providing cake appearance. Such excipients are usually expected to be in crystalline structure after lyophilization. Totally crystalline cakes are not commonly used for protein lyophilization, since the crystallization of all excipients will tend to remove any stabilizing effect of the excipients on the protein (10–12). For pharmaceutical protein products, a formulation termed "crystalline matrix" is widely used. In such a formulation, crystalline components are added at a relatively high level so that a crystalline matrix is formed for the amorphous components to collapse upon. In such a way, the crystalline component provides excellent cake appearance, good reconstitution characteristics, and ease in lyophilization. On the other hand, the amorphous component stabilizes proteins during processing and storage. Lyophilizing such a formulation allows partial collapse (also termed micro-collapse) without affecting cake appearance. As a result, the product can be lyophilized at a relatively higher product temperature if protein activity is not compromised. In this chapter, all of our discussion focuses on the crystalline matrix-type formulation.

A lyophilization process usually includes three steps. First, the aqueous solution that has been filled into containers (such as vials and trays) is frozen to lower than -40°C. Next, in the primary drying, the freeze-dryer chamber is evacuated and the shelf temperature is elevated to sublimate bulk water (also termed free water) out of the system. Finally, the shelf temperature is further increased to remove bound water by desorption. This is called secondary drying. The principles of the lyophilization process have been well described by Pikal (5) and Franks (1).

At Bayer Biotechnology, we usually develop a freeze-drying cycle in five steps. First, the formulation is characterized. Second, based on the understanding of the formulation, the process is optimized. Third, the range of the critical process parameters is found. Fourth, the process is scaled up and transferred to production. Fifth, the process at the production scale is validated and qualified. Here, we focus only on the formulation characterization.

In summary, in this chapter we will discuss the freeze-drying formulation characterization, which is the first and the most important step in developing a freeze-drying cycle. All of the characterization discussion will focus on a crystalline matrix type of formulation. In the discussion, we will follow the order given below:

- Utilize differential scanning calorimetry (DSC) to characterize the formulation for freezing and primary drying.
- Confirm DSC results with a freeze-drying microscope and determine the maximum allowable product temperature during the primary drying.
- Generate a water absorption/desorption curve for characterizing the secondary drying process.
- Conduct a moisture optimization study to determine target moisture content for developing secondary drying.
- Use MDSC to measure T_g to predict stability of the products.

UTILIZE DSC TO CHARACTERIZE FORMULATION

Basically, DSC measures heat flow as a function of temperature applied to a sample going through freezing, melting, crystallization, and glass transition. The thermal properties that can be measured by using DSC include eutectic crystallization