Microscopy

Freeze-dry microscopy improves pharmaceutical efficiency, cost and quality

Freeze drying, as a unit operation in the production of parenteral drugs, is becoming more prevalent because many of the new molecular entities coming out of discovery have less than two years of shelf life before they expire. Therefore, pharmaceutical formulators are using freeze drying (also known as lyophilization) to extend the shelf life of their drugs. However, formulators usually use large-scale machines to determine the right conditions for freeze-drying products and extending shelf life, which can makes freeze drying the most expensive and time-consuming step in the parenteral manufacturing process.

To minimize cost and wasted efforts, many companies developing new products find it in their best interest to optimize their lyophilization cycles by looking at other methods that get the job done, like freeze-dry microscopy. Freeze-dry microscopy is quickly becoming one of the most popular ways for pharmaceutical formulation labs around the world to efficiently and cost-effectively determine the optimal conditions of a formulation pre-lyophilization.

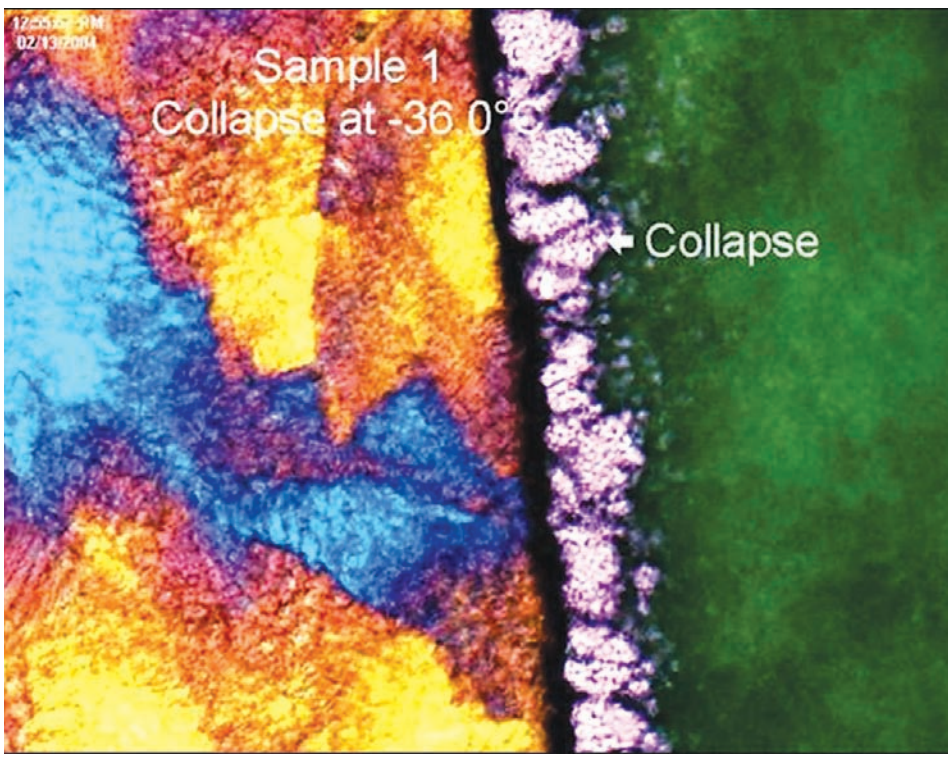

Figure 1 - T_c is best viewed via a polarized light microscope and thermal stage.

Freeze-dry microscopy

Freeze-dry microscopy focuses on the direct examination of freezing and freeze drying via a special microscope and thermal stage. The process's popularity stems from its ability to save pharmaceuticals more time, money and product than traditional trial and error techniques allow. Freeze-dry microscopy 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 it at less than optimal temperatures. We will begin our investigation of this practice by examining its technique followed by a careful inspection of the necessary equipment.

Traditional lyophilization process

Freeze-drying processes freeze the material and then reduce the surrounding pressure and add enough heat to allow the frozen water in the material to sublimate, or transition directly from the solid phase to gas phase. Freeze drying typically has three stages: freezing, primary freezing, and secondary freezing.

On larger-scale operations, typical in the pharmaceutical industry, freezing is usually done with a freeze-drying machine. 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 or the direct phase change from ice to vapour, without the product passing through the liquid state. During this phase, temperature is critical because, 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.

Critical-temperature regulation

Every formulation has a definitive critical temperature, after this point the formulation experiences processing defects during freeze drying and may be unusable. Maintaining temperature below the critical limits before the formulation goes through freeze-drying (or before the frozen water is removed) is imperative or the product can be ruined during the actual process, wasting time and money. 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 implement these same critical conditions on a macro scale, using less time and product to determine optimal pre-lyophilization conditions.

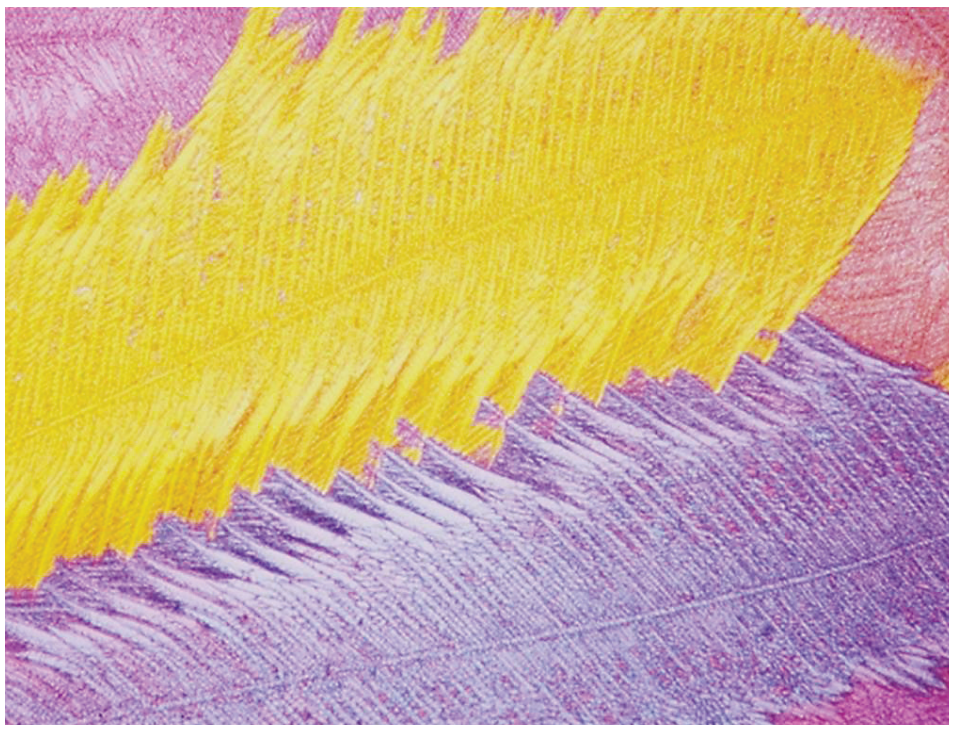

Figure 2- Dendritic ice morphology under crossed polars.

Figure 3 - An ideal thermal stage allows for numerous key capabilities.

Critical temperatures deciphered

Critical temperatures can be classified into three key areas, eutectic temperature (T_e), glass-transition temperature (T_g), and collapse temperature (T_c). T_e refers to crystalline systems and is measured by thermal or thermoelectric analyses such as differential scanning calorimetry (DSC). Exceeding this temperature will cause the material to melt during processing. T_g refers to amorphous systems and is also measured by thermal or thermoelectric analyses. In summary, eutectic or glass-transition temperatures determine maximum temperature that the formulated product can withstand during primary drying without the loss of structure.

Glass-transition temperatures are typically followed by the collapse temperature (T_c) as the sample is warmed. T_c 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 difficulty in re-constitution and poor product appearance. T_c is best viewed via a polarized light microscope and thermal stage (Figure 1). Let's examine the necessary components to achieve a complete and functional freeze-dry microscopy lab.

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, vacuum pump and appropriate imaging system to archive experiments. When purchasing a polarized-light microscope some key components need to be included. The optimal system includes a strong light source, preferably 12V/100W, a Bertrand lens, proper working distance objectives, and polarizer/analyzer. Polarized-light microscopy allows the researcher to visualize critical temperature. It also allows them to determine if their sample is crystalline or partially crystalline due to the birefringence of anisotropic crystals within the frozen matrix (Figure 2- showing dendritic ice morphology under crossed polars).

Equally important is the thermal stage (Figure 3). An ideal thermal stage allows for 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 systems, formulators can determine critical temperature before lyophilization begins, saving themselves product and profit losses associated with trial and error attempts to freeze dry. Formulators can predict how their products will react under different thermal conditions, pinpoint critical temperature, and optimize the lyophilization processes by getting it right the first time.

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 in money both in process development and in commercial manufacturing.

By Jeffrey McGinn, vice president and director of instrument sales, McCrone Microscopes and Accessories, a division of The McCrone Group.

The McCrone Group's College of Microscopy teaches a practicum on freeze-dry technique and lyophilization cycle development and optimization. More information is available at www.collegeofmicroscopy.com.