ABSTRACT: A cold plasma ionization device has been designed to monitor freeze-drying processes in situ by monitoring lyophilization chamber moisture content. This plasma device, which consists of a probe that can be mounted directly on the lyophilization chamber, depends upon the ionization of nitrogen and water molecules using a radiofrequency generator and spectrometric signal collection. The study performed on this probe shows that it is steam sterilizable, simple to integrate, reproducible, and sensitive. The limitations include suitable positioning in the lyophilization chamber, calibration, and signal integration. Sensitivity was evaluated in relation to the quantity of vials and the probe positioning, and correlation with existing methods, such as microbalance, was established. These tests verified signal reproducibility through three freeze-drying cycles. Scaling-up studies demonstrated a similar product signature for the same product using pilot-scale and larger-scale equipment. On an industrial scale, the method efficiently monitored the freeze-drying cycle, but in a larger industrial freeze-dryer the signal was slightly modified. This was mainly due to the positioning of the plasma device, in relation to the vapor flow pathway, which is not necessarily homogeneous within the freeze-drying chamber. The plasma tool is a relevant method for monitoring freeze-drying processes and may in the future allow the verification of current thermodynamic freeze-drying models. This plasma technique may ultimately represent a process analytical technology (PAT) approach for the freeze-drying process.

KEYWORDS: Freeze-drying, Process analytical technology, PAT, Cold plasma, Ionization, In-process monitoring

Introduction

Lyophilization, or freeze-drying, is the method of choice for biologicals stabilization. This predominantly three-phase process comprises formulation freezing, sublimation, and secondary drying. The first two phases are performed below the glass transition temperature (Tg'), and sublimation is also performed under vacuum to remove crystallized ice (1) (2). The product is warmed up during secondary drying in order to desorb unfrozen and sorbed water (3). Since its early development in the 1950s, freeze-drying has undergone many mechanical and automatism improvements, including sterilization, in-place cleaning, automatic loading and unloading, and process monitoring. Nowadays, freeze-dryers are essential equipment and require operators to demonstrate many competencies and skills.

As the complexity of biomolecules increases, the stabilization needs become critical. Recent biotech products require well controlled stability, and today, the freeze-drying process remains a unique stabilization process (4) (5). In the case of vaccines, antigenicity is linked to complex glycoprotein conformations or live organism preservation. Therefore, vaccine freeze-drying covers the most stringent formulation and process conditions. The complexity of the freeze-drying process depends upon the freezing behavior of formulations, thermodynamic thermal transfers, and gas flow dynamics under vacuum (6) (7) (8).

As the equipment, products and processes are becoming increasingly complex, the control of the freeze drying process is critical. This is achieved by controlling the following parameters:
During freeze-drying it is essential to monitor dependent parameters such as sublimation endpoint and product temperature. The sublimation endpoint is critical in two scenarios:

- If the secondary drying is launched before the sublimation endpoint, the product temperature may exceed $T_g$, causing some vials to collapse and an increased defect rate. This is a quality issue.
- If the secondary drying is delayed after the sublimation endpoint, the cycle will not be well optimized and some freeze-drying capacity may be wasted. This is a business issue.

With respect to product temperature measurement, new freeze-dyers often have automatic loading devices, which reduce the need for manual handling of vials. This is beneficial, as operators remain a major source of contamination. However, with automatic loading devices, the placement of product temperature probes is no longer possible, and product temperature profiles are no longer available, even if product temperature measurement is challenged. This results in a lack of information for producers, quality assurance officers, and authorities.

The aim of the process analytical technology (PAT) initiative, which was launched by the Food and Drug Administration in 2002, was to promote a better understanding and control of processes. As a result, on-line monitoring devices have been developed. PAT also aims to finalize the quality decision process during the manufacturing process through the assessment of critical process parameters, method development and scaling up, capability assessment, and the deployment and transfer to industrial scale.

However, the deployment of PAT with respect to freeze-drying faces the major barrier of lack of availability of compatible monitoring equipment. In fact, the extreme conditions of the freeze-drying process (vacuum, aseptic rules, and low temperature) render many devices inappropriate. Therefore, the quest for new monitoring devices to control the freeze-drying process with a high degree of assurance has become essential.

This section reviews the PAT tools currently used for monitoring the freeze-drying process with respect to the following criteria:

1. Global load monitoring. As freeze-drying is dependent upon heat and mass thermal transfer, some heterogeneities may limit control. It may be erroneous to rely on individual vial measurement to control the whole load.

2. Compatibility with automatic loading/unloading devices. The placement and removal of vials must not be impaired.

3. Compatibility with cleaning in place (CIP)/stopping devices. No leads should compromise the movement of shelves, CIP ramps, or nozzles.

4. Compliance with aseptic handling. There should be no source of contamination within the materials or during positioning.

5. Steam sterilization. The device must sustain repeated steam sterilization at a minimum of 123 °C and 2 bars for a duration of 3 h.

6. Leakage control. Placement of the device should not induce freeze-dryer leakage. It should also support at least a 5-$\mu$ bar vacuum, and measurement should be independent of equipment leak rate.

7. Simple integration into an industrial freeze-dryer. The device should be installed to current existing ports using triclamp flanges, and the data acquisition signal should be compatible with 21CFR Scada/recorders.

8. Calibration or correlation to primary methods (see Table I).

Few types of equipment fulfill all the success criteria (9) (10) (11) (12) (13) (14) (15) (16) (17) (18). For example, some can monitor whole loads but are hardly steam sterilizable or compatible with leak control. The moisture probe presented by Roy et al. (19) was promising, but failed to reach production scale as it could not sustain steam sterilization. The last two devices in
### TABLE I
Technology Comparison

<table>
<thead>
<tr>
<th>Success Factor</th>
<th>Monitor Global Load</th>
<th>Automatic Loading</th>
<th>CIP + Stoppering Device</th>
<th>Aseptic Handling</th>
<th>Steam Sterilizable</th>
<th>Leak Rate Control</th>
<th>Simple Integration</th>
<th>Calibration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature probe</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>+/-</td>
<td>YES</td>
<td>YES</td>
<td>+/-</td>
<td>YES</td>
</tr>
<tr>
<td>Wireless product temperature probe</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>+/-</td>
<td>YES</td>
<td>YES</td>
<td>+/-</td>
<td>YES</td>
</tr>
<tr>
<td>Conductivity probe</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>Microbalance</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>+/−</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>FTNIR product probes</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>YES</td>
<td>+/−</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>Pirani/capacitive differential pressure control</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>+/−</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>Moisture probe</td>
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<td>YES</td>
<td>NO</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>Pressure rise measurement</td>
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<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>NO</td>
<td>YES</td>
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</tr>
<tr>
<td>Mass spectrometry</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>NO</td>
<td>NO</td>
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</tr>
<tr>
<td>TDLAS</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>Cold plasma</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
</tr>
</tbody>
</table>

FTNIR: Fourier transform infrared.
TDLAS: Tunable diode laser absorption spectroscopy.
Table I could potentially be valuable for freeze-drying monitoring. However, a full cost-benefit analysis needs to be undertaken for each technique.

In the present study, we evaluate the cold plasma technique for monitoring freeze-drying on a pilot, scaling-up and industrial scale, with respect to comparison with other methods (microbalance, Pirani), reproducibility, product signature in scaling-up, and industrial lots monitoring.

Materials and Methods

Cold Plasma

This equipment is based on the inductive coupled plasma/optical emission spectroscopy (ICP/OES) technique, which is normally used in chemical and gas analysis (Lyotrack, Adixen, France). Although this type of instrument used to be larger, recent improvements have allowed size reduction. It is now similar in size to a pressure gauge. The device is composed of a quartz tube that directly contacts the lyophilization chamber, and it is connected to the freeze-dryer ports via an ISO-KF25 or triclamp flange. All parts of the device that make contact with the internal atmosphere of the freeze-dryer are constructed from stainless steel, quartz, or FPM (fluoroelastomer gasket), and can withstand sterilization conditions at 2.3 or 2.3 bars and 135 °C. A plasma sensor measures the ratio of water vapor to nitrogen under vacuum conditions (labeled as humidity in the software). The principle of the plasma sensor is portrayed in Scheme 1. A radio frequency source with a low power consumption (<15 W) creates a cold plasma in the quartz tube under vacuum below 3 mbar. The plasma remains active in the vacuum range from 3 to 0.005 mbar, which is compatible with the pressure range utilized in lyophilization processes. The light emitted by the plasma is characteristic of the gas present in the plasma and can be collected with an optical fiber and diffracted with an optical spectrometer. The optical spectrum is analyzed by the plasma sensor software, and the curve of humidity is displayed in real time. The humidity signal from the plasma ranges between 0 (no water vapor) and 1 (saturated with water vapor). Scheme 2 summarizes the integration of the device to the computer and recorder. Humidity values may also be transferred from the sensor via a 0–10-V analog output. This signal may be further integrated to a 21 CFR digital recorder.

The software delivers a graphical display of the plasma signal curve versus time (Scheme 2). The primary drying endpoint can be determined by signal treatment using an adjustable threshold or signal first derivative.

Microbalance

A microbalance was used as a reference method for sublimation monitoring in pilot freeze-dryers (20). This allows the direct measurement of mass transfer.
compared with Lyotrack, which measures vapor saturation in the chamber; the product probe monitors the heat and mass transfer balance. The microbalance is placed directly on the freeze-dryer shelf (CWS-40 Martin Christ, Germany). The microbalance servomotor, which is controlled by a personal computer interface with Labview® platform software, lifted vials every 5 min for weight loss monitoring and then replaced them on the shelf. The data were treated using Statistica 7.1 software (Statsoft, USA).

Freeze-dryer Load

In the pilot study, formulations were prepared with sucrose 2%, 5%, 10% w/w and mannitol 4% w/w (Merck, Germany) and water for injection (WFI). Type I glass vials (Forma Vitrum, Switzerland) of 3 or 9 mL volume were filled with 2 mL of the prepared solution and stoppered with bromobutyl stoppers (Helvoet, Belgium).

Freeze-dryer

Four freeze-dryers were used in our study:

- Pilot freeze-dryer, D25 Dry-winner (Heto, Denmark)
- Pilot freeze-dryer, Epsilon D25 (Martin Christ, Germany)
- Scaling-up freeze-dryer, Finn Aqua FCM30D (GEA, Germany)
- Industrial freeze-dryer GT500 (GEA, Germany)

The pilot freeze-dryers used were mounted with a Pirani ACG controller (BOC Edwards, USA) and a capacitive pressure gauge (MKS, USA). Data acquisition was performed with a DX200P recorder (Yokogawa, Japan). The data were treated using Statistica 7.1 software (Statsoft, USA).

Freeze-drying Cycle

The basic freeze-drying cycle was defined as following: Freezing below –40 °C for at least 1 h; sublimation at 0.080 mbar (106 mtorr), with a temperature ramp of 3 h to –21 °C and holding –21 °C for at least 18 h.

Results

Use of the Cold Plasma Sensor

Figure 1 presents a typical response of the cold plasma during a freeze-drying cycle. The plasma device was placed on top of the lyophilization chamber. The load was composed of 11,000 vials (eleven thousand).
limination end-point. A correlation to other end point assessment methods will be proposed later.

At 28 h, secondary drying was carried out with a shelf temperature rise and pressure dropdown. The pressure was pulled down to maximal vacuum in 4 h. At lower pressure (0.015 mbar), the relative composition of gas is changed. The sensor response detected this pressure drop, and the baseline signal shifted to 8.5% of relative saturation. When the shelf temperature was increased after 31 h, moisture was desorbed from the cakes and vapor flow increased. The sensor response increased to 16% of relative saturation and ultimately stabilized at 11% after 5 additional hours.

Positioning the Sensor

As the positioning of the sensor on the freeze-dryer may have an impact on the signal, three different positions were tested:

- on the lyophilization chamber port
- on the condenser port
- between chamber and condenser

The same freeze-drying cycle and load was used throughout (200 vials, filled volume 0.5 mL with sucrose 5%). A pilot freeze-dryer equipped with multiple ports was used for this study. The three different responses of plasma are plotted on Figure 2. When the cold plasma sensor was located on the chamber, the response signal was higher and more stable than when placed between the chamber and condenser. If the plasma sensor was placed on the tubing between the chamber and condenser, the signal was high, but was more influenced by changes in pressure. The measure was performed using a dynamic flow with controlled pressure. As long as the pressure was regulated by nitrogen injection, the gas content changed sensitively. Thereafter, the plasma response may be affected.

At the end of sublimation, the signal remained higher in the chamber than at the in-between position. When the device was settled on the condenser, no response to vapor was observed, indicating that this is not a suitable position for monitoring the lyophilization process. Setting on either the chamber or an intermediate position, therefore, seems the best location, but larger-scale testing and differ-

Figure 2

Plasma response in relation to positioning (+ chamber, - between chamber and condenser, △ condenser).
ent geometries will be necessary to optimize positioning.

**Limit of Sensitivity and Correlation with Microbalance**

The cold plasma sensor limit of sensitivity was established in the pilot study, when the minimal load that the plasma device could monitor was evaluated. In the same set of experiments, the plasma system was compared to other equipment used for freeze-drying monitoring. In the first instance, a microbalance was used to correlate the plasma sensor signal with a single vial. In order to track most of the vapor leaving the vial, the device was positioned on the tubing between chamber and condenser.

A design of experiment (DOE) plan was initiated using eight different freeze-drying cycles. The input process parameters were chamber pressure (10.10^{-6} bar; 200.10^{-6} bar), shelf temperature (−31°C; −11°C), and sucrose concentration (WFI 100%; sucrose 10% w/w). The studied responses were microbalance sublimation endpoint and plasma sensor sublimation endpoint.

The experiments were conducted according to a full factorial plan using three factors with two levels. The sublimation endpoints were determined by the first derivative of the signals (Figure 3). When only one vial was sublimed, the two methods for sublimation endpoint evaluation were well correlated; the R^2 factor is above 0.99 (Figure 4).

**Cold Plasma Sensor Comparison with the Pirani-capacitive Measurement and Product Probe**

The cold plasma response was compared to the Pirani-capacitive differential pressure measurement and the product temperature probe, other common devices for freeze-drying monitoring on a larger scale. The objective was to compare the sublimation endpoint with the three methods for freeze drying 1000 vials. The reference methods used were

- individual vial monitoring with a product temperature probe (Pt100)
- global load monitoring with a Pirani-capacitive differential pressure measurement

After 12.5 h of primary drying, the three signals simultaneously began to evolve. As the product temperature started to rise from −35 °C to the shelf tem-

<table>
<thead>
<tr>
<th>D.O.E. Pattern</th>
<th>Shelf temperature</th>
<th>Chamber pressure</th>
<th>Formulation dilution</th>
<th>Sublimation endpoint time</th>
</tr>
</thead>
<tbody>
<tr>
<td>- - -</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
<td>20:47</td>
</tr>
<tr>
<td>+ - -</td>
<td>1</td>
<td>-1</td>
<td>-1</td>
<td>15:06</td>
</tr>
<tr>
<td>- + -</td>
<td>-1</td>
<td>1</td>
<td>-1</td>
<td>27:50</td>
</tr>
<tr>
<td>+ + -</td>
<td>1</td>
<td>1</td>
<td>-1</td>
<td>12:28</td>
</tr>
<tr>
<td>- - +</td>
<td>-1</td>
<td>-1</td>
<td>1</td>
<td>16:55</td>
</tr>
<tr>
<td>+ - +</td>
<td>1</td>
<td>-1</td>
<td>1</td>
<td>12:36</td>
</tr>
<tr>
<td>- + +</td>
<td>-1</td>
<td>1</td>
<td>1</td>
<td>22:05</td>
</tr>
<tr>
<td>+ + +</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>11:45</td>
</tr>
</tbody>
</table>

**Figure 3**

Results obtained with the parallel testing of plasma sensor and microbalance.

![Figure 3](image-url)

<table>
<thead>
<tr>
<th>Sublimation endpoint with Plasma and microbalance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma endpoint = -0.0296 + 1.0296 x microbalance endpoint, R^2 = 0.99, (---) 95% confidence interval of regression.</td>
</tr>
</tbody>
</table>

**Figure 4**

Sublimation endpoint of one single vial determined by plasma sensor and microbalance; (o) Individual sublimation endpoint, (—) sublimation endpoint regression, plasma sensor endpoint = −0.0296 + 1.0296 x microbalance end point, R^2 = 0.99, (----) 95% confidence interval of regression.
temperature (–20 °C), both the Pirani and plasma sensor signals decreased. After 15 h 20 min, the product temperature reached the shelf temperature, indicating that the monitored vial had no more sublimation endotherm and the sublimation endpoint had been achieved for this vial. The product probe signal matched the plasma sensor signal. However, this correlation was not extensively studied because product temperature is dependent on temperature probe positioning inside the vial, and the probe may monitor heat transfer rather than mass transfer.

The Pirani signal exhibited a small sigmoid decrease up to 14.5 h, but the signal was perturbed by the nitrogen injections for pressure control, suggesting that this system is not ideal for the measurement of signal stabilization.

The plasma signal marked a deep sigmoid decrease from 1 to 0.1 arbitrary unit (au) after 15 h, where 1 au indicates that the atmosphere of freeze-dryer is only composed by water vapor and 0 au only composed by nitrogen. This signal continued to decrease until 18 h, when the signal derivative was less than 0.001 au per hour (Figure 5).

Reproducibility Between Batches

Another evaluation test was performed on a larger scale, utilizing up to 11,000 vials (3 mL) per load. The plasma sensor was positioned on the top of the chamber. The objective was to test signal reproducibility after three consecutive placebo runs under the following conditions:

- same equipment
- same load (vial number, formulation, filling volume)
- same freeze-drying cycle

As shown in Figure 6, the plasma responses were very similar from one cycle to another. The sublimation endpoint and the secondary drying signal were nearly the same between the three cycles. The plasma signal

![Figure 5](image-url)

**Figure 5**

Sublimation endpoint determination with three methods: Plasma sensor (+), Product temperature (–), Pirani-capacitive relative signal (○).

![Figure 6](image-url)

**Figure 6**

Evaluation of plasma sensor consistency during three consecutive lots under the same conditions (product, lot size, cycle, freeze-dryer). Lot 1 (—), Lot 2 (—–), Lot 3 (—·—).
Use of Plasma in Scale-up Freeze-dryers

This later study focused on the scaling-up of the lyophilization process. Scaling-up is a sensitive issue because freeze-dryer geometry may vary from one piece of equipment to another. In this study, the identical placebo product was scaled up from pilot (210 vials in a 0.2-m³ chamber) to scaling-up freeze-dryer (11,600 vials in a 3.5-m³ chamber). In both tests, the plasma sensor was positioned in the lyophilization chamber. As seen in Figure 7, although though the signal was relatively higher for the pilot freeze-dryer, the plasma responses between the two lot sizes exhibited a very similar shape. For the two cycles, the setting and the product were the same. The sublimation endpoint was similar between the two lot scales, but the sublimation lasted for 1 more hour with the pilot freeze-dryer. The secondary maximal signal, which corresponds to the sorbed moisture release, appeared at approximately the same time, with less than a 15-min delay for a scaling-up size.

Use of Plasma Sensor for Secondary Drying Monitoring

The secondary drying behavior of different formulations was compared under the same process conditions, using an amorphous sucrose formulation and a crystalline formulation of mannitol with some mannitol hydrate (a complementary study by X-ray diffraction showed some mannitol hydrate formation).

With the sucrose formulation, the plasma sensor signal showed a fast release of moisture upon desorption (Figure 8). Once the shelf reached the target temperature, the sorbed moisture was released and equilibrium was reached asymptotically. However, for crystalline mannitol the plasma sensor signal did not increase immediately, even though the target shelf temperature was reached (Figure 8). In the case of crystalline mannitol, the structure retained more moisture upon desorption, and the plasma sensor was able to track this behavior. The last placebo test was conducted on a full industrial scale, involving over 100,000 3-mL vials and a 3-day cycle. The plasma sensor was set at the top of the approximately 15-m³ freeze-dryer chamber. As shown in Figure 9, the...
plasma device remained sensitive under these conditions. When sublimation commenced, the signal rapidly reached saturation. After 20 h the signal started to decrease in a sigmoid shape, but increased again when the signal was close to stabilization. At this stage the shelf temperature was reasonably increased to accelerate the sublimation endpoint. The plasma signal slowed down as the sublimation of remaining vials stopped. At the sublimation endpoint the signal stabilized at a higher level than during scaling-up tests. At the beginning of secondary drying, the pressure was reduced to full vacuum, and the sensor signal increased because of proportional changes in chamber gas content. When the shelf temperature was raised, the plasma sensor responded to the release of sorbed moisture. After 6 h the signal started to return to its initial level during secondary drying, indicating the potential endpoint of secondary drying.

The cold plasma sensor demonstrated an ability to monitor industrial-sized lots. The persisting high moisture signal at the end of primary drying suggests that top chamber moisture remains high and that positioning would be best suited in the vapor flow pathway.

Discussion

In order to become accepted as a suitable in-process monitoring device, the cold plasma system has to demonstrate sensitivity, reproducibility, and correlation to existing methods. In this series of studies, a cold plasma system for the monitoring and validation of freeze-drying cycles was assessed at the research and development level, during scaling-up, and on an industrial scale.

As a research and development tool, this system provides a greater understanding of the kinetics of the freeze-drying cycle. The main application for this technology is an accurate determination of the sublimation endpoint, based on the quantity of remaining water molecules in the freeze-drying chamber. One of the challenges of freeze-drying validation is to prove consistency throughout process parameter testing and product quality attributes. The process parameters are either direct (shelf temperature, chamber pressure, moisture content) or indirect (product temperature). With the cold plasma sensor, a direct parameter is monitored for the full load of the freeze-dryer. In comparison, product probes only measure an indirect parameter (the endothermic effect generated by sublimation) on an individual vial within the load.

Within a freeze-dryer chamber, there are two ways to control pressure: either by injecting nitrogen to compensate the vacuum generated by the pumps, or by directly controlling the vacuum pumps with a valve. With nitrogen injection control, when the pressure decreases, the quantity of nitrogen is reduced in relation to the water molecules. The cold plasma sensor signal is therefore sensitive to changes in pressure, due to the relative quantitative measurement of water versus nitrogen inside the freeze-dryer. Secondary drying is often associated with a drop in pressure and subsequent modification in the water-to-nitrogen ratio, as the pressure control is achieved through the injection of nitrogen. The plasma signal should therefore be normalized in relation to total pressure in order to avoid this baseline signal shift during secondary drying.

The cold plasma device also allows gas dynamics inside a freeze-dryer to be evaluated. As shown in Figure 7, under non-saturated conditions, there is a clear difference between pilot and scale-up freeze-dryers with respect to nitrogen injection. As nitrogen molecules are heavier than water molecules and have a different thermal conductivity (nitrogen $0.146 \text{ W} \cdot \text{m}^{-1} \cdot \text{°C}^{-1}$, water $0.603 \text{ W} \cdot \text{m}^{-1} \cdot \text{°C}^{-1}$), the ratio of gases present in the freeze-dryer will affect the heat transfer between the shelf and the vial at low pressure. The gas composition will in turn influence the sublimation speed, resulting in a different cycle time. This explains why it is not equivalent to control the freeze-dryer pressure by closing and opening the vacuum.
pump valves or by injecting a gas. This phenomenon has a practical application for the study of pressure control and its impact on the freeze-drying process between different freeze-dryers. Indeed, the cold plasma system can help to fine-tune a good regulation of nitrogen inside the freeze-dryer.

The signal response of the system in relation to probe positioning confirms that water vapor repartition inside a freeze-dryer is not homogeneous during the cycle. With respect to chamber position, the signal slope shows a smaller decrease when it is located in the tubing between the chamber and condenser. This may be explained by the difference in volume between these two parts of the freeze-dryer and by the proximity of the coils to the separation tubing. At the beginning of the cycle, the first water molecules to escape from the vials travel randomly inside the chamber and bounce off the walls and shelves until they reach the condenser coils. As the shelf temperature increases, the system becomes saturated with water molecules and a gradient of water molecules is created between the condenser, in-between tubing, and chamber. This gradient of water molecules creates a diffusion flow from the chamber towards the condenser. Each water molecule escaping from a vial is directly taken by the flow of the others and pushed towards the condenser, where the cold coils induce condensation. This is confirmed by the low values of water inside the condenser. When the primary drying endpoint is reached, the flow of water molecules ceases and a sharp reduction in water molecules inside the tubing is noted. Equilibrium is finally reached when a quantity of molecules remain evaporating in the chamber (desorption phase).

Following the law of probability, the water molecules finding their way towards the condenser through the tubing, generally represent 1/30 of the chamber surface. Upon entering the tubing they diffuse towards the cold coils. As the coils have a lower temperature, a partial water pressure difference is created locally. An interesting application of the cold plasma sensor is to undertake humidity mapping of the freeze-dryer. If measurements are made at different points during the freeze-drying cycle, these data can be used for a numerical analysis of mass transfer during the process leading to a more precise modelization for the kinetics of mass transfer in larger freeze-dryers. Indeed, many models for freeze-drying have focused on the mass balance within the vial (21):

\[
Q_{in} = Q_{out} \\
Q_{in} = A_v \cdot K_v \cdot (T_s - T_p) \\
Q_{out} = \Delta H_{sub} \cdot (dm/dt) \\
A_v \cdot K_v \cdot (T_s - T_p) = \Delta H_{sub} \cdot (dm/dt) (1)
\]

where

\[
Q_{in} = \text{heat flow (cal/h or W)} \\
A_v = \text{internal surface of vial (m}^2\text{)} \\
T_s = \text{shelf temperature (K)} \\
T_p = \text{product temperature (K)} \\
Q_{out} = \text{mass flow (cal/h or W)} \\
\Delta H_{sub} = \text{enthalpy of sublimation (2,839,000 J/Kg)} \\
(dm/dt) = \text{mass transfer rate = sublimation rate (Kg/h)} \\
(dm/dt) = A_v \cdot dL/dt, \text{ with } dL/dt = \text{the position of the subliming front as function of time} \\
K_v = \text{heat transfer coefficient (W} \cdot \text{m}^{-2} \cdot \text{K}^{-1})
\]

Freeze-drying modelling should consider the global pathway of moisture from the vial to the condenser, and some authors have proposed the Knudsen formula to express the sublimation speed \( G_{sub} \):

\[
G_{sub} = \kappa P_{sat} \left( \frac{M^{1/2}}{2\pi RT} \right), \quad 0 < \kappa < 1 (2)
\]

where

\[
\kappa = \text{the coefficient of evaporation} \\
P_{sat} = \text{the saturated vapor pressure of ice} \\
M = \text{the molecular weight of water vapor} \\
R = \text{the gas constant} \\
T_{sat} = \text{the absolute temperature of sublimating ice}
\]

The effect of the condenser may be represented as follows:

\[
G_{sub} = \kappa (P_{sat} - P_s) \left( \frac{M^{1/2}}{2\pi RT} \right), \quad l > \lambda (3)
\]
where

\[ P_e' \] is the pressure in the condenser

\[ l \] is the distance from the sublimating ice to the condensing ice

\[ \lambda \] is the mean free path of vapor molecule between two collisions

The cold plasma sensor may allow the direct relative measurement of \( P_{\text{int}} \) and \( P'_e \), and the evaluation of the Knudsen coefficient of evaporation and \( G_{\text{sub}} \). These data may help characterize the gas flow kinetics within a freeze-drying unit. The sensor may also be an appropriate tool for modelling the vapor flow within a freeze-dryer.

On a pilot scale, the cold plasma system was shown to be sensitive to the sublimation of a single vial containing a volume of 0.5 mL. This is of particular interest, as the lyophilization process depends upon individual vial behavior when each vial is being processed individually. During the thermodynamic process, some vials may be delayed and lead to heterogeneity, which is a major concern. This is controlled through the cycle adjustment of stationary phases. The cold plasma sensor could therefore be applicable to homogeneity validation studies. The cold plasma device was also sensitive throughout the range of chamber pressure (from \(10.10^{-6}\) to \(200.10^{-6}\) bar), shelf temperature (from \(-31\) to \(-11\) °C), and formulation resistance to water flow (from null with pure water to high value with sucrose 10%). Because the sensor can measure the water vapor content in freeze-drying from 1 to 100,000 vials, it can be used to develop a freeze-drying cycle.

The correlation with existing methods of lyophilization monitoring was achieved by comparing the cold plasma sensor to microbalance and Pirani-capacitive systems. The correlation of the plasma sensor and microbalance was established with a single vial using a DOE plan comprising eight tests. For the sublimation endpoint monitoring, a correlation coefficient above 0.99 was obtained between microbalance and plasma sensor (Figure 10). The experimental design showed that the main interactions with sublimation speed were:

\[
y = \beta_0 + (\beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3) + (\beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3) + (\beta_{123} x_1 x_2 x_3) \quad (4)
\]

**Figure 10**

**Estimation of factor interactions with microbalance and plasma sensor using a full factorial plan.**

The experiment illustrated in Figure 3 demonstrated a good correlation for endpoint determination between microbalance and the plasma sensor. When an in-depth analysis of the parameters was performed, the main influencing parameters were found to be similar between microbalance and plasma sensor. Shelf temperature had the major impact on sublimation time, followed by pressure and formulation concentration. These conclusions about formulation are linked to the chosen range (0–10% sucrose). However, if the sugar concentrations were much higher (e.g., 40% sucrose), the influence would become predominant. It is unlikely that a product above 20% of dry material would be freeze-dried.

As expected for a thermodynamically closed system such as a freeze-dryer, the analysis of different monitoring devices shows the strongest correlation between shelf temperature and pressure. The advantage of microbalance in monitoring a freeze-drying cycle is the continuous measurement of the weight decrease inside vials. But as the plasma sensor is correlated with microbalance, the knowledge of endpoint for primary drying is sufficient to develop a robust freeze-drying cycle. The comparison of the plasma sensor with the Pirani-capacitance system confirmed a correlation, but the plasma sensor was far more sensitive to the water decrease inside the chamber. This correlation was hardly achieved because of the Pirani gauge’s signal lack of accuracy.

The plasma system can also determine the primary drying endpoint, and can help to establish the temperature slope between primary and secondary drying in order to avoid a huge exhaust of water during this
phase. If the ramp is too sharp, the glass transition of the product may be exceeded, resulting in cosmetic or stability problems. When we observed the plasma signal in the sublimation ending region, the system seemed to be sensitive to remaining sublimating vials in the load. Indeed, even a small increase in shelf temperature was detected by the plasma sensor. The first derivative of the signal is a good way to control process stabilization. Figure 11 illustrates the evolution of first derivative of the plasma signal. The proposed threshold of 0.01 relative units per hour was empiric and was determined through microbalance individual vial testing. On a larger scale the threshold should be adapted in relation to product specification and visual defect rate.

Reproducibility was assessed through three placebo tests under equal conditions and critical process parameters. As seen in Figure 6, a good signal superposition between the three cycles was recorded, indicating a good batch-to-batch reproducibility of the plasma sensor signal and its potential for consistency evaluation.

With respect to the product, even slightly different formulations have characteristic fingerprints with the cold plasma system. This system could therefore be potentially used to assess the identity of the product being freeze-dried. These data confirm that the plasma sensor is eligible in the PAT toolbox for freeze-drying as qualitative equipment.

One of the main benefits of plasma sensors is the ability to be mounted on almost any kind of freeze-dryers, irrespective of size. Indeed, scaling-up can be achieved (Figure 7), and only a few more hours are needed at the end of primary drying to assure a good transfer of the cycle towards the industrial freeze-dryer.

The kinetics of desorption during secondary drying can also be monitored using the plasma device (Figure 8). This is of particular interest when freeze-drying formulations containing hydrate crystal excipients. Indeed, our study with mannitol confirmed that plasma sensor was useful in monitoring hydrate form desorption once the activation energy has passed.

It is unlikely that product temperature probes will be placed on industrial freeze-dryers with Sterilization In Place (SIP), CIP and automatic loading and unloading. Therefore, to be fully compatible with production freeze-dryers, a monitoring device will require simple integration and to be steam-sterilizable. The installation of the plasma device is similar to a capacitive pressure probe with triclamp joints, and the output signal can be directly integrated to a 21CFR digital recorder. The plasma probe is steam-sterilizable; the parts in contact to the lyophilization chamber are stainless steel and a quartz tube. Cold plasma ionization is therefore an efficient alternative technique to product temperature probes.

Information provided by cold plasma sensors can be useful from a PAT perspective, as the freeze-drying cycle can now be followed with respect to indirect (shelf temperature, pressure and time) and direct (water quantity) parameters, thus allowing batch comparisons. Integration from a PAT perspective has the advantage of global load monitoring and the ability to

---

**Figure 11**

Cold plasma sensor signal (___) and signal first derivative to time (△) when the sublimation endpoint is reached.
evaluate product quality on the basis of in-depth process parameters monitoring.

The plasma sensor is already established as a qualitative system, but by using a calibration procedure it will be possible to transform the obtained values into a measure of water quantity within the freeze-dryer. A custom-built calibration bench was developed to recreate the atmosphere present inside a freeze-dryer. Plasma calibration involved searching for the maximum intensity of optical peaks corresponding to water vapor and nitrogen. A floating calibration was performed to ensure that two different units could be collected at the end the same signal. Using this bench, the percentage of water vapor to nitrogen has been adjusted from 0 to 100% over total pressure ranges from 0.005 to 0.5 mbar. However, the cold plasma sensor does not give any absolute measurements, and further investigations are required in order to find a fixed reference of moisture level. Furthermore, this calibration bench working under vacuum is complex to operate, and the calibration procedure will certainly require further developments.

Conclusions

The cold plasma sensor appears to be a promising tool for monitoring the freeze-drying process. Its main interest lies in the ability to control freeze-drying at each step of industrialization from robust cycle design to scaling-up testing, homogeneity validation, and industrial consistency lots. The main advantages of this method are the sensitivity to a single vial, global monitoring, and the noninvasive aspect. Other benefits include compatibility with automatic loading, aseptic handling, and sterilization. Reproducibility under the same conditions and correlation with other monitoring methods were established. The main limitation of the plasma sensor is the requirement for adequate positioning within the vapor flow pathway in larger freeze-dryers. Calibration will also require complex equipment capable of generating a vapor-saturated chamber under vacuum. The perspective is the evaluation of cold plasma ageing in the production environment. In the future, the sensor signal should be normalized in relation to total chamber pressure in order to gain an easier signal interpretation. Nevertheless, the cold plasma technology may fulfill the gap for freeze-drying monitoring equipment in the PAT field of activity.

Acknowledgments

Many thanks to Adixen and GSK Bio development teams for their transversal collaboration.

References


