collapse was suggested based on a drop in the thermocouple product temperature measurement data. However, the resulting product was still acceptable in terms of cake appearance and product quality. However, a further increase in the shelf temperature to 0 °C results in significant increase in product degradation, which is considered as the failure point for the product. The acceptable cake appearance from these cycles is mainly due to the presence of crystalline material in the cake, which was confirmed by the XRD data. Therefore, the incorporation of a crystalline bulking agent into a protein formulation can significantly increase the primary drying temperature without compromising the product quality. The final design space for primary drying was obtained as the combinations of conditions identified from theoretical modeling and the expanded conditions from experimental studies. This much wider design space (shelf temperature ranges from −25 to −5 °C) can build robustness into the process, facilitate a smooth scale-up, and provide a scientific basis for dealing with process deviations which are still within the design space.

The secondary drying temperature impact was also investigated. Temperature ranges from 30 to 40 °C were studied in terms of moisture level and other CQAs. A 30 °C cycle gave a slightly higher moisture level, while 40 °C secondary drying condition produced acceptable product quality even though there is a slight increase in protein degradation. Thus, the secondary drying is robust between temperature of 30 and 40 °C.

The shelf temperature cooling rate during freezing was also examined as it could impact the crystallization behavior of ice and bulking agent and thus affect the final product quality. Different rates between −0.1 and −0.5 °C/min were examined with other lyo parameters fixed. The cake appearance was not impacted; however, a larger increase in protein degradation was observed upon lyophilization with the fast freezing rate. Large number of smaller ice crystals can be formed at a fast freezing rate, which could cause a larger protein–ice interface and result in a higher degree of protein degradation. In addition to this, freezing rates above −0.5 °C may not be achievable at large scale largely due to limitations of the production dryer. In order to make any recommendation of freezing ramp rates, it is very important to use experimental data with nucleation temperature and/or SSA measurements, rather than the lyophilizer limitations alone.

Finally, robustness studies with the combinations of Lyo process temperature and chamber pressure were performed to assess product quality. Aggressive runs above the collapse temperature were used to generate the stability data and cycles with both primary and secondary drying temperature set points ±5 °C and pressures ranging from 50 to 200 mTorr were used to run beyond normal manufacturing conditions. The working cycle with manufacturing set points were used as a control, and all samples from these cycles were tested for long-term stability. It was found that the product quality attributes from these runs were well within the acceptable ranges upon storage, suggesting the process robustness. These stability data along with theoretical modeling are extremely useful to support cycle deviations from manufacturing lyophilizer.

These studies can form the basis for a thorough process and product understanding and support process validation. However, scale-down experiments may not