either frozen or refrigerated following lyophilization due to the poor thermal stability of the dried product [44]. Thus, the significance of each step in optimizing the product quality cannot be overlooked. Each stage of the lyophilization process is outlined below in greater detail.

Stage 1: Freezing

Destabilizing stresses associated with stage 1 of the lyophilization process may include degradation at the ice–water interface, increase in ionic strength or shifts in pH during the freeze-concentrate state, and potential cold denaturation and aggregation of the product during freezing.

The function of freezing is to convert water into ice and is usually carried below the T_g' or T_{eu} (eutectic temperature), or T_c (collapse temperature) associated with the formulation [33, 35]. The T_g' and Teu can be obtained using differential scanning calorimeter (DSC) while T_c measurements are obtained using a freeze-drying microscope. Ice crystal formation is impacted by super-cooling nucleation and the rate of freezing. Slower freezing rates result in larger ice crystal formation, while LN2 (liquid nitrogen) blast freezing will result in microcrystals being formed. Although larger ice crystal structures are generally easier to dry during primary drying due to their large pores, LVVs are usually blast frozen to minimize time in solution due to high liquid degradation rates. This results in the microcrystal structures and increased mass resistance during drying, in the end leading to more conservative and lengthy cycles to minimize cake collapse.

For subunit vaccines, an annealing step may add value to the process. Annealing allows the crystallization of a bulking agent (i.e., glycine, mannitol, etc.) and redistribution of ice crystals into a more homogenous cake morphology. Annealing may also help minimize the freezing inhomogeneities that could occur between a laboratory-, pilot-, and commercial-scale lyophilizer due to differences in particulates present since GMP manufacturing occurs in a low-particulate environment (Class 100) [45].

The reader is reminded that unwanted crystallization during storage could severely impact the stability of the product due to increased moisture content and corresponding lower T_g. Formulation selection, when utilizing a crystalline component, must be achieved in a way that allows successful crystallization during annealing [46]. This can be achieved by maintaining a ~2:1 ratio of crystalline to amorphous material [47]. Similarly, surfactants may be added to the formulations [40] due to the potential of the vaccine to degrade ice–water interfaces and also to prevent adsorption to glass surfaces, especially for subunit vaccines.

Stage 2: Primary Drying

The frozen water is sublimed in the primary drying step (i.e., lowering the chamber pressure and raising the shelf temperature). Primary drying optimization is often considered a challenging task due to that fact that the product temperature is indi-