10-3 mbar
• Silver heating block (ensuring high thermal conductivity)

With these components, formulators can determine the critical temperature before lyophilization begins, saving themselves product and profit losses associated with trial and error freeze dry attempts. Formulators can predict how their products will react under different thermal conditions and pinpoint the critical temperature so they can get lyophilization right the first time.

**Praxair: A Case Study**

Praxair, Inc. is a global Fortune 300 company that supplies atmospheric, process and specialty gases. Two years ago, one of the company’s facilities started using freeze-dry microscopy to determine primary drying temperatures on small samples of product for lyophilization cycle optimization and to predict substance stability and reaction during R&D. Praxair scientists typically work with samples of product from Pharma and Biopharma to determine lyophilization parameters like freeze rates, shelf and product temperatures and sublimation rates. Determining these factors enables Praxair to optimize their processes to attain ideal moisture levels and shelf life for lyophilized products.

Praxair began using freeze-dry microscopy to minimize the amount of product used for determining critical temperatures and lyophilization cycle optimization for formulations.

“Although we use lyophilization literature and industry standards to work with formulations at the correct temperatures, every sample is different, and we sometimes lose product and have to do more trial and error work,” says Praxair biologist Lori Otten. “Also, some Pharma clients do not give outside labs complete formulation information until confidentiality agreements are completely worked out.”
Without key formulation information, Otten says she and her colleagues could be delayed in working on portions of product because they did not want to damage the sample.

Otten’s facility invested in a freeze-dry microscopy system to better support the key stages of the lifecycle of biologic and pharmaceutical products of their clients. “Freeze-dry microscopy helps us better capture the process conditions and understand our clients’ products, and therefore better meet their needs.”

“It’s a precise, simple procedure that gives you a precise temperature when you can run your cycles at,” adds Otten. “What took days to do, we now complete in an hour and use less product to do it.”

The Future of Formulation
Freeze-dry microscopy, as part of a complete thermal-analysis study, is an invaluable tool in the characterization of the thermal properties of any formulation. The process enables pharmaceutical companies to save a significant amount of time and money both in process development and in commercial manufacturing. Freeze dry-microscopy is an excellent research tool for cycle development from drug discovery through Phase II clinical trials.

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References