Lyophilized formulations are common within the biopharmaceutical area, especially when associated with LVV. Selecting the optimized formulation either through DoE or empirically, as described above, needs to meet the GTPP and the necessary CQAs related to a lyophilized product (i.e., reconstitution time, moisture level, cake appearance, etc.) [32–34]. Additionally, a technology transfer protocol and a robust manufacturing process will need to be completed to ensure success of the program. The fundamentals and guidelines associated with lyophilization formulation development are described in greater detail below.

Lyophilized Formulation Development

When starting lyophilization development, the critical properties to understand include but are not limited to the glass transition temperature (T_g, T_g), collapse temperature (T_c), the phase behavior of the formulation (amorphous vs. crystalline), liquid degradation rates of the product, and overall yield and stability requirements [33, 34].

Given the fact that the lyophilization process generates a variety of stress on the vaccine product, the formulator must balance these to achieve an optimized product. Some of the common stresses include pH shifts during freezing, freezing method, and type of ice crystals formed during the freezing process, potential phase separation, and in certain instances cold denaturation of enveloped viruses in the freeze concentrated state [14, 16, 36, 37]. In addition to identifying the proper formulation to mitigate product stresses, the formulation scientist should also examine the actual lyophilization conditions to improve product success. Both the formulation and lyophilization cycle are inter-connected [32, 38–41]. Factors impacting formulation selection have been detailed above, while factors impacting lyophilization are described below. In general, lyophilization consists of three stages: freezing, primary drying, and secondary drying [42]. Each stage generates its own stress on the vaccine product and should be explored independently and in conjunction with the others. The reader is reminded that basic lyophilization consists of a drying chamber with a heat transfer fluid circulated through shelves, a condenser that is connected to the drying chamber through the spool piece, and a refrigeration/heating/vacuum system to control the temperature and pressure of the cabinet. During a scale-up process, environmental conditions associated with the formulation process, shelf temperature, radiation effect, chamber pressure, process time, and process monitoring capabilities could account for scale-up differences between laboratory-, pilot-, and commercial freeze-dryers [43]. Furthermore, it is recommended that the significance of these aspects must be studied early in program development to avoid delays in scale-up and technology transfer.

Furthermore, the highly labile nature of LVV renders freeze-drying a challenging task. An example is the respiratory syncytial virus (RSV) which has been shown to be highly thermolabile even when stored with excipients at refrigerated and frozen conditions. As a result, the lyophilization cycle will result in poor drying yields and stability profile with minimal process changes resulting in significant potency losses. Similarly, Zostavax® a live enveloped virus manufactured by Merck® is stored