Part II

Common Drying Technologies

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Advances in Freeze Drying of Biologics and Future Challenges and Opportunities

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6.1 Introduction

Freeze drying, also termed lyophilization, has been utilized for desiccation and stabilization of compounds (pharmaceuticals and biopharmaceuticals) for several decades [1, 2]. One of the earliest references to freeze drying of labile compounds can be found in the context of serum and biologics in 1935 by Flosdorf and coworkers [3], which were subsequently followed by numerous publications of drying of plasma, viruses, bacterial cultures, etc. [4–10]. Several examples of the application of freeze drying of foods (fish, meat, potatoes, tofu, etc.) have been actually dated to several centuries earlier than drying of pharmaceuticals. Freeze drying is the most widely employed technique for drying of pharmaceuticals and represents the gold standard to which other drying technologies are compared. A number of reviews on freeze drying can be found in the literature for practitioners engaged in formulation, process development, and scale-up of pharmaceutical compounds [11–15]. The focus of this commentary is to provide a summary of the evolution and advances in pharmaceutical freeze drying, with an emphasis on the drying of biologics. We will review the approaches ("best practices") for the stabilization, tools for process monitoring and control, and methods for characterization of the physical matrix and the active compounds in the dried state and current challenges and future opportunities in freeze drying.

6.2 Where Are We Now?

At least 475 prescription medicines (new molecular entities and biologics) have been approved by the US FDA since 2000 [16]. Based on our analysis of drug products in development vs. commercially available (using PharmaCircle), over 650 marketed drug products are lyophilized. There are hundreds of biologics in development for several therapeutic indications that include monoclonal antibodies (mAbs), antibody drug conjugates (ADCs), enzymes, clotting factors, fusion proteins, and emerging modalities in the areas of cell and gene therapy. A significant fraction of biologics in development exhibit instability in aqueous

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environments, thereby presenting the need for the removal of water to improve their shelf life. Biologic drug substances and drug products are lyophilized in a variety of containers, including glass bottles, vials, dual-chamber systems (syringes, cartridges), and trays [2].

During freeze drying, water is converted to ice during the freezing step, ice is removed via sublimation during the primary drying step, and the unfrozen water is removed by desorption during the secondary drying step. Typically, freezing, primary drying, and secondary drying are conducted at different shelf temperatures. In addition, during the drying steps, the chamber pressure within the freeze dryer is controlled to a predetermined set point. There are also examples of lyophilization processes where both primary and secondary drying could be combined into a single step. While lyophilization is intended to preserve and stabilize a biologic, freezing and drying lead to dramatic alternations in the environment of a protein, thereby causing the development of a variety of stresses, which can compromise the pharmaceutical stability of the protein [17–20]. The stresses during freezing include low temperature, ice, and freeze concentration. The low temperature has been implicated in protein unfolding during cooling/freezing and is described as cold denaturation [21–25]. Ice formation leads to the greatest change in the physical environment and leads to concentration of the solutes, a process known as freeze concentration (or cryo-concentration). Freeze concentration, in turn, could lead to separation of the amorphous phases [26, 27] and/or crystallization of solutes from the cryo-concentrate [28–31]. Solute crystallization of components, specifically bulking agents such as mannitol or glycine, is intended and advantageous and utilized to reduce the duration of the drying [11, 15]. The unintended crystallization of formulation components such as buffer salts [32–34] or cryoprotectants [35, 36] can be damaging and are often encountered in the case of poorly formulated biologics. Ice crystallization presents a new interface (ice–freeze concentrate interface) and has been shown to cause protein unfolding [37, 38]. The overall effect of ice is governed by the degree of supercooling and the freezing rate, which in turn affect the size and number of ice crystals. The structure of the frozen matrix leaves its imprint on the dried matrix and determines the rate of drying [39]. Larger ice crystals are formed during slow cooling, which lead to larger pores on ice sublimation and facilitate faster drying. Fast cooling leads to the formation of smaller ice crystals and consequently smaller pores during primary drying and poses a greater resistance to water vapor transport during drying. In addition, the appearance of ice has been thought to also impose a mechanical stress on proteins [40, 41]. Removal of the unfrozen water during drying further stresses proteins and leads to additional degradation during drying [19].

6.3 Current State

6.3.1 Rational Formulation Design: Keeping It Simple

Over the years, several guidelines have been described for rational/systematic formulation development as opposed to trial-and-error (or empirical)

approaches [2, 11, 42]. A general approach is to include a buffer (*to maintain the pH*), a cryo-/lyoprotectant (*to ensure stabilization during freezing and drying*), and a surfactant (to protect from ice–aqueous interface) in a protein formulation and can be easily extended to a number of modalities ranging from mAbs to ADCs to vaccines. In such a scenario, the choice of components and composition should be such that the buffer salt and stabilizer do not crystallize during freezing and drying. Examples of formulation platforms based on a thorough understanding of factors causing protein destabilization (physical and chemical) as well as thermal and phase behavior of the formulation exist across the pharmaceutical industry, with the approach being to keep formulations "simple" and "lyophilization friendly." Poorly designed formulations can significantly extend the duration of drying from a couple of days to a week or longer. In addition, the use of trial-and-error or empirical approaches in formulation and/process development can lead to conservative and less efficient processes.

A buffer is included to control the pH during freeze drying and in the reconstituted solution. Commonly utilized buffer systems in biopharmaceuticals include glutamate, histidine, sodium citrate, sodium (or potassium) phosphate, succinate, and Tris buffers [43, 44]. Acetate-, carbonate-, or imidazole-based buffers are avoided due to their volatile nature. Since buffer crystallization during freezing is well documented in the literature for the more commonly utilized buffers such as phosphate and succinate buffers [32, 45, 46], an understanding of the phase behavior can be utilized in the design of the formulation such that the required buffer capacity and pH are maintained during processing. A recommended strategy is to utilize a high weight ratio of the non-crystallizable (that is, the cryo-/lyoprotectants such as sucrose and trehalose) formulation components to that of the buffer [11, 15].

Freeze-dried formulations of biologics containing a low solids content (1%) also include a bulking agent to increase the cake mass and to prevent blowout during drying. Bulking agents could be crystallizable such as glycine and mannitol, which crystallize during either freezing or subsequent annealing and provide a scaffold on which the amorphous formulation components can be dried above the critical temperature (collapse temperature) [1, 2]. Widely utilized bulking agents, one example being the disaccharide sucrose, remain amorphous during freeze drying. Bulking agents can confer stabilizing effects and prevent protein instability if they remain in the amorphous state. Amino acids (glycine), disaccharides (sucrose, trehalose), polyols (mannitol, sorbitol), and polymers (dextran, Ficoll, polyethylene glycol, polyvinylpyrrolidone) exhibit protection during freezing (cryoprotectant) and drying (lyoprotectant). Crystallization during processing can cause destabilization (by the removal of the stabilizer from the same phase as the protein) and has been observed in the case of trehalose [35, 36] and sorbitol [47, 48], which have been shown to crystallize during prolonged annealing or storage at temperatures above the glass transition temperature of the freeze concentrate (T'_g). Mannitol is not recommended as a stabilizer due to the risk of crystallization either during processing or on storage. The crystallization behavior of mannitol is influenced by a formulation composition (inclusion of salts, ratio of mannitol to non-crystallizable component) and processing conditions (rate of cooling, freezing temperature, annealing temperature and time).

In the context of cryoprotection by sugars such as sucrose, it appears that the extent of stabilization is dependent on the pre-freeze solution concentration of the sugar, with 0.2–0.5 M providing effective stabilization [1, 11, 49]. A weight ratio of the stabilizer to protein of at least 1 : 1 is recommended for ensuring good stability during drying and/or storage and ~5 : 1 for optimal stability. In a study on aggregation during storage of several protein (recombinant cytokines and fusion proteins, molecular weights between 19 and 185 kDa), the physical stability increased monotonically with an increase in the disaccharide concentration over a sugar to protein mass ratio (R) of 0.5 : 4 (or a molar ratio of 56 : 541). The authors also observed that when the sugar to protein weight ratio was ~1, the native structure retention and structural relaxation time (indicator of global mobility in the dried matrix) reached a maximum. Local mobility measurements based on the mean-square amplitude of motion using neutron backscattering correlated with aggregation rate constants at all the compositions [50, 51]. Sucrose and trehalose are widely utilized as stabilizers in protein formulations. While there are reports of no differences in protein stability in sucrose vs. trehalose formulations, there are also reports where sucrose-based formulations exhibited greater stability (by a factor of two) than in trehalose-containing formulations. Even though a greater global mobility was associated with the sucrose-based formulations than in trehalose formulations, a greater decrease in the fast dynamics (a measure of local mobility) was observed in the sucrose formulations when compared with the trehalose systems [52]. Local mobility may exhibit better coupling with stability during storage at temperatures far below the T_g. Similar effects on local mobility were observed in observed in protein–sugar systems containing small amounts of antiplasticizer (glycerol or sorbitol). It is hypothesized that the inclusion of glycerol decreased the free volume in the protein–sucrose glasses, limiting motion and thereby slowing down local mobility [53]. Similar observations have been documented in disaccharide glasses containing sorbitol [54]. The inclusion of plasticizers presents an additional option in the design of freeze-dried formulations.

Very often, other salts in addition to the buffer salts are included as formulation excipients to prevent precipitation of the protein or to reduce the viscosity of highly concentrated proteins. The overall effect of solubility and viscosity is dependent on the nature of the ionic species and the protein/protein interactions. Examples of salts included in the formulations include sodium chloride and arginine hydrochloride, among others [55, 56]. In addition, salts have been shown to decrease the collapse temperature [57, 58] and can also exacerbate the pH shifts in the event of buffer crystallization [32, 34]. While the inclusion of salts results in a formulation that could need conservative processing, there are approaches one could utilize to counter the reduction in the collapse temperature of salt-containing system. In such scenarios, it is advised to utilize a sucrose–mannitol combination such that sucrose provides the necessary stabilization during processing and storage, while mannitol can crystallize and enable drying at higher temperatures, thereby reducing processing times. Such a strategy based on the use of mannitol–sucrose combination at mannitol to sucrose ratios ranging from 2 to 4 ensures mannitol crystallization and can be of

practical benefit in the lyophilization of low protein concentration formulations (≤50 mg/ml) in the absence or presence of salts.

Since proteins are prone to adsorption and aggregation at interfaces (air–liquid, air–solid, ice–freeze concentrate), surfactants are included to ensure protein stability during formulation, fill, and finish operations as well as during freeze/thawing and reconstitution. Nonionic surfactants (fatty acid esters of sorbitan polyethoxylates such as Polysorbate 20 and 80) are commonly included at low concentrations (0.01–0.1% w/v, which is above the chemistry, manufacturing, and controls (CMCs) value) to prevent surface denaturation [59]. Polysorbates are prone to oxidative or hydrolytic degradation. Oxidative degradation in polysorbates can be mitigated by the inclusion of an antioxidant, chelator, or radical scavenger (ethylenediaminetetraacetic acid (EDTA) or methionine) [60, 61]. An alternate surfactant to polysorbate is poloxamer 188 (Pluronic F68), which is also approved for parenteral administration by the FDA [62]. We note that platform-based formulations may not always provide optimal stabilization for biologics such as cell-based and viral vector-based therapies and would require more extensive pre-formulation and formulation prescreening.

6.3.2 Process Design and Monitoring

6.3.2.1 Freezing

There is a common agreement that freezing step defines the properties of lyophilized material [2]. The temperature at which ice crystallization occurs (ice nucleation temperature), the rate of conversion of water into ice, formulation composition, and the ratio of stabilizer to the active ingredient dictate post-lyophilization stability of drug product. Due to the stochastic nature of ice nucleation, the thermal history of each individual vial may be different, leading to potential variability in product quality.

Annealing, as was demonstrated by Searles et al. [63], could minimize the differences in thermal history, increase the pore size (determined by the ice crystal size), and increase the sublimation rate [64]. During annealing, the product temperature is increased above the T'_g, thereby facilitating ice crystal growth (that is, increasing in the size of the larger ice crystals at the expense of smaller ice crystals, and the number of ice crystals, described as Ostwald ripening). The farther the product temperature from the glass transition temperature, the faster the annealing process. In the case of systems where the bulking agent is crystallizable, the eutectic melting (or secondary melting) temperature defines the upper limit for the annealing process. Thus, annealing may not be applicable to formulations exhibiting a high T'_g. Besides ice recrystallization (devitrification), annealing also promotes crystallization of the bulking agent(s) and significantly improves sublimation rates by enabling more aggressive drying [65]. An increase in the mobility during annealing could, however, induce phase separation, which may not be favorable for some products, limiting application of the annealing process [63, 66].

An alternative method of "erasing" the difference in structure due to ice nucleation variability is through the application of controlled ice nucleation. During controlled ice nucleation, product temperature is reduced below the melting

temperature of the formulation, and after a predetermined equilibration, ice nucleation is induced by an "ice fog" injection [39, 67–69], quick depressurization of the drying chamber post-pressurization [70], reduction of the chamber pressure [71, 72], or ultrasound [73–76]. Similar to annealing, controlled ice nucleation has been shown to reduce heterogeneity in drying rates across a shelf of vials, typically resulting in the reduction of primary drying duration.

There are differences in the ice structure that forms on freezing induced via controlled ice nucleation vs. annealing post uncontrolled/spontaneous freezing. During stochastic freezing, ice typically nucleates at the bottom of a vial, and the ice front propagates from the bottom toward the top of the solution. It is, therefore, not unusual to observe a "concentrated film/layer" at the surface of the lyophilized cake because of the freezing process suggesting phase separation. Annealing, performed after initial ice crystallization, could promote additional phase separation. It is not surprising, therefore, that the initial resistance of a cake generated from a process utilizing annealing is higher than that of the cake formed during stochastic freezing. During controlled nucleation, ice typically forms at the surface of the liquid material (top of the vial) and propagates toward the bottom of the vial, pushing freeze concentrate to the bottom of vial. Therefore, it is not unusual to observe a ring of more dense material at the bottom of the cake produced using controlled nucleation. One can expect some differences in the distribution of formulation components within the cakes produced using annealing vs. controlled ice nucleation methods. In theory, some differences in the stability behavior could be result of differences due to the freezing method. Therefore, both methods should be utilized during freezing process design to identify the most suitable method for the product.

To the best of the knowledge of the authors, annealing is more often employed during freezing than controlled ice nucleation. The reason for this is quite simple. The commercial dryer design does not need to be modified for implementation of annealing as long as the software allows inclusion of multiple steps for freezing. The implementation of controlled ice nucleation requires either modification of the dryer to enlarge the port for quick depressurization (in the case of pressurization/depressurization-induced freezing) or the purchase of a separate unit to generate a sterile ice fog. Controlled ice nucleation is available in laboratory and large-scale dryers available from most lyophilizer manufacturers. The potential benefits, challenges, and costs of the technology must be considered in determining the implementation of the technology. The biggest obstacle is the finding the "right product," which could not be manufactured without the use of controlled nucleation. Examples of such products exist: virus as an active ingredient in animal vaccines [77], whose survival could be only guaranteed by utilization of controlled nucleation (as opposed to annealing). The implementation of controlled nucleation for freeze drying of biologics appears to be limited in at least two scenarios. The first example is of products containing high potency drugs (low-concentration active pharmaceutical ingredient (API)) in a formulation consisting of a crystallizable bulking agent with a stabilizer and where annealing can be successfully utilized. Crystallization of the bulking agent creates a matrix that can withstand significantly higher temperatures (up to 40 °C during primary drying) without any negative impact on product quality.

Thus, faster drying is available without the need for modification to the dryer (as would be needed for controlled ice nucleation). The other scenario exists in the case of formulations containing high protein concentrations where aggressive drying can be utilized (typically above the T'_g) [78]. In this case, freeze drying above the T'_g or even above the microscopic collapse temperature could reduce drying heterogeneity without affecting the product quality. There appears to be a limited need for application of controlled ice nucleation for lyophilization of such products. Thus, due to the limited number of products that could significantly benefit from controlled nucleation, implementation costs, additional validation activities, and regulatory implications, implementation of controlled nucleation method has not progressed as fast as one would assume despite the obvious benefits of the technology. In the opinion of the authors, it may be another decade before controlled ice nucleation is routinely employed for commercial manufacturing.

6.3.2.2 Product Temperature Measurement

Product temperature measurement is likely the oldest and is most directly linked to product quality. Thermocouples and resistance temperature detectors (RTDs) were the preferred tools for the measurement of product temperature [79]. In the experience of the authors, RTDs were mostly employed for monitoring of bulk freeze drying (product filled in trays) since the measuring element is typically >3 mm and is not suitable for temperature measurement in small volumes. Thermocouples were more suited for vial-based freeze drying. Both types of sensors are easy to calibrate and provide important information on process status (product temperature) at the point of contact. Since measurements are limited to the location of temperature sensors, the use of multiple sensors at critical locations (identified from the experience, for example, corners and center of the shelf) is a typical strategy for successful process control. If the sensors are positioned accurately, they provide information on product temperature relative to the critical temperature and also enable detection of the end point of freezing and primary drying. Product temperature sensors in combination with batch monitoring systems (discussed below) are powerful tools for the process control. While they are useful tools in process monitoring and control, the misuse of temperature sensors, due to wrong placement, could lead to variability in temperature measurement, which could complicate operator's decision on the state of process. Temperature sensors are invasive and influence the drying behavior in vial being monitored [64] since they could serve as nucleation sites. This results in the nucleation of ice at higher temperature in sensor-containing vials during the freezing ramp, lowest cake resistance, and shorter drying duration when compared with most vials (non-sensor-containing vials). Controlled nucleation or annealing could minimize the differences in product resistance, but sensor-containing vials may still not be representative due to the additional heat received by product from the sensors (encountered to a greater degree in the edge vials). During commercial manufacturing, product temperature placement is typically utilized in only edge vials due to sterility concerns. Since edge vials are not representative of the entire batch (due to the additional radiant heat received by the product from the chamber walls and door), the determination of the primary drying

end point could be challenging. For these reasons, temperature measurement devices were abandoned for the most commercial processes, especially when automatic loading systems were introduced into manufacturing. Wireless sensors, especially those that do not introduce heat into the product (for example, Tempris® probes) [79], could reintroduce direct temperature measurement as a valuable tool for the process monitoring. Since product temperature history is directly linked to product quality, temperature sensors could serve as "true" process analytical technology (PAT) tools. Miniaturization of wireless sensors, addition of robotic systems for sensor placement in commercial lines, and modification of existing control systems allowing process decision making based on sensor feedback in combination could increase the probability of implementation of temperature measurement into routine commercial processes.

6.3.2.3 Pressure Rise Test/Manometric Temperature Measurement

The pressure rise test (PRT) is a batch monitoring processes that has been well known for decades, but is also a test whose potential was never completely utilized. PRT is performed, both during primary and secondary drying, by the closing valve between the chamber and condenser and monitoring the resulting chamber pressure increase. The shape of the pressure increase curve could provide very valuable information about the product as well as the process: the average batch product temperature at the sublimation surface, vial heat transfer coefficient, cake resistance, sublimation rate, and end point of primary drying [80]. Manometric temperature measurement (MTM) was one of the first PAT tools for process control at the time of its introduction in 1997. The lack of reliability of fast response valves (that is, fast or quickly closing valves) limited the application of MTM for process control. MTM, however, is still considered a valuable method for the detection of end points of primary and secondary drying. In the opinion of the authors, multiple factors such as availability of software, experience of drug product manufacturers with PRT, and availability of hardware (which is typically built into the design of modern freeze dryers) will help maintain PRT as a valuable option for the process control.

6.3.2.4 SMART Freeze-Dryer™ Technology

The introduction of the SMART™ freeze dryer was a revolutionary step in freeze-drying process control [81]. It provided an inexperienced operator the tool(s) to conduct close to optimal freeze drying. The starting point of process design using SMART technology is providing information about the product (solids content, critical product temperature, nature of product [amorphous or crystalline]), container closure (internal and external vial diameters), and number of vials and initiating the process without providing the exact set points for process parameters. Based on the product properties, the software selects or identifies an appropriate freezing process by introducing an annealing step, if needed, and maintaining the product below the T_g' during the vacuum initiation step. During primary drying, by periodically closing the valve between the chamber and condenser for a short duration coupled with application of the MTM method, the product temperature at the sublimation surface is estimated, and cycle parameters are adjusted to maintain product below the

critical temperature. By integrating the sublimation rate, estimated at each point of MTM test, the software predicts the end point of primary drying and moves process from primary to secondary drying. During secondary drying, the program continues testing the status of drying by comparing the increase in the pressure during PRT to a predetermined value, which is linked to target residual water content of product. Once the estimated water content is less than the target water content, the software moves process to the final holding step. Thus, there is no interaction between the operator and freeze dryer during entire process. In theory, each step of the process could be the optimal as long as the input parameter (such as pressure increase during testing) is accurate. In reality, it has been reported that [82] the predictions of sublimation rate and product temperature are not accurate after completion of approx. 50% of primary drying, especially for high solids content formulations. In the experience of the authors, product temperature predictions become less reliable after the completion of approx. about 65% of primary drying and is accompanied with underestimated of primary drying durations for high protein concentration formulations. In addition, pressure increase during PRT results in a small (up to 1 °C) increase in the product temperature, which could be critical if the cycle is operating at the edge of failure. Again, fast-closing valves (necessary for the successful execution of PRT) are still not available for the commercial dryers. A combination of the multiple factors listed above (*less accurate sublimation rate and product temperature predictions, pressure increased coupled with product temperature increase, and unavailability of fast-closing valves at commercial scale*) may be responsible for the lack of implementation of the SMART technology for commercial manufacturing. In addition, the current "rule of thumb" (or do we say, requirements?) for lyophilization process description in the regulatory submissions and batch records does not provide the flexibility that is built into the SMART approach.

6.3.2.5 Application of Pirani Gauge for the Control of Primary Drying

In the past, Pirani and similar thermal conductivity-type gauges were popular for pressure monitoring and process control in laboratory and commercial freeze dryers. While some of these gauges were stable to steam sterilization, the other failed after being subjected to only a few sterilization cycles [79]. Due to their sensitivity to gas composition in the dryer chamber, Pirani sensors were replaced by capacitance manometers (CM), which are currently the preferred pressure sensors for process control. The sensitivity of the Pirani gauge to water vapor is the basis for the "comparative" pressure measurement method. In this method, the difference between Pirani and CM readings is constantly compared and serves as a major input in determination of the drying end point. In theory, at the end of primary drying, when there is no water vapor in the drying chamber, the pressure difference should be zero at all pressure set points. This, in turn, could be utilized to advance to the secondary drying step. In reality, there is always an offset between Pirani gauge and CM sensor reading, which can be determined during an initial calibration in a dry and empty chamber. The midpoint of the Pirani decay curve has been identified as the point at which almost all primary drying could be considered complete [83]. The algorithm for the "comparative"

pressure method is well developed and tested, at least, on laboratory-scale dryers. The Pirani sensors are inexpensive and relatively robust, thereby providing a reliable tool for process control. In the opinion of the authors, the lack of flexibility in the description of lyophilization processes based on the regulatory submission requirements limits the use of comparative pressure measurement for routine commercial manufacturing. Since the primary drying duration in regulatory dossiers and batch records is typically fixed, any change in the primary drying duration is considered as a deviation.

6.3.2.6 Application of Mass Spectroscopy for Process Control

The benefits of application of mass spectroscopy (MS) to lyophilization process control have been known for at least a few decades [84]. It is an accurate method for the detection of gas composition in the drying chamber, which could be employed for the detection of end points of primary and secondary drying steps. However, the technology was not routinely used due to high cost of equipment and the footprint. Recent advances in miniaturization of mass spectrometers as well as the associated cost reduction have enabled the installation of mass spectrometers, or of the lesser expensive residual gas analyzers (RGAs), on commercial freeze dryers. To the author's knowledge, several RGAs are currently installed on large-scale dryers. The purpose of an RGA is to enable the detection of silicone oil leaks [85]. The authors believe that in the near future, RGAs will be routine utilized in freeze-drying process control. One application of RGAs is in the measurement of sublimation rate. In principle, if the molar ratios of gases in the chamber during primary drying are measured by an RGA, one could estimate the rate of generation of water molecules due to sublimation at a known flow rate of nitrogen (used for the pressure control). By estimating the sublimation rate, one could then calculate the average product temperature at the sublimation surface, which could then serve as an input parameter for process control. Unlike PRT/MTM, this method does not require periodic interruption of the process, and therefore, in theory, it could be more suitable for implementation in combination with the SMART technology.

6.3.2.7 Heat Flux Sensors as PAT Tools

Heat flux sensors (HFSs) have been recently proposed as PAT tools for lyophilization process control [79]. The sensors are attached to the shelf surface at certain locations and allow measurement of the vial heat transfer coefficient, product temperature, and sublimation rate without direct contact with the product. When placed at representative locations across the freeze dryer, the inclusion of HFSs presents the opportunity to control each step of lyophilization. For example, they could be employed for the detection of crystallization end point of excipients during freezing [86] or of the end points of primary and secondary drying (will require extremely sensitive of sensors). HFSs can also be utilized for the control of heat flux from the shelf to the product during freezing to manipulate the ice structure (and consequently specific surface area) to form larger pores. Linked to the programmable logic controller (PLC), information from all HFSs could serve as inputs into process control and potentially used for the development of the next generation of SMART technology. In spite of their

potential, it appears, to the best of our knowledge, that HFSs are currently not yet installed on any commercial dryers due to incompatibility with automatic loading systems. If HFSs can be embedded into the shelves at certain locations during shelf fabrication process (to minimize interference during operations), they could serve as powerful tools for process monitoring and control.

6.3.2.8 Pressure Decrease Method

Recently Pisano and coworkers [87] described a method for control of primary drying by periodically closing the controlled leakage valve and applying the algorithm for data extraction, termed as a pressure decrease test (PDT). During this test, the valve that controls chamber pressure by nitrogen injection is shut off for a short duration, resulting in a decrease in the chamber pressure. The PDT algorithm enables calculation of the vapor flow rate produced by sublimation, cake resistance, and vial heat transfer coefficient. A steady-state primary drying model is utilized to calculation of the product temperature. The decision to move the process to the next step is facilitated by these inputs. Thus, unlike the PRT/MTM method, a slight drop in the product temperature during PDT allows more effective execution of the process at the edge of failure. While the input parameters for the primary drying model generated from the PDT method are similar to those from the MTM method, it appears to be more easily implemented in commercial dryers. The leakage valve is fast closing and relatively cheap, and implementation of the method does not require dryer modification since it is currently an included feature in most modern dryers. Incorporation of the PDT method into SMART approach, in the author's opinion, appears to be more reliable when compared with the MTM method. The authors, however, are not aware of any successful commercial implementation of PDT to date.

6.3.2.9 Tunable Diode Laser Absorption Spectroscopy (TDLAS)

Tunable diode laser absorption spectroscopy (TDLAS) [88] is a near-IR (NIR)-based optical method for the measurement of mass flow rate during drying. The optical hardware is placed in the duct between the chamber and condenser with the laser beam positioned at a 45° angle to the direction of the flow. In the opinion of the authors, TDLAS is currently the only reliable noninvasive methodology that has been tested on large-scale dryers. However, it requires modification of existing freeze dryers in order to mount the hardware. TDLAS has been demonstrated to be very useful tool for calculation of the product temperature at the sublimation surface [89], vial heat transfer coefficients [90], and cake resistance [91]. Generation of these input parameters during an active process without any intervention and the subsequent application of the algorithm based on the steady-state primary drying model enable process control process in a similar manner to the SMART technology. The implementation of TDLAS into SMART will bring lyophilization process control to the next level.

6.3.2.10 Emerging Analytical Tools for Process Monitoring and Control

Raman and infrared (IR) spectroscopy have been utilized for monitoring of the entire freeze-drying process. While this approach provides important information on the state of excipients (phase behavior) [92, 93] or in estimation

of the product temperature without contact [94], these technologies are limited to observation of the edge vials, which are not representative of an entire batch. In the past decade, IR cameras have been integrated into freeze dryers to enable online process monitoring [95]. One must track the advances in these technologies, which in the future may provide options that could be applied in development of a new generation of process control.

6.3.2.11 Modeling of Freeze-Drying Process

When efficient process monitoring and control based on critical attribute such as product temperature are not available, modeling of the freeze-drying process could assist in design of optimal drying process. To the best of our knowledge, no reliable models exist for freezing in vials at laboratory and commercial scales. Robust primary drying models have existed at least three decades and are routinely utilized for process design, scale-up, and analyses of deviations [96]. The most accessible (user-friendly) secondary drying model was published recently [97] and can be easily implemented into the existing control systems (if input parameters are known). In the author's opinion, the use of modeling is essential for the process design. However, modeling efforts must be coupled with advanced PAT tools and advanced algorithms to design and transfer the most efficient lyophilization process.

6.3.3 Tools to Monitor Dried Products

Characterization of the formulation prior to and post freeze drying is critical for formulation and process development. The discussion on characterization here is limited to the dried state. Several tools are available to monitor the physical and chemical stability of the biologic along with the matrix into which the drug is lyophilized.

6.3.3.1 Structure of the Biologic

Chemical degradation of proteins during processing and on storage is determined using liquid chromatography-mass spectrometry (LC-MS) [98]. Physical degradation (non-covalent aggregation) is investigated using UV spectrometry, light scattering, size exclusion chromatography, analytical centrifugation, and IR spectroscopy. Fourier transform infra-red (FTIR) has been widely utilized to assess the secondary structure during storage for at least two decades [52, 99]. Raman spectroscopy is utilized to characterize the phase behavior of the matrix (containing crystallizable components) [100] as well was the secondary structure of proteins during storage [101, 102]. More recently, solid-state hydrogen–deuterium exchange mass spectrometry (ssHDX-MS) and solid-state photolytic labeling mass spectrometry (ssPL-MS) were employed for the characterization of formulation and process-induced conformational changes in lyophilized proteins [103, 104]. Deuterium uptake using ssHDX-MS of several mAb formulations determined post-lyophilization correlated with the physical (aggregation) and chemical instability storage. The authors of the study presented ssHDX-MS as promising approach to obtain an early readout of differences in long-term stability between several formulations and therefore

accelerate formulation screening and selection [105]. In the past several years, the range of modalities with the biologics portfolios has expanded with the introduction of cell and gene therapy-based formulations. The arrival of new modalities presents new challenges for formulation and analytical scientists to develop tools to monitor structural changes in cells and viral vectors in the dried state and the consequent impact on product properties.

6.3.3.2 Characterizing Matrix Contributions to Stability

The toolbox for the analytical characterization of the excipient matrix continues to expand. Calorimetric (*differential scanning calorimetry, isothermal microcalorimetry*), spectroscopic (*IR, Raman, frequency modulation spectroscopy for headspace water analysis*), diffractometric (*ambient and variable temperature X-ray diffractometric*), gravimetric (*dynamic vapor sorption*), chemical, and combinations thereof (X-ray diffractometry- differential scanning calorimetry (*XRD-DSC), thermogravimetry, DVS-Raman*) are routinely utilized for the characterization of product quality attributes (thermal, water content and uptake, phase behavior, etc.) [106]. Scanning electron microscopy, Brunauer-Emmett-Teller (BET)-based N2 or Kr sorption–desorption measurements, and mercury porosimetry enable assessment of drying behavior and product morphology. A number of recent publications on the use of X-ray CT illustrates the growing interest in understanding cake structure and morphology and their relationship with product properties (drying behavior, appearance, reconstitution) [107, 108]. Combination of data generated using specific tools (BET analysis of nitrogen or krypton adsorption isotherms + electron spectroscopy for chemical analysis, ESCA/X-ray photoelectron spectroscopy (XPS)) can also help resolve the contribution of the specific surface to the overall degradation in the dried state [109, 110].

While lyophilization provides a great improvement in the stability of proteins by reducing molecular motion in the dried state relative to that in solution, there is still sufficient molecular mobility (global and local, as a function of temperature, water content, formulation composition) for degradation to occur [111]. Consequently, characterization of molecular mobility has been a topic of great interest for at least three decades. Specifically, there have been efforts to understand if there exists coupling between dynamics (primary or α-relaxations vs. secondary or β-relaxations) in the amorphous glasses and pharmaceutical stability [112, 113]. Motions directly coupled with viscosity are described as global, primary, or α-relaxations where a structural relaxation time, τ, is utilized to describe the time constant for such motions. The reciprocal of the structural relaxation time provides an estimate of global mobility. Such relaxations slow greatly below the glass transition temperature. The stability of several proteins was determined above and below the glass transition temperature where optimal stability was observed far below the T_g. Co-lyophilization of proteins with a high-T_g glass-forming solute appeared to provide good stability, though in other cases it did not yield optimal stabilization [50]. Therefore, there is a consensus that a high T_g is not a sufficient predictor of molecular mobility [114]. Dynamics in glasses are governed by fragility, thermal history, and T_g. The difference between the T_g and the storage temperature is not directly related to molecular

mobility since a high-T_g formulation could exhibit higher mobility due to a difference in fragility. While α-relaxations greatly slow down at temperatures well below the T_g, molecular mobility is determined by secondary or β-relaxations, which represent fast dynamics. Local mobility characterized using neutron scattering [52, 112, 115] or solid-state nuclear magnetic resonance (NMR) [116, 117] has been implicated in degradation of human growth hormone (hGH), insulin, mAbs, and other proteins. Several techniques are employed to characterize the molecular mobility and include differential scanning calorimetry (DSC), dielectric analysis (DEA), thermally stimulated current (TSC), isothermal microcalorimetry (IMC), NMR, and neutron backscattering. In some of the cases, the limitation to the use of the techniques may either be due to lack of easy access or long measurement times (days). There have also been efforts to develop surrogate measurements to neutron backscattering such as low frequency Raman scattering [118], which appears to rank-order formulations and therefore distinguish between even slightly different formulations.

There are also other contributors to stability beyond molecular mobility and include phase separation [119, 120], effective solid-state pH (characterized via Hammett acidity function) [121, 122], and specific surface area [109, 110]. Traditionally DSC is utilized for detection of phase separation in polymer-based systems but is of limited use in protein-rich systems. Instead, Raman line mapping and solid-state NMR have been utilized to detect phase separation, where ssNMR has been successful at detecting phase separation in domain sizes smaller than the Raman line mapping capability [111]. A combination of ESCA and BET was utilized to evaluate dried proteins (mAbs and vaccines) covering a 30-fold range of specific surface areas, generated through foam drying, freeze drying, and spray drying [109, 123]. A strong correlation is observed between degradation rate and fraction of protein available at the surface when compared with surfactant-containing systems. In a subsequent study using hGH, aggregation on storage correlated with percentage protein at the surface in formulations containing sucrose, trehalose, or hydroxyethyl starch (HES) as stabilizers, suggesting that surface degradation accounted for significant fraction of total degradation [110]. One must exercise caution in interpreting stability data for freeze-dried products or those containing lower or higher specific surface areas (SSAs) (foam dried, spray dried, spray freeze dried, etc.) and consider the contribution of surface degradation and others to overall destabilization.

6.3.3.3 Looking Beyond the Biologic and the Formulation Matrix

In addition to monitoring and characterizing the drug product, the volume of the container above the drug product (headspace) is also monitored [124, 125]. Headspace analysis, based on laser absorption spectroscopy, is utilized for nondestructive measurement of gases (oxygen, carbon dioxide, water vapor) during container-closure/packaging development, during stability study, and for in-line testing during manufacturing. In addition, foreign particles (examples: metal, glass) are detected via X-ray-based analysis and aid in the identification of non-formulation components/contamination during visual inspection.

6.4 Current Challenges

6.4.1 Understanding Protein Degradation in the Frozen State and Dried States

The physical environment of a protein undergoes dramatic changes leading to the development of several stresses that affect protein stability. The three key stresses include low/cold temperature, ice formation, and freeze concentration [18]. Freeze concentration, in turn, could facilitate second-order reactions, crystallization of solute (buffer or cryo-/lyoprotectants), phase separation (amorphous–amorphous separation), and redistribution of solutes. The formation of ice leads to the development of a large ice–aqueous interfacial area. Proteins have also been shown to adsorb and unfold at the ice–freeze concentrate interface.

The formation and growth of ice crystals could also lead to entrapment of the solution (freeze concentrate) phase, which is dependent on the conditions of freezing (geometry of the crystallization front, progression rate of the ice–freeze concentrate interface, and macroscopic viscosity) [126]. A concentration gradient at the ice–freeze concentrate interface as opposed to the bulk environment could create heterogeneity in composition and could have implications on protein stability. One could invoke ice-induced solution entrapment as an alternate mechanism for protein degradation. Entrapment of the freeze concentrate could cause partitioning of proteins into a quasi-liquid layer (QLL) on the ice crystal surface (also described as a liquid-like layer below the ice melting temperature) as opposed to the bulk freeze concentrate. The microenvironment experienced by the proteins in the QLL (could be different w.r.t. local acidity) when compared to the bulk freeze concentrate. While entrapment of solutes by ice has been investigated in binary and ternary systems using optical refractometry, FTIR, and confocal Raman microscopy, its effect on protein degradation is unknown. Typical protein formulations are multicomponent systems containing protein, buffer salt, cryoprotectant, and surfactant. The evolution of the freeze concentrate and consequently the spatial heterogeneity would be much more complex than in the "simpler" binary and ternary systems. Lastly, the phase transformation of water to ice also leads to a volume expansion and presents the potential role of mechanical stresses and elevated local pressure in protein destabilization. Additional ice crystallization (also described as devitrification) occurs during warming above the T_g' of the frozen solution. Thus, proteins could experience a dual stress imposed by ice formation during freezing and warming. We note that only limited information exists on the contribution of mechanical stresses to protein degradation during freeze/thawing [40, 41]. Considering the diversity of biologics modalities that formulation scientists are currently encountering (mAbs, fusion proteins, cell and gene therapy-based therapeutics), there are numerous future opportunities to improve our understanding of the freezing stresses on protein stability.

Addition of excipients such as disaccharides provides stabilization in the frozen state (cryoprotection) as well during and post-drying (lyoprotection). Components such as surfactants minimize degradation during freezing and reconstitution. Chemical stabilization is achieved through appropriate selection

of pH, buffer, and antioxidant. Some formulation components can also provide protection from physical and chemical degradation. In the context of degradation and stabilization in the dried state, thermodynamic and/or kinetic stabilization mechanisms are invoked. The "water substitute hypothesis" is a thermodynamic stabilization mechanism, and the "glass dynamics hypothesis" offers kinetic stabilization [114]. Several commentaries are available invoking either mechanism or making the case for thermodynamic vs. kinetic stabilization [54, 112, 115, 127–130]. It has been argued that stability data can be interpreted via either mechanism and remains a hot topic for investigators. It has been pointed out that the lack of agreement on stabilization mechanism(s) could be attributed to either incomplete analyses of the stability data or to inadequate characterization tools. The use of H/D exchange, solid-state tryptophan fluorescence spectroscopy, low frequency Raman scattering, and solid-state NMR may help resolve contributions to degradation and stabilization. Furthermore, in several cases, experiments may not be appropriately designed to investigate the contributions of thermodynamic vs. kinetic stabilization where both mechanisms could be in play [114]. The identification of other contributors to instability such as degradation at the solid–air interface points to the complex nature of solid-state protein degradation/stabilization.

6.4.2 Process Inefficiency

In organizations engaged in the manufacture of a large number of lyophilized products, there may be very often limited capacity for bringing new products onboard. In such cases, optimization of legacy processes, which are often empirical, inefficient, and suboptimal, becomes a critical activity. The key reason for this inefficiency is likely due to utilization of conservative approaches (an accepted tradition in the past and sometimes even in the current times) to develop robust process. In such conservative approaches, the product temperature during drying was typically maintained below the T'_g, and soak time during primary drying was of extended duration to account for any unexpected process variations, thereby resulting in long process durations. Freeze drying above collapse temperature (microscopic or visual) was mostly uncommon. Recent advances in freeze drying above the collapse temperature [65, 131–134] suggest that formulations (of both biologics and small molecules) can be freeze-dried well above the T'_g, microscopic collapse temperature, and even above visual collapse temperature with retention of quality during the shelf life. The key benefit of freeze drying above the collapse temperature is the short drying duration, which in turn helps bring down operational and utility costs and creates additional manufacturing capacity.

We provide a few critical activities to ensure design of efficient processes:

(i) Determine maximum allowable product temperature (not always collapse temperature) drying above which could affect long-term stability and other critical quality attribute (CQAs) (such as reconstitution time).
(ii) Determine freeze-dryer capabilities (minimum controllable pressure and maximum sublimation rate) as a part of equipment qualification.

(iii) Implement reliable PAT tools to make timely decision on the process (freezing, primary drying, and secondary drying end-point determination).
The outputs of these activities when combined with modeling enables the design of the most efficient cycle, possibly executed at the edge failure. The risk of failure, however, could be reduced by implementing the best PAT tools available to date (TDLAS, Tempris, PRT, comparative pressure measurement, mass spectrometry, etc.). As discussed earlier, poorly designed formulations, at least in our view, is a key contributor for the design inefficient drying processes. Formulations with $T'_g < -40\,^{\circ}C$ were not that uncommon in the past. Even the best efforts and tools for process optimization would yield relatively long freeze-drying processes. In such cases, reformulation based on best freeze-drying practices may be the best option for operational efficiency.

6.5 Vision for the Future

While lyophilization remains the gold standard for the preservation of biopharmaceuticals, it must continue to evolve and innovate to provide process monitoring and control options and support the needs presented by new modalities.

6.5.1 Advances in Container-Closure Systems

The majority of current container systems, used for lyophilization, are glass vials, typically type 1. They exhibit a low extractable and leachable profile and are relatively inexpensive. It appears that fogging (or hazing) behavior could be associated with less expensive glass vials and was eliminated with the use of (more expensive) vials with a hydrophobic coating [135, 136]. Vial breakage poses serious challenges when considering possible product contamination due to broken glass and consequently with the cleaning of drying chamber. Crystallization of excipients such as mannitol [137–139], sodium chloride [140], and dibasic sodium phosphate [141] during freezing or drying was implicated. A modified freezing protocol (slower cooling) or reduction of fill volume could minimize this negative phenomenon. The introduction of mechanically strong and chemically durable Valor® vial enables the use of larger fill volumes. In addition, it could possibly facilitate faster filling rates and therefore reduce the ambient temperature hold of temperature-sensitive drugs, unless shear stress induced by accelerated presents stability challenges. Despite higher cost, superior mechanical strength, and chemical inertness, glass containers with hydrophobic surfaces may become more popular in freeze drying of biopharmaceuticals for more expensive drug products.

Convenience in administration of drugs presents a critical driver for the use of dual-chamber systems (vials, syringes, cartridges) in spite of the additional processing challenges (presented by the need for siliconization, stopper assembly, loading and unloading, stoppering in more controlled environments, filling of the diluent, etc.). The market share of dual-chamber-based systems has

increased steadily and is expected to continue to grow unless processing costs and costs of goods exceed projected costs. Plastic containers present an alternative to glass-based systems and could be of practical benefit if the challenges associated with the diffusion of water vapor and oxygen during storage could be resolved.

Stoppers with vent(s), to facilitate water vapor removal during drying, will continue to remain the most popular closure systems for use in lyophilization. Most likely, igloo-type stoppers will prevail over two-leg stoppers due to reported better machinability and handling behavior during filling at commercial scale [142, 143]. Advances in rubber formulations will continue to evolve to ensure elasticity for handling, limit permeability to water and oxygen, and also target the lowest extractable and leachable profiles. In the opinion of the authors, stoppers with hydrophobic coatings will become more common due to two advantages: (i) reduction in the sticking of stoppers to the shelf during stoppering process and (ii) decrease in leachables.

6.5.2 Dryer Design

6.5.2.1 Laboratory-Scale Dryers

Currently, modern laboratory-scale freeze dryers can be equipped with a variety of tools, including wireless temperature sensors (for example, Tempris), HFSs, TDLAS, mass spectrometer, and comparative pressure measurement to control the process based on the critical temperature(s) and detection of the end points of primary and secondary drying. In addition, as described earlier, a few technologies are available to control ice nucleation during freezing and to minimize the differences in product resistance between laboratory and commercial products. Some manufacturers also offer freeze dryers fabricated with large ducts between the chamber and condenser, thereby minimizing the resistance to mass flow and facilitating a relatively robust pressure control at high sublimation rates. The edge effect, typical for current design of freeze dryers [144], can be also minimized by controlling the chamber wall and door temperatures, an option that is also offered by manufacturers. Refrigeration systems are designed to maintain low shelf temperatures during freezing (for example, to crystallize sodium chloride, if needed) or maintain low condenser temperatures (<−60 °C) even at the peak of sublimation. SMART Freeze-Dryer technology [81] is also available and aids in optimal process control based on critical temperature(s) and desired product properties. The next generation of SMART Freeze-Dryer-like process control software could become available in the recent future, thereby providing the option(s) for most efficient process design at laboratory scale, available for users with a range of lyophilization experiences. A typical laboratory-scale freeze dryer can process up to 2000 × 2 ml vials (or 500 × 20 ml vials). In addition, some of laboratory-scale dryers can be converted into steam sterilized units with clean in place (CIP) capabilities. For very low batch size products (for examples, cell and gene therapy, personalized medicines), advanced laboratory-size dryers can be used to conduct the manufacture of clinical materials.

In summary, one can conclude that development scientists have access to most advanced and well-equipped dryers to deliver the most optimal lyophilization

cycles. The energy efficiency of process, however, is very low (<2%), which should be a target for the future improvements of laboratory dryers at reasonable acquisition costs for the end user [145].

Due to the high costs associated with production of biologics, very often only limited quantities of drug substance/drug product may be available in the early stages of product development. Therefore, process development could be challenging and could be alleviated by the use of appropriate replacement materials (surrogates) with similar thermal properties, drying behavior, and cake resistance such that 10% of the vials could contain the active material, with the remainder being the mimic material-containing vials. An alternate option has been provided by freeze-dryer manufacturers through the development of miniaturized versions of laboratory-scale freeze dryers. LyoCapsule and Micro Freeze dryer are examples of commercially available units and are equipped with a variety of options (HFSs, TDLAS, controlled ice nucleation, etc.) depending on the manufacturer. With this option, cycle development and scale-up activities can be conducted using a few vials [146]. The drying chamber is small, which allows accurate measurement of thermal events, such as crystallization of excipients, which is easily monitored in dryers equipped with the HFS [86]. One could expect an increase in sensitivity of analytical tools (HFSs) used in micro freeze dryers in near future. With continued innovation in the design of the next-generation miniaturized dryers, one could foresee dryers combining the capabilities of HFSs (as in differential scanning calorimeters), computational fluid dynamics (CFD) modeling, and conventional dryers. The best-case scenario would be such that a small batch of vials could be lyophilized with the ability to accurately detect and characterize thermal events during freeze and drying. If controlled nucleation becomes widely used at commercial scales, micro freeze dryers may become essential tools for the process development and scale-up.

6.5.2.2 Commercial-Scale Freeze Dryers

The conceptual design of currently available commercial freeze dryers has not significantly evolved in the past four decades. The large chambers are reinforced to withstand pressures up to 2 bar (1500 Torr, 0.2 MPa) and operate in vacuum as low as 1 Pa (1E-5 bar, 7.5E-3 Torr) and temperatures from −60 °C to 127 °C. The chamber is heated to >120 °C during steam sterilization, vacuum dried, and tested for leaks. Cooling post steam sterilization could be a rate-limiting step toward initiation of loading of the dryer. Some commercial freeze dryers are equipped with a jacket through which a heat transfer fluid (typically water) is circulated to expedite the cooling from the sterilization temperature. In most cases, the lowest temperature that wall could support is the temperature above the freezing point of water. To the best of our knowledge, jacket cooling does not appear to be utilized during freeze drying. Thus, during the drying of low collapse products (shelf temperature is typically <−25 °C), the wall and door temperatures are in equilibrium with the ambient temperature outside the dryer and the shelf temperatures causing a significant edge effect. In the experience of the authors, the edge effect (defined as the ratio of heat received by edge vials to the heat received by center vials) could reach 1.5, at least at the beginning of drying process [147]. Calculations performed using the primary drying model [96] with the

cake resistance values taken for 10% sucrose [148] revealed that edge effect of 1.5 could result in differences in primary drying time between edge and center vials in a range from 20% (collapse temperature of $T_c = -38\,^{\circ}C$) to 35% ($T_c = -15\,^{\circ}C$). The shelf temperature and pressure were varied between −34.5 to 23.0 °C and 30 to 50 mTorr, respectively. In theory, edge effect could be eliminated if wall and door temperature would be held close to the product temperature during the drying process. While currently it is very expensive to maintain jacket temperature below 0 °C, technology innovations may enable, in near future, the design of freeze dryers with the minimal edge effect.

The consistency of product quality attributes strongly depends on uniformity of heat transfer through the shelves of freeze dryer. In current commercial freeze dryers, the difference in the shelf surface temperature is typically within 3 °C. Some of current freeze-dryer manufacturers already produce relatively thin shelves with heat transfer coefficient similar to those in laboratory dryers [149]. Improvements in shelf design targeting enhancement of heat transfer could minimize challenges in process scale-up from laboratory to the commercial dryers.

Condenser design is critical to the performance of lyophilizer. Suboptimal condenser design could limit achievable sublimation rates [150]. For many years, manufacturers have utilized a "rule of thumb" in designing condensers such that the ratio of the condenser surface to the shelf surface should be at least 1. Using low condenser temperatures (≤−70 °C) [150] may not necessarily be beneficial since it creates a heterogeneity in ice distribution across the condenser (with greater ice deposition at the entrance to the condenser). Ideally, ice distribution should be uniform within the condenser. Specially designed baffles can be utilized to direct the water vapor flow within the condenser. An alternate option is to section off the condenser to create surfaces with different temperatures and could be assessed in future designs. The direct simulation Monte Carlo (DSMC) technique could be applied to model vapor flow in the condenser chamber and predict ice deposition on the condenser surface [151].

The geometry of duct between chamber and condenser is critical, and it could impact the flow rate from the chamber to condenser. For example, modifications of location of the baffles increased the flow rate by at least 50% [152]. On the other hand, introduction of a CIP pipe into the duct resulted in notable (about 20%) decrease in the mass flow rate [153]. Basic engineering principles suggest that longer and smaller duct requires a larger pressure gradient to maintain a certain sublimation rate. Recent advances in CFD enable tuning of duct geometry (diameter, length, valve configuration) and condenser performance (temperature) based on the target sublimation rate [154]. While TDLAS is a valuable PAT tool, it requires an increase in the duct length to position the sensors. Dryer manufacturers also offer a very short duct equipped with a mushroom valve that enables very high sublimation rates. As an alternative to TDLAS, one can also employ differential pressure measurement across the duct for the estimation of sublimation rate [155].

Three process parameters are typically controlled during commercial manufacturing: shelf temperature (inlet temperature of heat transfer fluid, in most cases), chamber pressure, and time. Conventional commercial freeze dryers are

equipped with CM, Pirani gauge, flow meter to control pressure by injecting nitrogen, and valve between the condenser and chamber providing the capability to conduct the PRT. Some "modern" freeze dryers are also equipped with mass spectrometer, TDLAS, or internal antennas for wireless temperature measurement. All tools described here are readily available and provided as customizable feature/options on the acquisition of new dryers.

While shelf temperature control is typically ±3 °C, chamber pressure control may not be so accurate. The pressure can vary from the set point by 1 mTorr or could be as high as 30 mTorr depending on the load and stage of drying. The parameters for proportional–integral–derivative (PID) controller are typically set by manufacturer for the empty and clean dryer during or prior factory acceptance test. Due to significant differences in the gas composition during drying, constant values of PID input parameters may not be suitable for all stages of process, causing large pressure fluctuations either at the initial stage (when sublimation rate is the highest) or toward the end of primary drying depending on the tuning of the controller. To cover these fluctuations and assure achievable process tolerances for the commercial process, one would need to perform robustness study targeting a wide product temperature design space, which could lead to unnecessarily conservative process [156].

Another variable during lyophilization process is the condenser temperature, which could peak during initial sublimation step, resulting in pressure control failure. Manufacturers account for the potential of such an occurrence and typically overengineer a refrigeration system adding cost to the dryer.

Given current experience in manufacture of freeze dryers as well as advances in computational modeling and PAT tools, one could expect, in near future, that equipment manufacturers could offer following improvements to the design of commercial lyophilizers:

- Temperature-controlled walls and doors of freeze dryers.
- CFD-led design to optimize configuration of duct/condenser for higher throughput.
- Flexible PID inputs, adjustable to sublimation rate, to drastically reduce pressure fluctuations (ideally, to keep pressure within 1 mTorr from the set point).
- Adjustable refrigeration system to match peak (or maximum) sublimation and reduce power consumption.

In addition, an ideal freeze dryer could be equipped with all available PAT tools to date: *comparative pressure measurement (Pirani vs. capacitance manometer), PRT, mass spectrometer, tool for direct or indirect sublimation rate measurement (TDLAS, PDT, pressure gradient through the duct etc.), wireless temperature sensors, and IR camera*. The combined cost of all listed tools could be less than the cost of a single drug product lot, given the cost of biologics and other parenteral products. With the target to design the most efficient process and based on current experience and equipment capabilities, the ideal freeze dryer should be able to deliver normalized sublimation rate >1.5 kg/m^2. A combination of all tools listed above would be of practical benefit only if the freeze-drying cycle duration can be flexible and most optimal for every single product lot. The combination of inputs from variety of sensors will ensure that the cycle yields a product with

the required quality. Implementation of product temperature sensors would also assist in the analysis of process deviations when other methods fail to assess the actual product temperature during cycle in relevance to the proven product temperature design space and will, potentially, enable real-time product release.

6.5.3 Redefining Product Appearance/Elegance

The appearance of the lyophilized drug product (cake, powder, etc.) is the one of most important quality attributes. Since freeze drying is performed at low temperatures, an educated customer expects uniform elegant cylinder, which in most cases is a white cake. This is probably fair expectation if manufacturers include mannitol or glycine as bulking agents and the concentration of active ingredient in formulations is smaller relative to the other excipients. Nonuniform cakes in such formulations are usually evidence of inefficient manufacturing process that could be accompanied with elevated moisture and exhibit faster degradation. In most cases, however, the presence of bulking agent enables the use of very aggressive cycles without affecting product quality [65, 134]. Moreover, systematic investigation of effect of collapse on protein stability [132, 157, 158] revealed greater protein stability in collapsed cakes where collapse had occurred during drying when compared with that in the cakes that collapsed during storage. A recent commentary describes scenarios where a nonideal (an inelegant) cake appearance has no impact on product quality and is an inherent characteristic of the product (either due to formulation, drug product presentation, or freeze-drying process) [159]. It has also been previously documented that product appearance may not play a significant role in product stability if the residual water content is within specification and other attributes such as reconstitution time are not affected [133].

Recent advances in analytical techniques provide the capability to conduct of 100% inspection by NIR or headspace analysis at the commercial scale. This, in turn, could provide the opportunity/flexibility for real-time product release if the PAT tools demonstrate that process was within the validated design space and that the residual moisture is within the validated range. In such a scenario, cake appearance may not matter.

6.5.4 "Intelligent" Formulation and Process Design

Through molecular modeling, we can design molecules (more well behaved from the perspective of viscosity, less aggregation prone, etc.), which can be matched with most effective stabilizers and other components to design "intelligent" or "lyo-friendly" formulations. In silico modeling, similar to the published approach [160], can be used as a first step of molecule design. While there is limited literature on the use of modeling to understand the interactions between proteins, carbohydrates, and other excipients during freezing and drying [161–163] and there are no robust models to date for protein–excipient interaction, one can employ best practices in formulation design for lyophilized products [1, 2, 11, 164] to identify combinations of excipients and compositions that provide optimal stabilization [51, 54, 129]. Low frequency Raman scattering

and hydrogen–deuterium exchange MS (HDX-MS)-based methods have been utilized to rank-order formulations during initial formulation screening and have demonstrated the potential to predict long-term stability. Low frequency Raman scattering is not available as a routine screening tool yet, but will be available for formulation scientists in the recent future [118]. HDX-MS-based characterization (described earlier) has been utilized extensively within both academic and industry settings and shows great promise for inclusion into routine formulation screening and analytical toolboxes. Once an optimal formulation targeting the highest glass transition temperature (T'_g, using the Fox equation) has been identified, the currently available primary drying model [96] coupled with best practices in the development of freezing and secondary drying steps [15] can be utilized to design an efficient process cycle, which can be confirmed experimentally. In silico tools, best practices in formulation and process development, and modeling should be utilized in modern formulation and process design. The lessons learned in the formulation development of proteins such as mAbs, ADCs, and fusion and PEGylated proteins could be extended to newer modalities (cell and gene therapy), but may need additional formulation development efforts coupled with the use of alternate technologies (such as spray freeze drying, foam drying, and other methods described in the subsequent chapters of this book).

6.5.5 How Could Alternate Drying Technologies and Freeze Drying Coexist?

6.5.5.1 Alternatives to the Current Batch-Based Vial Drying

Continuous Vial Freeze Drying Given the heterogeneity of the current batch vial freeze-drying process (in the absence of annealing or controlled ice nucleation), continuous vial freeze drying could provide a reasonable alternative offering shorter drying cycles per unit (vial) and, possibly, smaller equipment footprint. Currently, two concepts of continuous processing are available: spin freezing rotary drying [165] and suspended vial drying [166].

During spin freezing, the vial is rotated at about 3000 rpm and quickly cooled by nitrogen flow, forming a thin layer (less than 2 mm) of product [165]. IR radiators provide uniform energy input to the product by rotating the vials during drying. NIR is used to confirm the end of the drying process. The drying time is ≈1 hour even for low collapse temperature products. While visual inspection of the vial with product (the product is a shell along the walls and bottom of the vial as opposed to a conventional cake/cylinder) could be challenging, NIR confirms that residual moisture is around the target. This method could be used for products that require very fast freezing (>5 °C/min).

In the second concept based on drying of suspended vials [166], ice nucleation occurs at a high temperature (ensuring low degree of supercooling) by induction of vacuum such that the nucleation temperature is the same for all vials. Then, the vials are transferred to the drying chamber and transported inside the chamber in a continuous manner while remaining suspended. The heat is supplied by radiation and convection, evenly for each vial. By induction of controlled nucleation and elimination of the edge effect, the drying time could be reduced by factor of 3.

To date, there is no commercial continuous manufacture of biologics in vials. In the opinion of the authors, continuous vial freeze drying has the potential to replace traditional freeze dryers, especially when a small footprint is needed and lot size is relatively small.

Spray Freeze Drying Another alternative to vial drying is spray freeze drying (atmospheric spray freezing followed by vacuum drying in either rotating drum or on vibrating shelfs), which could produce relatively large particles (≈0.3–1 mm). These particles (pellets) are still mechanically strong resulting in excellent flowability, but are small enough to allow almost 40X faster drying when compared with vial-based drying [167, 168]. Production of dry pellets provides flexibility in manufacturing of pharmaceutical products by powder filling of targeted doses using previously dried bulk. Spray freeze drying can also be conducted in continuous mode [169].

6.6 Summary

There have been significant efforts in the use and development of rational formulation and process design of biologics when compared with trial-and-error approaches utilized as recently as 10–15 years ago. The emphasis on moving away from empirical strategies for freeze drying has been coupled with the advances in frozen and dried product characterization, equipment design and characterization, process control, monitoring, and characterization approaches and container-closure options for lyophilization. The range of therapeutic biologic compounds requiring removal of water for long-term stabilization has also expanded to cover mAbs, fusion proteins, ADCs and other conjugated proteins, bispecific compounds, vaccines, and cell and gene therapy modalities. In several cases, the advances in alternate drying technologies (discussed in the subsequent chapters of this book) have presented options to stabilize compounds without the need to formulate to counter the damaging effects of ice formation and the accompanying changes in the environment of the protein. In spite of the limitations associated with freeze drying either from the challenges presented by ice formation or equipment costs and maintenance or process inefficiency, lyophilization will continue to serve as the gold standard to which other drying methods will be compared. The challenges presented by newer modalities will provide opportunities for freeze drying (vial- and non-vial-based) and other drying technologies to continue to develop innovative strategies for stabilization and manufacture of biologics and meet the needs of the patients and the healthcare industry.

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Tributes

Dr. Michael (Mike) Pikal (1939–2018)

Michael J. Pikal, Professor Emeritus in the Department of Pharmaceutical Sciences at the University of Connecticut (UConn) and the world's leading expert in pharmaceutical freeze drying, passed away in 2018. A collaborator of Dr. Pikal, the late Felix Franks had aptly described Dr. Pikal as the undisputed "King of Pharmaceutical Freeze Drying."

A physical chemist by training, Dr. Pikal began his career in the pharmaceutical industry as a scientist at Lilly Research Laboratories in the early 1970s after undergraduate work at St. John's University (Minnesota), graduate work at Iowa State University, postdoctoral fellowship at Lawrence Livermore Laboratory, and an assistant professor stint at the University of Tennessee in Knoxville. At Lilly, he began his groundbreaking research in the science of freeze drying after having been introduced to the technology by Alan MacKenzie. Over the next four decades, he published several key papers on lyophilization formulation and process design/characterization (including principles governing heat and mass transfer and models for primary and secondary drying) and stability of pharmaceuticals. While at Lilly, he was also selected as a Fellow of the American Association of Pharmaceutical Scientists (AAPS) and was a recipient of the Ebert Prize for a manuscript published in the *Journal of Pharmaceutical Sciences*.

After a distinguished career at Lilly, Dr. Pikal joined the Department of Pharmaceutical Sciences at UConn in 1996 where he went on to build a world-class program in lyophilization. He mentored more than 40 PhD students, postdoctoral fellows, and visiting scholars, who engaged in finding answers to more fundamental scientific questions as well as resolving practical challenges. Dr. Pikal emphasized the need for utilizing systematic approaches to solving formulation, processing, and stability challenges instead of "throwing a patch on them and hoping they'll go away." While at UConn, another feather was added to his cap following his appointment as the first Pfizer Distinguished Endowed Chair in Pharmaceutical Technology in 2005. He was the driving force and a critical contributor in establishing research consortia such as the Center for Pharmaceutical Processing Research (CPPR), the National Institute for Pharmaceutical Technology and Education (NIPTE), and the National Institute for Innovation in Manufacturing Biopharmaceuticals (NIIMBL). Dr. Pikal was also instrumental in the development of a technology roadmap for lyophilization within the LyoHub consortium to survey the current state, develop the vision for the future, identify the critical gaps in practices and barriers in implementation, and define the goals for the development of new lyophilization methods. In 2009, he was the recipient of the AAPS Distinguished Pharmaceutical Scientist Award, recognizing his achievements that have made a lasting impact on freeze drying and pharmaceutical sciences. In recognition of his contributions, the *Journal of Pharmaceutical Sciences* published a special issue highlighting his key scientific accomplishments in pharmaceutical sciences. While the scientific community will sorely miss Dr. Pikal, we also celebrate his contributions and accomplishments to freeze drying.

Dr. Alan MacKenzie (1932–2018)

Dr. Alan Mackenzie, who was one of the pioneers in the field of lyophilization, also passed away in 2018 after over five decades of stellar contributions to freeze drying. His undergraduate work and doctoral work in chemistry were completed at the Imperial College in London, and he subsequently moved to the United States on a King George VI Memorial Scholarship. While in the United States, he held appointments at the American Foundation for Biological Research, the University of Wisconsin, and the University of Washington and also served as the past president of the Society for Cryobiology. He was a contemporary and collaborator of J. D. Davis, Samuel Goldblith, Basile Luyet, Harold Meryman, Don Rasmussen, Louis Rey, W. W. Rothmayr, and Terence Rowe. The collaborations led to the publication of over 60 manuscripts, including seminal work on freezing and freeze drying of aqueous solutions, foods, pharmaceuticals, cells, and tissues. We note that the current design of the freeze-drying microscope in many of our laboratories is credited to Dr. MacKenzie's efforts to improve the first-generation microscope designed by Dr. Louis Rey and documented in publications in the early 1960s.

Dr. MacKenzie's seminar on freeze drying at Eli Lilly became Dr. Mike Pikal's first introduction to lyophilization, who in turn went on to become the world's foremost expert in the science and technology of freeze drying. Dr. MacKenzie along with Dr. Mike Pikal and Dr. Felix Franks (from Cambridge University) were a powerhouse within the field of freeze drying. Their collaborations with peers such as Dr. Steven Nail (from Upjohn Laboratories and now at Baxter Biopharma) led to a number of innovations in freeze drying. Dr. Mike Pikal paid a tribute to Dr. Mackenzie in an email to Graham MacKenzie shortly before his passing: "Alan was a friend for about 40 years and was the one who got me interested in freeze drying. He has made great contributions to the science of freeze drying, and all of us who knew him will miss him greatly." While his passing is a tremendous loss, we believe that his outstanding contributions to freeze-drying science will continue to inspire the current and future generations of researchers.

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