Advances in Process Analytical Technology in Freeze-Drying

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Introduction

Rational design and utilization of freeze-drying processes are essential to minimize their impact on drug product quality and assure consistent clinical performance. Development of the formulation and lyophilization process should focus on the product quality attributes. The International Conference on Harmonization (ICH) guidance Q8 (R2) suggests that the product quality cannot be tested in a product by using limited off-line measurements, instead it should be built in by design of the formulation and manufacturing process. This guidance lays the foundation for a quality-by-design (QbD) paradigm, which stresses the scientific understanding of formulation and processing factors on product quality and also the ability to assure product consistency [25, 26]. Process analytical technology (PAT) is a vital part of the implementation of QbD. Based on the in-line real-time measurement of critical process parameters (CPPs), the manufacturing process can be monitored and further controlled with appropriate feedback mechanisms. PAT can also facilitate the trending of the process operations to support continuous improvement efforts. Even though multiple off-line analytical techniques, such as chromatography and

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spectroscopy, can give useful information about the quality of the final product, these technologies do not directly provide real-time process information. Here, we mainly focus on the online monitoring devices used in the freeze-drying process.

QbD design of a robust freeze-drying process relies on the thorough understanding of the impact of the formulation and process parameters on product quality. The critical formulation factors include the properties and amount of the drug and each excipient used and their interactions [31]. The CPPs include, but are not limited to drying shelf temperature, chamber pressure, freezing/heating ramp rate, etc. Process optimization depends on understanding each freeze-drying stage, monitoring CPPs and controlling them whenever necessary.

Due to the extreme conditions used in the freeze-drying process (low temperature, high vacuum, and high temperature for sterilization), only limited PAT devices are compatible with the harsh environment. During the early development history in freeze-drying, there was not much understanding about the required technology and visual inspection was mainly used to guide process development. The Pirani gauge was later employed to monitor or control the pressure during the drying process, and it is still being explored for regular use in good manufacturing practice (GMP) environment. Also the thermocouple was implemented to monitor product temperature, which has greatly accelerated the development of lyophilization field. Lately, more advanced tools such as manometric temperature measurement (MTM), tunable diode laser adsorption spectroscopy (TDLAS), and near-infrared (NIR) are being developed to monitor the product temperature, moisture level, and sublimation rate during lyophilization. These new technologies have a great potential in monitoring and controlling the freeze-drying process.

Based on their design applications, PAT tools for lyophilization can be categorized to the following four types:

1. Product temperature monitoring

The product needs to be fully frozen and should be dried below a critical temperature (collapse temperature for amorphous system or melt temperature for crystalline system) to ensure an elegant cake appearance. In addition, the product quality is heavily influenced by the thermal history which the product has experienced during the sample preparation process [35]. Therefore, it is critical to monitor and control the product temperature during the freeze-drying process. Both wired product temperature probes such as thermocouples and resistance temperature detectors (RTDs), and wireless probes such as the temperature remote interrogation system (TEMPRIS) have been developed for this purpose.

2. Primary drying endpoint

The endpoint of primary drying is the point at which sublimation rate is low enough to suggest that primary drying is almost complete. At the end of primary drying, the cake structure should be retained without any collapse, and it should not melt back even after exposure to ambient temperature [23]. This structural retention indicates that most frozen water has been removed by sublimation and the cycle can be progressed into secondary drying to further remove the “bound” water. Pirani gauge,

MTM, TDLAS, and plasma emission spectroscopy (Lyotrack) can be used to detect the primary drying point.

3. Moisture content

Since the freeze-drying process is essentially a water removal process, it is of great importance to constantly monitor residual moisture. NIR-based technology has been developed for this purpose, and can be used to provide a complete inspection of all the vials from the same batch. In addition, TDLAS has been developed to monitor the sublimation rate, which can also be used to track the amount of water removed and monitor the moisture content.

4. Changes in molecular structure

Recently, spectroscopic methods such as Raman and NIR were developed for monitoring of water–ice phase transition and mannitol polymorphism transition. In addition, protein conformational changes and the protein–excipient interactions during the freeze-drying process can also be studied. These techniques can give more insights into protein conformational stability and the lyoprotectant–protein hydrogen bonding interaction in real-time during the dehydration process.

This chapter intends to provide a comprehensive review of latest PAT tools for freeze-drying, with emphasis on suitability for a large-scale manufacturing process where PAT implementation and sterility concerns become critical. First, each of the latest process monitoring devices is reviewed in terms of the major applications and limitations. Second, all these techniques are summarized based on their capabilities, practical advantages, and scalability to a large-scale freeze dryer. Finally, the current most commonly used PAT tools are discussed, and future implementation of promising PAT tools is presented.

Process Analytical Technology

Product Temperature Probe

It is well understood that product temperature is the critical parameter of interest for an effective freeze-drying process [15]. In general, during the primary drying stage, the product temperature should be maintained below the maximal allowable critical temperature [19]. Frequently, thermocouples are used to monitor this parameter during the freeze-drying cycle at the laboratory or pilot scale. At a large scale, thermocouples or RTDs may also be used, the latter of which is preferred due to its mechanical robustness and effective sterilization. Lastly, thermocouples are used to determine the endpoint of primary drying [18–20].

During the primary drying stage, there is a difference between the shelf and product temperature, which is due to the heat absorbed by the sublimation process. The endpoint is the point at which the product temperature equilibrates with the shelf temperature. In the example shown in Fig. 1, the product temperature remains quite

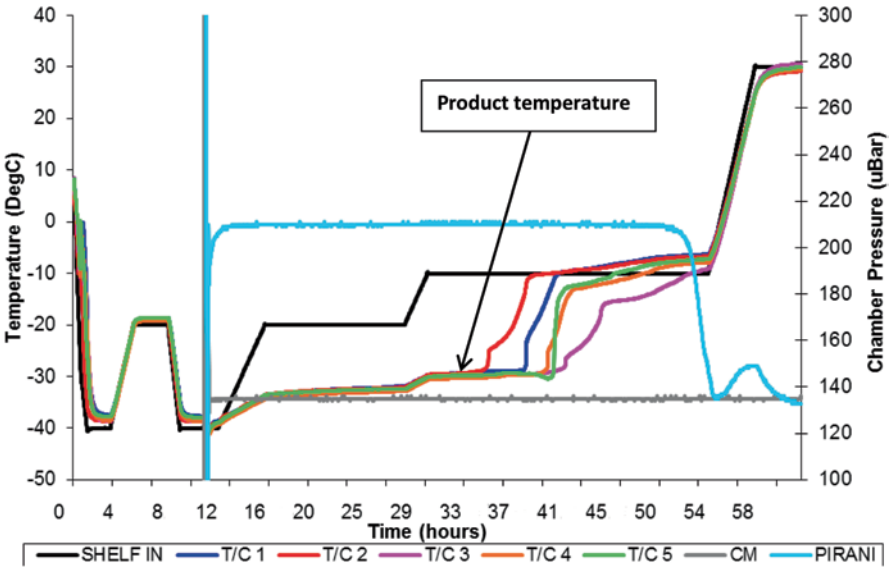

Fig. 1 Typical freeze-drying cycle showing thermocouple trending and endpoint during the primary drying stage of the cycle

low around −30 °C during the early stage of primary drying, and then it quickly merges with the shelf set point at about 40–52 h, depending on the product location. It can be concluded that primary drying has completed at this point as sublimation process has ended.

Even though thermocouples are a useful tool to determine the primary drying endpoint, caution must be taken in the validity of the data. It is well described in the literature that product vials with thermocouples placed within are not representative of the rest of the batch [20]. This is mainly due to the invasive nature of thermocouple placement, as they are inserted directly within the product in order to obtain best results.

The main challenges associated with using a thermocouple to monitor product temperature are:

1. Placement within the product vial
2. Impact on ice nucleation and cake resistance

The ideal placement of the thermocouple should be center-bottom of the product touching the bottom of the vial [28]. Thermocouples placed too high will lead to an early indication of the completion of primary drying step, as the thermocouple tip will lose contact with ice at an earlier stage, indicating that all ice has been removed, even though there would still be ice remaining in the vial. In order to compensate for this, the use of a Pirani gauge to determine endpoint would be an option [20] or, as it has been suggested, a conservative approach would be to add between 10 and 30 % of time to the primary drying step [31].

The most important impact of thermocouple presence is on ice nucleation and in turn cake resistance. Simply stated, invasive product temperature measurement techniques impact the freezing of the product. This is the main reason for the vial with the thermocouple not to be considered as being representative with the rest of the batch [9, 20]. The presence of thermocouples in the product interferes with the ice nucleation process in that the thermocouple itself acts as a nucleation site. The nucleation temperatures of the product in the thermocouple containing vials are often much warmer than that of the rest of the batch, resulting in a lower degree of supercooling, larger ice crystals and, in turn, a lower resistance to vapor flow during primary drying as compared to its non-thermocouple containing neighbor.

Lastly, other challenges include placement of thermocouples during scale-up in a commercial freeze dryer, which poses issues of mechanical and sterility nature. Thermocouples are difficult and impractical to use in a manufacturing environment as they are often incompatible with stoppering and auto-loading systems. Wireless technology may be an option to deal with this challenge.

Wireless Product Temperature Probe

In order to address the pitfalls of the thermocouple use at the commercial scale, the TEMPRIS was developed. The TEMPRIS (IQ Mobil Solutions GmbH) consists of eight sensors, an interrogation unit, and a computer system with CarLog software [28].

TEMPRIS is an invasive technique, where the probe is placed within the product to monitor product temperature, similar to a traditional thermocouple. The major concern associated with this system is the size of the probes themselves, as it has been suggested that the probes should be smaller in size [19]. A maximum product fill depth of $\leq$0.4 cm was required when using the TEMPRIS probes [28]. It was reported that the correct placement of the TEMPRIS probes as shown in Fig. 2 was crucial in order to obtain reliable temperature profiles and endpoint monitoring, and a larger tip resulted in a more sensitive measurement of ice sublimation [28].

Studies have been performed to examine the performance of the TEMPRIS monitoring system and to compare to a traditional 36-gauge thin wire thermocouple. Using a 25-mg/mL sucrose solution and a LyoStar II lyophilizer equipped with MTM, it was found that the product temperature profile gave a similar trend as the traditional thermocouple measurement, which was also used during the same cycle. The study showed that the product temperature data generated from a center vial using the TEMPRIS monitoring system were in good agreement with both thermocouple data and predicted data using MTM. The TEMPRIS data also provided a more representative bias between edge and center vials as compared to that of a thermocouple [28]. Similarly, agreement was noted between TEMPRIS and thermocouples for a system consisting of 50 mg/mL mannitol/sucrose solution (10:1). In addition, the TEMPRIS probes indicated a more representative primary drying endpoint in that they all displayed a delayed increase in temperature during the end of primary drying in comparison to traditional thermocouples [28].

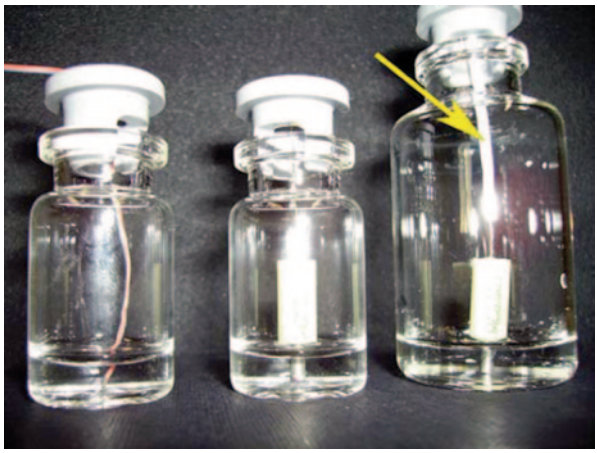

Fig. 2 Correct placement (*bottom center*) of a thermocouple (*left*) and two TEMPRIS sensors (5 and 20 mL vial, *right*) (Adapted from [28])

Another benefit of this approach includes the adaptability at scale, where due to the wireless technology, the TEMPRIS may be used with an automatic loading system. Second, the fact that the probe may be placed anywhere on the shelf enables understanding variation in product temperature across the shelf and chamber [19].

Disadvantages are the same as for thermocouples and RTDs, where product vials with probes are not representative of the entire batch [19]. The product temperature data generated with TEMPRIS would not be representative due to its influence on ice nucleation. In addition, the industry is moving towards noninvasive methodologies of measuring product temperature during freeze-drying process.

Pirani Gauge Versus Barometric Endpoint Determination

A Pirani gauge is commonly used to monitor the primary drying process. It consists of a metal wire (often platinum) open to the applied pressure. The wire is heated by a passing current and cooled by the surrounding gas, and the gauge wire temperature is dependent on the rate of heat loss to the surrounding gas. The rate of heat loss is directly proportional to pressure, and thus this measurement is based on the thermal conductivity of the gas present in the chamber [18]. The gauge can give accurate result from 10 to 10^{-3} Torr, but only for the gas against which it is calibrated (normally nitrogen). Since the Pirani gauge is sensitive to the chemical composition of the gases being measured, its use could result in inaccurate pressure measurements in the chamber due to the gas composition changes (i.e., from water vapor to nitrogen) during drying. However, as described later, a Pirani gauge is commonly used with a capacitance manometer for the detection of the primary drying endpoint.

The Pirani gauge is an inexpensive and small tool that can withstand steam sterilization without loss of performance [20]. In addition, it can be easily installed in a large-scale lyophilizer, which makes it popular for process monitoring. However,

with prolonged usage, heat transfer via radiation can become significant due to the increasing emissivity of the filament; some periodic maintenance/replacement may be needed to ensure accuracy.

A capacitance manometer (CM, also called MKS Baratron) is commonly put in the drying chamber to measure and control the chamber pressure during lyophilization [18]. The CM device is made of a metal diaphragm placed between two fixed electrodes, with one side being evacuated to high vacuum to serve as a zero reference pressure, and the other side being exposed to the chamber pressure. Since a change in device output voltage is directly proportional to the applied pressure, the CM is able to measure the absolute pressure independent of the gas composition, which is different from the Pirani gauge [19]. In addition, CM can give a stable output within a very wide pressure range (0–760 Torr), and can withstand steam sterilization. Therefore, the CM is commonly used for chamber pressure measurement in the freeze-drying process.

The joint use of a Pirani gauge with a CM has been employed to determine the endpoint of primary drying for the entire batch. At the early stage of primary drying, essentially all of the gas in the drying chamber is water vapor, and thus the Pirani gauge gives about 60% higher result than CM reading because thermal conductivity of water vapor is about 60% higher than that of nitrogen [22]. At the late stage of primary drying, the gas composition in the drying chamber changes from mostly water vapor to mostly nitrogen, and thus the Pirani gauge will show a sharp drop in pressure and finally merge with CM readout as shown in Fig. 1. This sharp transition and the small pressure differential (ΔP) between Pirani and CM is an indication of the completion of primary drying. Once the primary drying endpoint has been reached, ΔP will be close to zero and remains constant. The $\Delta P = 0$ criteria could be used as a reliable detection of endpoint of primary drying, and this ΔP between Pirani and CM can be used as a control tool if a feedback loop is programmed to allow the automatic progression to the next drying step.

The drying process can also be monitored by taking vials out of chamber at different stages of primary drying using a "sample thief." A systematic study was performed for both 5% sucrose and mannitol systems, and residual water was measured either by gravimetric or Karl Fischer method [20]. Figure 3 shows the Pirani and CM pressure trends and percent residual water profile during primary drying for 5% sucrose (a typical amorphous system). At the onset, midpoint, and offset points of pressure drop in the Pirani gauge, the residual water content is about 25, 9, and 5%, respectively. When the sample vial is warmed to ambient temperature, the midpoint vial cake (taken out at about 48 h) showed collapse behavior, suggesting that residual moisture present is high enough to depress T_g to be below 25 °C. However, the offset point gives acceptable cake structure due to sufficiently low moisture. For the 5% mannitol system, the onset, midpoint, and offset points of pressure drop from the Pirani gauge give about 9, 5, and 4% residual water, respectively. Also, all three points give good cake structure for this crystalline system [20].

It is often helpful to define the endpoint of primary drying based on the Pirani transition alone. After primary drying is complete, most "unfrozen" water has been removed. However, the product still contains a relatively high level of residual water

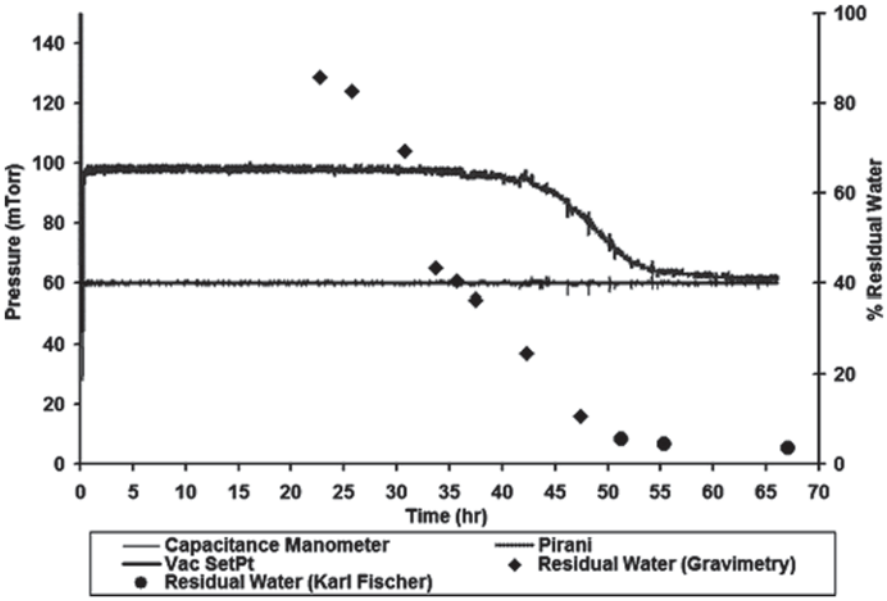

Fig. 3 Pirani versus differential capacitance manometer and percent residual water profile during primary drying for 5% sucrose (Adapted from [20])

(about 5–20% on a dried solid basis). Since the remaining "bound" water will be further removed during the secondary drying stage, a criterion of < 10% residual water may be considered to define the endpoint of primary drying [20]. As shown in Fig. 3, the midpoint of Pirani pressure drop gives low residual water < 10% for both sucrose and mannitol systems, thus the midpoint of the Pirani transition can be considered a good indicator of the endpoint of primary drying, at least for sucrose and mannitol [20]. Of course, the offset point of Pirani drop with longer primary drying time can also give acceptable result.

Manometric Temperature Measurement

It has been suggested that MTM may be used as an alternative method to monitor product temperature during primary drying [17]. The product temperature can be predicted by closing the isolation valve between the chamber and condenser for approximately 25 s to allow the pressure rise to be recorded over this time period. Due to the nature of the process, MTM is more likely employed at laboratory and pilot scale [19, 33]. When compared to invasive methods such as thermocouples, RTDs, or TEMPRIS, MTM offers a noninvasive product temperature measurement, which would be more representative as all the product remains intact and untouched during the freeze-drying process. As discussed in earlier sections, the use of sensors in the product results in nonrepresentative lyophilization behavior, in comparison to vials not containing any sensors [27, 33].

The principle involves fitting the MTM equation (Eq. 1) to the pressure rise data, generated from opening and closing the isolation valve, by nonlinear regression analysis [33].

$$P(t) = P_{\text{ice}} - (P_{\text{ice}} - P_0)\exp\left[-\left(\frac{3.461\text{NAp}T_s}{V(\hat{R}_p + \hat{R}_s)}\right)t\right] + 0.0465P_{\text{ice}}\Delta T\left[1 - 0.811\exp\left(-\frac{0.114}{L_{\text{ice}}}t\right)\right] + X_t \quad (1)$$

where P_{ice} is the vapor pressure of ice (Torr) at the sublimation interface; P_0 is the chamber pressure (Torr); N is the total number of filled vials; A_p is the inner cross-sectional area of the vial (cm^2); T_s is the shelf temperature (K); V is the chamber volume (L); $\widehat{R_p} + \widehat{R_s}$ is the total area normalized product and stopper resistance (determined by the fit); L_{ice} is the ice thickness (cm); ΔT is the temperature difference between ice sublimation interface and bottom of vials; and X is a constant (Torr/second).

Fitting Eq. 1 to the pressure rise data yields a vapor pressure of ice, P_{ice}, and the $\widehat{R_p} + \widehat{R_s}$ total resistance of product and stoppers. The MTM product temperature can be calculated from the vapor pressure of ice (P_{ice}) by Eq. 2.

$$\ln(P_{\text{ice}}) = \frac{-6144.96}{T_p} + 24.01849 \quad (2)$$

where T_p is the product temperature (K) and P_{ice} is the MTM fitted vapor pressure of ice.

When considering the application of MTM to collect product temperature data during the primary drying stage of the cycle, it should be noted that the product temperature is measured from pressure rise data, calculated using a CM and applying Eq. 1. As the pressure rise measurement is a function of the full chamber over a time period of 25 s, it does not discriminate between vials at specific locations within the freeze dryer. The calculated product temperature using the MTM equation is therefore an average of the entire batch [24].

Studies were performed to understand the comparability between product temperature measurement using thermocouples and MTM [34]. Sample of 5% sucrose was used with a target product temperature of −40 °C, which is well below the sucrose collapse temperature of −32 °C. It was found that the temperature gradient was negligible during the drying step, the comparative thermocouple and MTM measurements were in good agreement. A second experiment using 5% glycine suggested that the average product temperature was heavily skewed towards the coldest vials (interior or center vials as opposed to edge vials). Interior vials form the majority of vials when considering the overall array on a given shelf. As the edge vials display higher temperatures due to the influence of radiative heat transfer, this should be taken into account from thermocouple placement.

MTM limitations include the fact that the product temperature is an average temperature with bias towards the coldest vials and a minimum ice sublimation area is required for accurate results, estimated by a Q value of ≥ 0.23. For a laboratory freeze dryer with a 50-L chamber, the minimum area would be 150 cm^2 [34]. MTM is only useful for 2/3 of the primary drying step due to the high degree of drying heterogeneity towards the end of primary drying [19].

Benefits of using MTM are clear in that it provides a product temperature profile by noninvasive means, arguably resulting in a more representative profile. This principle could prove to be a powerful PAT tool. It has been observed that MTM is effective at product temperatures as low as −45 °C, which covers the majority of freeze-dried formulations. MTM has also been used to measure dry layer resistance (Rp), where it was shown that for 5 % glycine, 5 % mannitol, and 5 % sucrose formulations, R_p data were in good agreement with actual data (vial thermocouple method) when using a thermal shield to remove the influence of atypical radiation [34]. When measuring without a thermal shield, it was noted that the R_p values were consistently lower than actual data.

MTM was also examined as a method to measure the vial heat transfer coefficient (K_v) and sublimation rates in different conditions with and without thermal shields. Using an FTS Dura-Stop/Dura-Top freeze dryer, it was found that K_v was in good agreement with the traditional gravimetric method for 5 % sucrose when using a thermal shield such as aluminum foil. Conversely, without the thermal shield, the K_v measurement was consistently higher than for the gravimetric method, which was supported by data generated for multiple concentrations of mannitol, sucrose, and glycine [33].

SMART™ freeze dryer was developed using MTM [32]. The system was designed to enable the scientists to arrive at an optimized cycle in one run, once certain parameters were entered into the software prior to initiating the cycle (maximum allowable temperature, amorphous or crystalline, concentration, fill volume, vial geometry, vial type, number of vials, etc.). In short, the cycle is adjusted in real time (i.e., shelf temperature and chamber pressure), while maintaining the product temperature below the maximum allowable temperature.

Lastly, it has been reported that MTM has applications in the secondary drying step, where it has been shown to be able to predict average moisture content across the batch and the secondary drying endpoint [19, 32]. Residual moisture measured experimentally using Karl Fischer was measured for vials of freeze-dried sucrose (5 %) and glycine (5 %), where the samples were taken using a sample thief. In another experiment, the residual moisture was predicted by using a series of pressure rise steps (MTM method), where the rate of desorption was estimated using the ideal gas law [19, 32]. Good agreement was found between the residual moisture data generated from both methods.

Overall, MTM is a useful tool for the design and monitoring of the primary drying step, even with a number of limitations that need to be considered. It was also found that MTM was useful in measuring desorption rates and therefore residual moisture during the secondary drying step. However, implementation at commercial scale may be difficult due to the nature and requirements of the tool.

Tunable Diode Laser Adsorption Spectroscopy

TDLAS can be used to continuously measure trace concentrations of selected gases for various applications, and has recently matured into the lyophilization field as a process-monitoring tool. The TDLAS control unit provides two coupled outputs from the same laser, which are connected to two fiber optic collimators mounted on the duct connecting the drying chamber and the condenser. The diode laser light is transmitted through the gas mixture containing water vapor, and the beam's wavelength is adjusted to water vapor absorption lines to accurately measure the absorption [5]. The concentration of water vapor was measured based on the Beer–Lambert's law, and the gas flow velocity can be obtained from the Doppler-shifted water vapor absorption spectrum.

TDLAS was used to measure sublimation rate during the lyophilization process on both a laboratory and a pilot scale freeze dryer [5]. The time integrated sublimation rate obtained from TDLAS was compared to a gravimetric determination of the total weight of water removed based on the mass difference before and after the sublimation. The ratio of ''gravimetric/TDLAS'' measurements of water sublimed was 1.02 ± 0.06, suggesting that this in-line tool can be used for accurate measurements of total amount of water removed. In addition, the application of TDLAS for endpoint detection has been studied for 5% mannitol runs in both laboratory and pilot scale freeze dryers. As shown in Fig. 4, the sublimation rate was about 0.5 g/s during the early primary drying stage, and then gradually decreased to almost zero at the end of primary drying. During secondary drying, a bump in the baseline mass flux was observed with a maximum value of 1.0×10^{-2} g/s, and then dropped to zero at the end of secondary drying. Therefore, the TDLAS can also be used to detect the endpoints of primary and secondary drying for the product run.

TDLAS can be used for a rapid determination of vial heat transfer coefficient based on the sublimation rate profiles. Traditionally, several cycles are needed to be performed at different pressures in order to obtain the pressure-dependent vial heat transfer parameters. Since the sublimation rates can be frequently measured by TDLAS, the pressure effect can be incorporated into one cycle by using step-changes in chamber pressure, which makes it much more efficient to measure vial heat transfer parameters. Kuu et al. showed that both the contact parameter K_{cs} and the separation distance ℓ_v can be rapidly determined using the sublimation rate continuously measured by TDLAS within a short cycle. The determined K_{cs} and ℓ_v values closely fit both the sublimation rate and product temperature profiles, suggesting the validity of the approach employed [11]. Note that the TDLAS measured sublimation rate is the average of all vials in the whole batch, and thus the measured vial heat transfer coefficient using TDLAS is also the average value.

In order to evaluate the drying heterogeneity of vials, position-dependent vial heat transfer coefficients (K_v) were studied using TDLAS during sublimation tests with pure water [29]. The K_v data obtained from TDLAS were found to be comparable with K_v data obtained by the traditional gravimetric procedure. Edge vials were found to run at higher temperatures, which results in that K_v of edge vials was

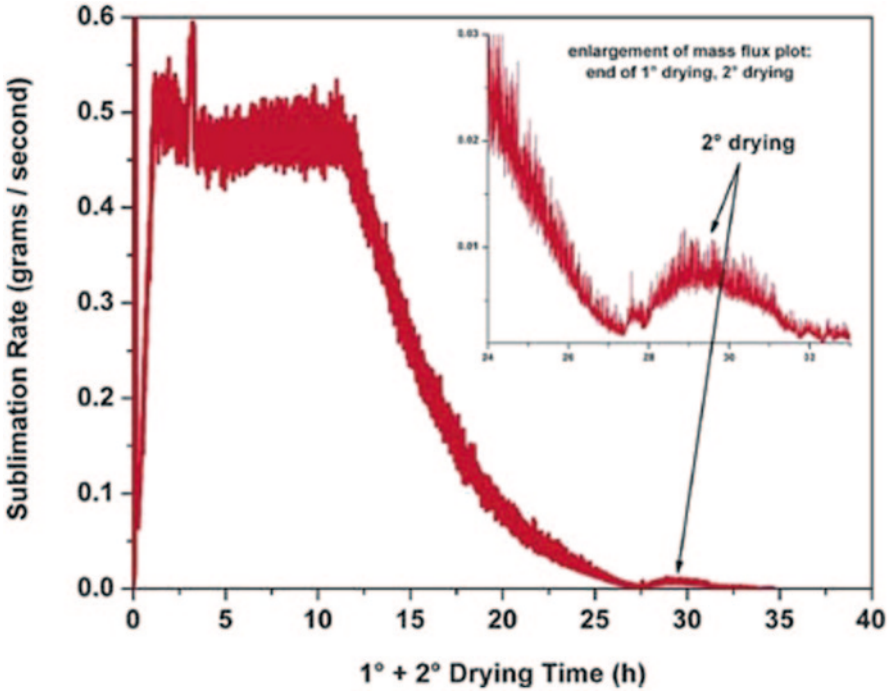

Fig. 4 Mass flow temporal profile over time for a 5% mannitol formulation in a pilot scale freeze dryer (Adapted with permission from [5])

about 20–30% higher than that of center vials. The combination of TDLAS mass flow rate measurements and the heat and mass transfer model based on a weighted K_v were employed for nonintrusive, real-time product temperature determinations. It was demonstrated that product temperatures calculated from TDLAS mass flow data were in excellent agreement with thermocouple data in the center vials during product runs with 5, 7.5, or 10% (w/w) sucrose, mannitol, and glycine, respect tively. TDLAS product temperatures for all freeze-drying runs were within 1–2 °C of "center vial" steady-state thermocouple data [29].

TDLAS has also been applied to the measurement of dry layer mass transfer resistance. By combining the TDLAS and the pore diffusion model, the effective pore radius of the dry layer can be estimated from the sublimation rate and product temperature profiles measured during primary drying. This method does not require solution of the complex heat and mass transfer equations, and has been demonstrated with product runs with 5% mannitol, sucrose, lactose, 3% mannitol plus 2% sucrose, etc. [12].

In addition to the process monitoring, effort has been made to achieve automatic product temperature control to reduce the primary drying time. Kuu et al. developed computer programs to determine the optimal shelf temperature and chamber pressure while ensuring the product temperature profile is below the target temperature

[10]. TDLAS was used to continuously measure the sublimation rate during 5% mannitol run, and the program was able to quickly perform the calculations based on heat and mass transfer equation. It was demonstrated that maximum product temperatures were controlled slightly below the target temperature, and a cascading temperature-ramping cycle is the most efficient cycle design [10].

Unlike thermocouple and other spectroscopic methods mentioned below, TDLAS gives the behavior for the whole batch. Overall, TDLAS shows a great potential to be used as an online monitoring tool for freeze-drying process, especially during manufacturing runs where the application of thermocouples is not possible. However, TDLAS also has some constraints. First, the integration of the technique to freeze dryer is not straightforward. The retrofitting of an old freeze dryer may be quite challenging, and the technique can only be applied to a freeze dryer with a long duct to allow absorption measurement at an angle (typically 45°) to the gas flow velocity vector. Second, the technology requires sophisticated calculations based on the gas concentration and gas flow velocity profile in the duct from the raw data. Therefore, depending on the duct size and configuration, the gas flow dynamics may be quite different between dryers. Spray balls and clean-in-place (CIP) nozzles in vapor duct at large scale may also impact calculations. In order to better understand these factors and facilitate process scale-up, more studies on large-scale manufacturing dryers are required.

Plasma Emission Spectroscopy (LyoTrack)

Lyotrack is a new online device developed to measure the humidity, and thus can be used to determine the endpoint of primary drying for the full batch. Lyotrack relies on inductively coupled plasma/optical emission spectroscopy (ICP/OES). This technology is adapted to the freeze-drying environment to measure water vapor concentration during the drying process [19]. The device is composed of two parts: the ICP plasma generator and the optical spectrometer. The ICP part creates a high power radio frequency wave, and the gas present in the freeze/dryer chamber forms cold plasma. The electrons from the gas atoms and molecules in the chamber are excited to a discrete higher energy state by absorbing this radio-frequency energy. The unstable excited atom will relax to its initial energy level and emit light. The optical spectrometer can thus identify the water vapor in the chamber based on the characteristic wavelengths of the emitted light.

Mayeresse et al. have systematically studied the Lyotrack using different formulations. The results indicate that Lyotrack can be used to determine the endpoint of primary drying with high sensitivity and good reproducibility [16]. The impact of probe location on the signal sensitivity was also studied, and it was found that the Lyotrack was sensitive to gas composition when it was placed in the chamber and in the duct connecting the chamber and the condenser but not in the condenser. The gas composition profile with drying time was similar when two lyophilizers of different scales were used for the same product, suggesting that the device can be used as a characteristic tool in process scale-up.

Hottot et al. compared five different types of sensors for the detection of the sublimation endpoint in syringe configuration [6]. Results showed that the Lyotrack and Pirani sensors provided similar results, with clear and precise sublimation endpoint determination. The Lyotrack sensor gave better signal-to-noise ratio than the Pirani gauge, which is possibly due to the signal disruption to Pirani caused by air injection for pressure regulation.

De Beer et al. simultaneously implemented four newly introduced PAT tools to monitor freeze-drying processes [2]. It was found that a combination of Raman and Lyotrack allows the monitoring of nearly all critical process aspects. Raman spectroscopy can give insight into the product behavior during freezing stage and also the bound water removal dynamics during secondary drying, whereas plasma emission spectroscopy gives information about the drying process.

The Lyotrack is simple to integrate into a lyophilizer via a tri-clamp flange due to its portable nature. Also, it is steam-sterilizable thanks to its durable construction. However, it also has some limitations. The humidity signal from the Lyotrack ranges between 0 (no water vapor) and 1 (saturated with water vapor), and it is difficult to transform this qualitative value into a measure of water quantity. In addition, Lyotrack involves ionization of the gas present in the chamber and the formation of free radical, and there is a potential risk to the stability of the freeze-dried product. In a recent study, oxidation of human growth hormone (HGH) was found to be significantly increased (about 12%) upon using Lyotrack in the drying chamber to freeze-dry HGH and HGH/sucrose formulations [20]. Thus, this technology could compromise the product quality of a molecule which is sensitive to oxidation. Moreover, Lyotrack is relatively expensive as compared to a Pirani gauge, and both sensors give very similar gas composition profiles, therefore, there is no strong advantage of using Lyotrack instead of the Pirani gauge for the primary drying endpoint determination.

Near-Infrared Spectroscopy

NIR is an effective tool for the understanding of the lyophilization process and evaluation of lyophilized pharmaceuticals. The NIR spectrum has a frequency range from 4000 to 12,500 cm^{-1} (800–2500 nm), and is highly sensitive to vibrational motions of the hydrogen atom in different molecular environments. Due to its ability to penetrate glass and plastic containers, NIR spectroscopy can also be used as a fast and noninvasive method to monitor the lyophilized product [1]. NIR has been not only used to determine the residual moisture in lyophilized samples but also evaluated for in-line process monitoring during freezing and drying stages. In addition, it offers many unique advantages such as the monitoring of protein conformational change and excipient morphology conversion during the drying process. NIR can therefore be a valuable tool for speeding the development of formulations and lyophilization process.

Off-Line Determination of Residual Water in Freeze-Dried Solids

Water exhibits strong absorption in the NIR region around 970, 1190, 1450, and 1940 nm [36]. Many efforts have been made to evaluate NIR spectroscopy as a fast nondestructive approach to determine the residual moisture content in the lyophilized products. Residual water was determined in freeze-dried sucrose samples using NIR diffuse reflectance spectrometry by scanning through the bottom of the glass vial [7]. A good correlation ($r^2 = 0.97$) was observed between residual water determined by traditional Karl Fischer method and NIR. The impacts of cake porosity, buffer concentration, and surfactant on NIR signal were also studied for a lyophilized monoclonal antibody system [14]. These factors do not have significant effect on the residual moisture when the cake thickness and diameter was much larger than the NIR penetration depth. However, it was found that the disaccharide concentration has a large impact on the NIR determined moisture content, which results from the strong NIR absorbance of disaccharide at the same wavelength as residual water. Therefore, the NIR method for moisture quantification is highly sensitive to the drug formulation, and a calibration curve needs to be developed against the standard Karl Fischer method. It was reported that the calibration curve developed for one formulation cannot be used for another drug concentration [13]. Even for a given formulation, samples from different batches could give differences in the correlation between NIR and Karl Fischer. Therefore, it is critical to develop robust calibration curves incorporating all relevant factors and manufacturing process variables.

In-Line Process Monitoring for Protein Conformation and Protein–Excipient Interactions

NIR has also been used as in-line PAT tool to monitor the lyophilization process since water and ice give strong signals in NIR spectra. De Beer et al. have studied the performance of a NIR probe directing to the sidewall of a vial for mannitol formulation [4]. Chemometric tools were used to extract useful information from the large raw data sets, and it was found that mannitol starts to crystallize when ice crystallization is complete. Since sublimation finishes at the bottom center of the vial, the drying endpoint can also be measured by the NIR tool. However, NIR may underestimate the drying endpoint as compared to the endpoint measured by Lyotrap method.

Another application of NIR spectroscopy is the differentiation of mannitol hydrate and surface water. The different crystalline mannitol polymorphs can be distinguished via NIR in the 4330–4450 cm^{-1} spectral range, and a nondestructive method was developed to determine the amount of metastable mannitol hydrate and surface water in lyophilized products [3]. This study indicated that NIR could be employed to monitor the formation of mannitol hydrate during lyophilization process.

NIR was recently used as an in-line process analyzer for monitoring protein unfolding and protein–lyoprotectant interactions during drying [21]. The amide A/II band near 4850 cm^{-1} was monitored along with the water absorbance band near 5160 cm^{-1}. This amide A/II frequency was found to correlate well with the water absorbance intensity during protein dehydration in the absence of protein unfolding, whereas deviation from the linear relationship was found to be related to protein unfolding. For sucrose formulations, the amide A/II frequencies decrease immediately after sublimation, suggesting an increase in protein–sucrose hydrogen bond interaction. This approach could provide more insights about protein conformational stability and the lyoprotectant interaction with protein at real time during the dehydration process.

Even though NIR has demonstrated some utility for in-line process monitoring, the technique has several limitations. First, NIR is based on the detection of a single vial in the edge, and only small part of the cake is measured by the NIR probe. Therefore, the result measured is not representative of the entire batch. Second, this technique requires an unobstructed view of the NIR probe to the contents of the vial and the insertion of a fiber-optic cable into the chamber, which makes it difficult to integrate into a manufacturing scale lyophilizer. While NIR has low potential as an in-line PAT tool for freeze-drying, it could find off-line applications as a nondestructive method for high throughput residual water measurements.

Raman Spectroscopy

Since water and ice are weak Raman scatterers, Raman spectroscopy is becoming popular for monitoring formulation characteristics during the freeze-drying process. De Beer et al. have studied Raman as the PAT to monitor the endpoint of primary drying. The Raman probe was placed above the product vial in the drying chamber. The Raman technique gave a significantly lower endpoint of primary drying, which was due to the inappropriate placement of Raman probe [3]. However, Raman spectroscopy was able to provide information about the endpoint of freezing and the mannitol solid-state conversion throughout the entire process. It was reported that a combination of Raman and Lyotrack allows the monitoring of nearly all process aspects. Raman spectroscopy can provide insights into the product behavior during freezing stage and also the dynamics of bound water removal during secondary drying. Conversely, Lyotrack gives information about the primary drying endpoint [4].

Recently, Raman spectroscopy was used for in-line monitoring of a microscale freeze-drying process [8]. The effect of cooling rate and annealing step on the solid-state formation of mannitol was studied. Principal component analysis was used to qualitatively analyze the solid-state forms of mannitol, while classical least-squares regression analysis was used to estimate the solid-state form ratios of each polymorphism. The results showed that mannitol hemihydrate emerged and subsequently transformed to more stable forms during the secondary drying step. Similar to NIR, the Raman measurement is based on one vial, and it is not representative of

the whole batch. In addition, since it requires a special arrangement of the Raman probe, it will be difficult for an in-line application in an industrial lyophilizer.

Impedance Spectroscopy

During this literature review, a potential new technology to measure product temperature was identified. An impedance spectroscopy method was introduced as a minimally invasive method of measuring product temperature during the freeze-drying process [30]. This approach was shown to be useful to monitor the freezing step, but at this point in the early development of the technology, it cannot monitor the product temperature during primary drying. The experimental setup consists of placing planar electrodes on the external vial wall, and these electrodes are then coupled to a high impedance analyzer. The impedance measurement is converted to temperature using an algorithm [30]. The placement on the external vial wall does not influence the nucleation event of the freezing process. If this technology evolves to monitor the product temperature during primary drying, it will significantly improve its value to lyophilization cycle monitoring. However, similar to the thermocouple approach, this system must be applied to the vial manually, which means that it would be a challenge to apply to commercial process monitoring.

Summary

Table 1 summarizes the major advantages and disadvantages of each PAT tool discussed above. It gives an overview of each PAT tool's capabilities and its practical application and scalability to large-scale freeze dryer. The major limitations associated with each tool are also listed in the Table.

Conclusions and Future State

Based on the QbD requirements, an ideal PAT tool for freeze-drying should be able to meet the following needs: (1) monitor the product temperature during *all* steps of freeze-drying process including freezing, primary drying, and secondary drying, and the temperature measured should be representative of the whole batch; (2) determine the endpoint of major steps including primary drying and secondary drying; (3) monitor the sublimation and determine the residual moisture during primary drying and secondary drying; (4) provide real-time product quality information such as the protein structure and excipient phase transition; and (5) integrate to different types of lyophilizer from laboratory scale to GMP manufacturing scale.

Table 1 Summary table for assessed online PATs

Criteria	Thermo-couple	TEM-PRIS	Pirani versus CM	MTM	TDLAS	Lyotrack	NIR	Raman	Impedance spectroscopy
Sensitive to placement positions	+	+	−	−	−	−	+	+	+
Measure product temperature	+	+	−	+	+	−	−	−	−
Detect endpoint of primary drying	+	+	+	+	+	+	−	−	−
Monitor moisture level	−	−	−	−	+	−	+	−	−
Monitor molecular structural change	−	−	−	−	−	−	+	+	−
Representative to the whole batch	−	−	+	+	+	+	−	−	−
Noninvasive (no direct product contact)	−	−	+	+	+	+	+	+	+
Simplicity of integration and use	+	+	+	+	−	+	−	−	+
Application to large-scale dryer	+	+	+	−	+	+	−	−	−

(+) indicates the ability to monitor the considered process aspect, while (−) means the non-applicability of the tool
TEMPRIS temperature remote interrogation system, *MTM* manometric temperature measurement, *TDLAS* tunable diode laser adsorption spectroscopy, *NIR* near-infrared *CM* capacitance manometer

Upon review of PAT technologies available to date, we found that not a single PAT tool currently exists to cover these broad requirements, possibly due to the harsh conditions of the freeze-drying process (deep vacuum, low temperature during freezing, high temperature during sterilization, and sterility requirement at GMP environment). Most PAT tools only meet one or two criteria listed above, and we currently have to rely on the combination of several PAT tools in order to achieve this ideal state. In addition, while more options are available as PAT tools for application in a small-scale lyophilizer, there are fewer PAT tools which can be used in GMP commercial manufacturing scale lyophilizer. Therefore, further development of these existing and new PAT tools to cover these gaps is strongly needed.

Product temperature monitoring with thermocouples will still be used to develop a safe lyophilization cycle. Traditionally, thermocouples or RTDs have been used to monitor product temperature in real time during technology transfer and scale-up. Over the years, thermocouples have even been used to control freeze-drying cycles during commercial manufacturing. However, thermocouples are less than ideal due to their effect on the nucleation process and potential sterility challenges when used in a GMP environment. Other approaches such as TEMPRIS or the new impedance spectroscopy system may be a more advanced means to measure product temperature, but they are not yet ready to replace thermocouples due to their limitations. The invasive TEMPRIS yielded data not representative to the other vials in the chamber, and the impedance system is limited at this point to the freezing step. MTM can measure average product temperature for the whole batch and it is very useful for development; however, MTM is limited to laboratory and pilot freeze dryer and is not scalable to a large-scale manufacturing dryer.

From a primary drying endpoint perspective, the Pirani gauge remains the best option for reasons outlined earlier in the chapter. Lyotrack can be a good option when used with a stable system which is not susceptible to oxidation degradation. A Pirani gauge is less expensive than Lyotrack, while it can provide very similar gas composition profiles. In addition, the Pirani gauge may also be used as a PAT control tool if a feedback loop was programmed to continuously measure the ΔP between the Pirani and the CM and allow the automatic progression to next step when $\Delta P = 0$ is reached at the endpoint of the primary drying.

Several new PAT tools such as TDLAS, NIR, and Raman have been developed in the past decades, and they have provided insights about the water removal and process transition. Among them, TDLAS is the most promising PAT tool as it can meet several criteria for lyophilization. First, it can measure sublimation rate during the primary drying and secondary drying process, and has the potential to monitor the residual moisture in the cake during secondary drying. Second, it can be used to determine the endpoint of both primary drying and secondary drying based on the change in sublimation rate. Third, based on the steady-state heat and mass transfer model, the signal measured can be used to obtain the average product temperature during primary drying, which will give the behavior for the whole batch. In addition, it can provide information about the vial heat transfer coefficient and dry layer mass transfer resistance. Research is currently ongoing to assess TDLAS for

other applications and thus far, even with certain limitations, this system looks very promising.

In summary, the end of thermocouple as an application to lyophilization does not appear to be anytime soon. There are other systems available to monitor the product temperature profile, which is ultimately the critical parameter during lyophilization. In terms of primary drying endpoint, tools such as a Pirani gauge and MTM are excellent for pilot scale applications; a Pirani gauge can also be used in commercial manufacturing. To date, it appears that TDLAS is the most promising PAT tool used during the freeze-drying process. TDLAS is applicable to more than one step of the freeze-drying process. It can be used to monitor product temperature during primary drying and determine primary drying endpoint, and also has the potential to control the secondary drying step by monitoring the residual moisture in the cake in real time. Development of new PAT tools for lyophilization is still needed in order to meet the multiple QbD requirements. The most applicable PAT tools will be able to monitor and control multiple steps of the cycle and also assure the product quality attributes in real time.

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