to determine the ideal surfactant and concentration as different proteins are best stabilized by different surfactants.

It has been well reported that proteins undergo structural changes during dehydration processes [4, 18]. The main driving force of this conformational change is the loss of polar water molecules, which make hydrophobic amino acid residues hide inside the folded native protein structure. When polar additives like sugars are added into the formulation, they can replace the water and maintain a polar environment. These "water replacement" sugars have been successfully used to maintain the native structure of proteins during lyophilization. The most effective water-replacing sugars are nonreducing sugars, like sucrose and trehalose. While water-replacing sugars have been used as bulking agents in many commercial formulations, they generally have lower collapse temperatures and can require longer drying times in the absence of crystalline bulking agents. When selecting a water-replacement sugar it is prudent to investigate other relevant aspects of the sugar, including the glass transition temperature in a frozen state and its stability at the targeted pH.

The diluent used for reconstitution of a lyophilized formulation is another factor to optimize during formulation development. The most common diluent is commercially available water for injection (WFI), while other commercially available diluents such as normal saline can be used. Although not ideal due to its reducing nature, 5% dextrose in water (D5W) can also been used as a diluent. Bacteriostatic WFI or bacteriostatic normal saline, both containing 0.9−1% benzyl alcohol as a preservative, have also been used as diluents. These diluents are often used when reconstituted products are susceptible to bacterial contamination. For example, a multidose formulation filled into a cartridge has been effectively used in pen injectors. However, whenever diluents containing preservative(s) are introduced to the formulation, additional compatibility studies are required to ensure protein compatibility. For some products requiring specific configurations, other custom diluents can also be recommended or packaged with the lyophilized vials.

Lyophilization Cycle Development

Once a good lyophilized formulation is developed with optimized excipients, it is important to develop an efficient drying cycle that can achieve a pharmaceutically elegant lyophilized cake with a desired moisture content capable of rapid reconstitution.

The first step to optimizing a lyophilization cycle is to characterize the frozen formulation with thermal analyses, e.g., subambient differential scanning or lyophilization microscopy, to better understand relevant physical changes that may occur in a frozen state. Crucial information can be obtained through these characterizations, such as glass transition temperature of the unfrozen fraction (T_g'), possibility of recrystallization upon annealing, the devitrification temperature, eutectic melting temperature, and freezing point depression. Based on these data, along with infor-