



## Research paper

## Mass spectrometry in freeze-drying: Motivations for using a bespoke PAT for laboratory and production environment



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## A B S T R A C T

Mass Spectrometry has commonly been used in the semi-conductor industry where maintaining a clean environment with minimum contaminants under high vacuum is crucial for successful manufacturing. Since the technology's early usage for pharmaceutical manufacturing in the 1980s, particularly in the freeze-drying environment, much has changed. The focus of the current work is aimed at asking some key questions regarding the maturity of the technology, its challenges and importance of having an application-specific instrument for quantitative process analyses applied to freeze-drying. Furthermore, we compare the use of mass spectrometers in early installations from the '80s with recent experiences of the technology in the production and laboratory environments comparing data from different MS technologies. In addition, the manuscript covers broad application of the technology towards detection of and sensitivity for analytes including silicone oil and Helium. It also explores the option of using MS in detecting water vapor and nitrogen concentration not just in primary drying, but also in secondary drying. The technology, when purpose built, has the potential for use as a robust, multi-purpose PAT tool in the freeze-drying laboratory and production environments.

## 1. Introduction

Freeze-drying involves removal of solvent such that the molecular structure of the active ingredient of the drug is least disturbed, thus providing a dried drug product that is quickly and completely rehydrated upon addition of the solvent. The process requires freezing on temperature controlled shelves of a freeze-dryer. The heat transfer fluid circulating through the shelves is often a silicone-based oil which can withstand temperatures down to  $-55\text{ }^{\circ}\text{C}$  to  $-60\text{ }^{\circ}\text{C}$  and on heating to  $121\text{ }^{\circ}\text{C}$ . It is imperative that the circuit remains completely closed at all times since the oil itself is not sterile. Service life of freeze-dryers can sometimes exceed three decades. A simple preventative maintenance operation of the shelf hoses can risk batch contamination unless materials and connections are carefully chosen. A typical freeze-dryer cycles through large local thermal stresses with operating temperatures ranging from  $-50\text{ }^{\circ}\text{C}$  to  $121\text{ }^{\circ}\text{C}$  and pressures ranging from 5 Pa

(37.5 mTorr) to 0.2 MPa (1500 Torr). Moreover, the shelves of a freeze-dryer move during Clean in Place/Sterilize in Place (CIP/SIP) cycles, loading/unloading, and stoppering of vials. Although rare, failed preventative maintenance operations or even the thermal or mechanical stresses could lead to micro cracks that eventually leak silicone oil. Initially, these cracks are too small to be observed during preventative maintenance or by the human eye. Coupled with the value of the products manufactured in each batch of a freeze-drying cycle, testing product integrity is of paramount importance.

Mass spectrometry (MS) is a powerful detection technique that allows sensing species concentrations down to ppm levels of contaminants in a closed environment. Here we investigate the possibility of using mass spectrometry and applying it towards contaminant detection and freeze-drying process analysis. While freeze-drying and PAT tools used for the same purpose remain the core scope of the manuscript, the authors would like to bring to the attention of the reader the

**Abbreviations:** CM, capacitance manometer; FBRM, focused beam reflectance measurement; Fluorescence, fluorescence spectroscopy; GC-MS, gas chromatography-mass spectrometry; ICP-MS, inductively coupled plasma-mass spectrometry; Laser Headspace, NIR laser measurements tuned typically for oxygen or carbon dioxide; LC-MS, liquid chromatography-mass spectrometry; MIR, mid infrared spectroscopy; MS, mass spectrometry; MTM, manometric temperature measurement; NIR, near infrared spectroscopy; RGA, residual gas analyzer; RTD, resistance temperature detector; TDLAS, tunable diode laser absorption spectroscopy; SIMS, secondary ion mass spectrometry; TOC, total organic carbon content; Turbidity, sensor to measure the amount of light (NIR) scattered from particulates in suspension; UV, ultraviolet spectroscopy; WFI, water for injection

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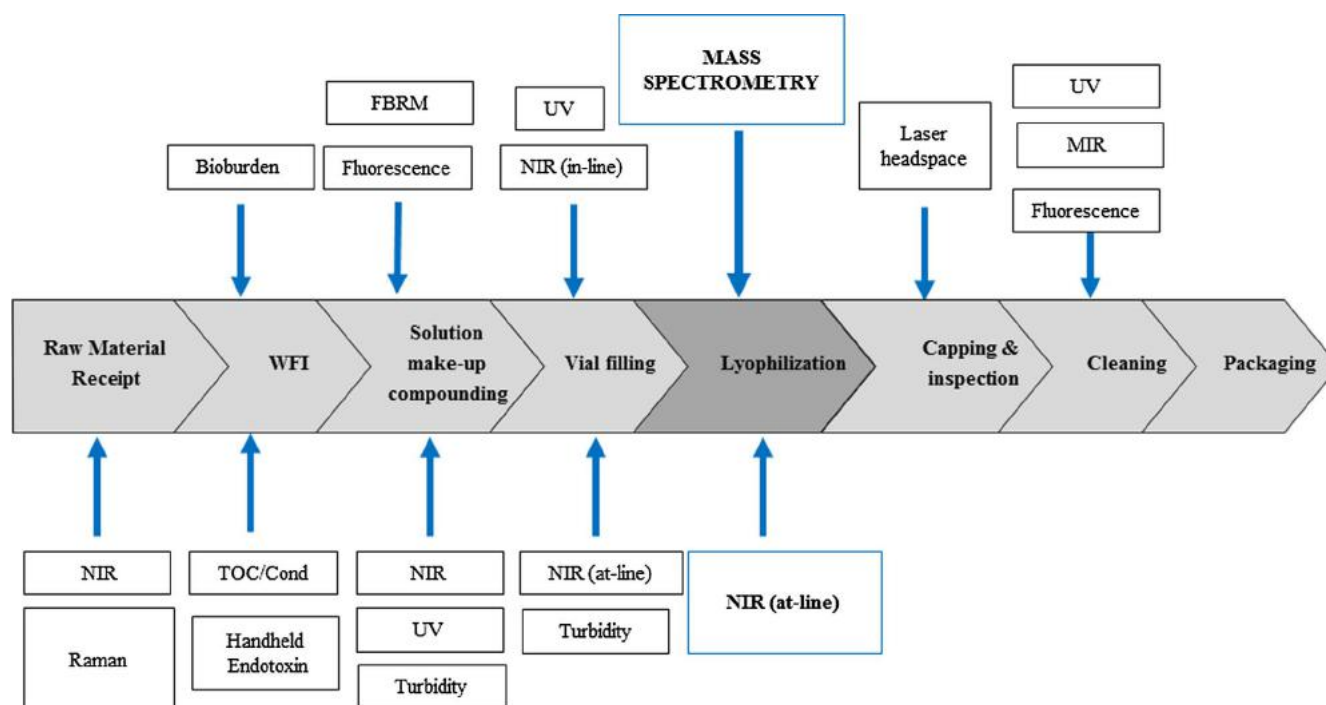


Fig. 1. Overview of PATs used In Aseptic Manufacturing.

different PAT tools used both upstream and downstream of the freeze-drying unit operation. The following section covers a range of PAT tools used in aseptic manufacturing.

### 1.1. A brief overview of some PATs used in support of aseptic manufacturing

Process Analytical Technology (PAT) as described by the Food and Drug Administration (FDA) – a definition:

The FDA considers PAT to be a system for designing, analyzing, and controlling manufacturing through timely measurements (i.e., during processing) of critical quality and performance attributes of raw and in-process materials and processes, with the goal of ensuring final product quality. It is important to note that the term analytical in PAT is viewed broadly to include chemical, physical, microbiological, mathematical, and risk analysis conducted in an integrated manner [1].

Fig. 1 provides a summary of some of the different PAT tools used at various stages of aseptic processing and manufacture. For the sake of brevity as the article is centered on mass spectrometry, we only briefly touch on some of those tools used immediately before, during and after lyophilization.

As illustrated in Fig. 1, there has been a conscious effort to identify and implement PATs useful to operations from non-invasive raw material receipt, analysis of water for injection, formulation and compounding, filling, lyophilization, capping, and cleaning to final packaging. Below, we briefly explain the processes immediately upstream and downstream of lyophilization. It is beyond the scope of the manuscript to provide a detailed review of each topic. The interested reader can refer to the identified literature referenced below.

#### 1.1.1. PAT for vial filling

During filling, both invasive and non-invasive spectroscopies can be used to ensure correct content, concentration and uniformity. Filling lines can be connected to non-invasive accessories that can make infrared measurements through flexible hosing. The content of filling tanks can be measured directly or non-invasively through ports fitted with sapphire windows or glass depending on the wavelength region being used by the spectroscopy. Turbidity sensors are also used in this area. Their working principle relies on being able to measure scattered

light from particulates in suspension and can provide a measure of when complete solution has been attained.

#### 1.1.2. PAT for lyophilization

Over the years, several PAT tools have been designed and implemented for process analysis and detection from freezing to end of primary drying. It is beyond the scope of this paper to review these PATs in detail; the interested reader can find them in Refs. [2–13]. While primary drying remains the longest and most energy-intensive step, the process is completed only after a suitable secondary drying step that allows sufficient desorption of the unfrozen water. Previous reports describe models that were developed to predict residual moisture in secondary drying [14–17], however as can be seen in Fig. 2, there are very few sensors that can provide process relevant information in early secondary drying. Furthermore, there are few or no known quantitative options for monitoring late secondary drying as highlighted in Fig. 2. This technology gap will be challenged in the current work by using a mass spectrometer to detect, quantify and provide real-time process analysis late in secondary drying.

Near Infrared spectroscopy (NIR) can be used to non-invasively measure residual moisture within lyophilized cakes contained in their sealed glass vials after they have been unloaded from a lyophilization chamber (spectral regions of interest are typically 1350 to 1460 nm and 1800 to 2000 nm for water); thus, permitting rapid verification that lyophilized cakes are within specification, this also supports high volume sample evaluations during development projects and in production. Commercially available NIR systems have been developed that are capable of 100% testing of residual moisture at line speeds of hundreds of vials per minute.

#### 1.1.3. PAT for capping & inspection

Typically, the type of PAT used at this stage are headspace analysers that can measure the headspace gas in a vial and provide a measure of what the gas composition is. This is particularly important for oxygen sensitive products, oxygen absorbs at 760 nm; carbon dioxide can also be measured and has an absorbance around 2000 nm. This type of analyser is based on a NIR laser system and can examine vials that are made of amber or clear glass. Headspace measurements can also reveal

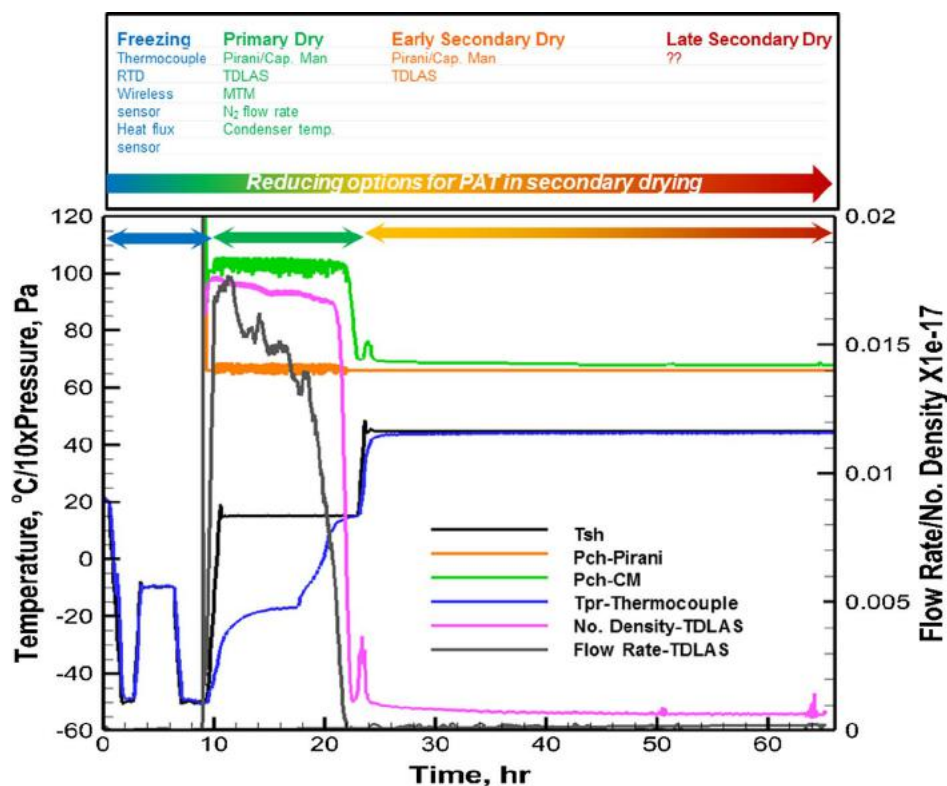


Fig. 2. Common PATs used in Lyophilization Process monitoring - CM: Capacitance manometer, MTM: Manometric temperature measurement, RTD: Resistance temperature detector, TDLAS: Tunable diode laser absorption spectroscopy, Pirani gauge.

information on vial integrity, that is, detecting if a vial is sealed properly. In the following section, we review mass spectrometry, the technology instrumentation involved, and its application in freeze-drying.

## 1.2. Mass spectrometry

Mass spectrometry, one of the major and rapidly developing analytical techniques of our time, is based on the conversion of chemical species into gas-phase ions, followed by sorting of the newly created ions by their mass-to-charge ( $m/z$ ) ratio and their subsequent detection. The output of the measurement is a mass spectrum, which typically shows the detector response for each  $m/z$  value, just like optical spectrometry shows intensity of light as a function of wavelength or frequency. The mass spectrometry data can also be presented in its reduced form as a histogram of each ion in the sample [18–20].

### 1.2.1. Residual gas analyzers vs. mass spectrometers

An interesting subgroup of mass spectrometry instruments with very specific purpose are the so-called Residual Gas Analyzers (RGAs) used for measuring the partial pressure of gas species in a vacuum system. They are small, robust, low-cost devices that are produced in two different ways. The simplest is an open design, which does not require independent pumping and has the RGA assembly mounted on the vacuum process to be monitored. The necessary condition is that the vacuum regime of this process is compatible with the operation of the RGA. More complex is the closed design that allows standalone operation as an independent mass spectrometer equipped with its own vacuum system (usually external). Standalone RGAs are typically connected to the process chamber to be monitored, through an orifice or valve [21].

### 1.2.2. RGA Instrumentation, design and operation

Current RGAs on the market use electron impact ionization with quadrupoles as mass analyzers. This makes them compact, easy to

operate, withstand higher pressures and their mass resolution can be adjusted simply by changing electrical voltages. The simplicity combined with the low cost, low pumping requirements, small footprint and almost no low  $m/z$  cut-off, make quadrupoles ideal mass analyzers for RGAs. There are two types of detectors used in RGAs and often the unit is equipped with both. For higher currents, the Faraday cup detector provides many advantages, including good quantification of the current produced by ions that discharge on its surface, good durability, and longevity. For very low limits of detection, electron multipliers are typically used when operating at low sampling pressures (about  $10^{-4}$  Pa). Electron multipliers require more frequent servicing and are prone to contamination and degradation over time [21–23].

Traditional RGAs typically have some imposed limitations on performance, such as lower accuracy or mass range [24]. Fig. 3 shows the schematic of a single quadrupole mass spectrometer and its operation. The gaseous sample is brought into the ion source (from left to right in Fig. 3) where the gas atoms or molecules collide with electrons emitted from a heated filament. The most commonly used electron energy is 70 eV, much higher than the ionization threshold. Upon collision with the sampled gas, there is conversion of neutral molecules or atoms of the sample into gaseous ions. Although many different processes are possible, the most typical is a loss of electron from the sample species, which results in creation of a positively charged cation radical. Depending on the chemical properties, the cation radical might fragment into smaller ions. All positively charged species are extracted from the ion source by electrostatic force, which is provided by optimized voltage on the ion optics elements and are sent to a quadrupole mass filter for further analysis.

The quadrupole analyzer functions as a scanning low and high band filter. For each combination of direct and alternating voltages that are aspirated on the quadrupole rods, only ions with relatively narrow-distribution of mass-to-charge ratio can pass through at a given time. The voltages on the quadrupole rods are scanned from low to high values, which allow ions to be scanned from low to high mass-to-charge

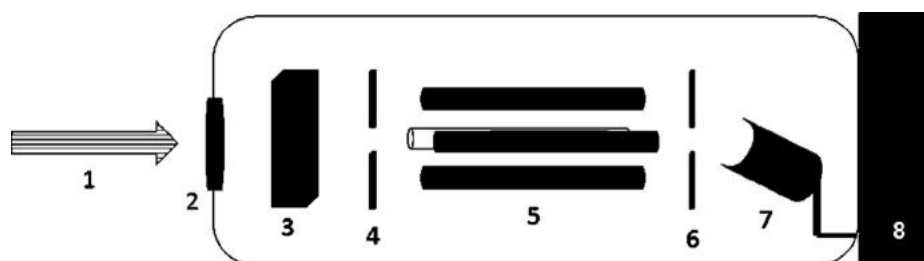


Fig. 3. Schematic of a quadrupole mass spectrometer (1) Direction of the sampled gas entry (2) Vacuum inlet port (aperture); (3) Ion source; (4) Electrostatic lens; (5) Quadrupole mass analyzer; (6) Electrostatic lens; (7) Detector and (8) Data and control system (outside vacuum).

ratio. Typically, a unit resolution is set, which means that quadrupole filtering allows roughly one mass unit to pass at the same time. Lower or higher resolution can be used as needed, although higher resolution results in lower ion transmission efficiency and translates into lower sensitivity.

The ions filtered by the quadrupole analyzer and successfully passed through are attracted towards a detector (component 7 in Fig. 3). In the case of a Faraday detector, they collide with a metal plate and discharge on its conductive surface. The discharge creates a current, which is monitored by the detector circuitry and measured as an analytical signal. The mass spectrum is recorded from lighter ions and continues towards heavier ions. The width of the peaks produced by the detector is given by the resolution of the quadrupole mass filter, while their area depends on the number of ions that reached the detector. Finally, the obtained mass spectrum is typically post-processed and recorded by a suitable data acquisition system. Below, we briefly describe the role of mass spectrometry in freeze-drying.

### 1.3. Mass spectrometry in freeze-drying

The application of mass spectrometry to freeze-drying dates back to the early 1980's with work done by Jennings [25]. Jennings showed that a spectrometer can be used to identify chemical composition inside a freeze-drying chamber by monitoring partial pressure of water vapor and nitrogen as process gases. However, it was noted that the operating pressures needed to be as low as  $1.33 \times 10^{-2}$  Pa ( $10^{-4}$  Torr). Later, Nail and Johnson [26] used a mass spectrometer on an Edwards production dryer to show that there was an order-of-magnitude change in the response at the end of primary drying. Fig. 4 (left) shows an image of a Fisons instrument from an installation dating back to 1994. These systems were extremely large and are examples of systems adapted from other applications, often more expensive, useful for qualitative determination, but not for quantitative analysis. Since then, there have

been some notable contributions to the use of mass spectrometry in freeze-drying [27–30,3,6].

While the use of mass spectrometry in freeze-drying found early adopters, its wide-spread use, like many other technologies in the pharmaceutical industry, has been limited until more recent advances in the technology took place. Considerable time has gone in formulating means to quantify leaks into a freeze-dryer from an unclassified area [31]. Very little is done today to quantify the presence of leaks inside the chamber of a freeze-dryer from a non-sterile source. One such source could be the heat transfer fluid used inside the shelves for controlling product temperature. There are limited guidelines for such procedure. For example, according to the FDA's document on Freeze-Drying inspection [32]:

*“As in any vacuum chamber, leakage can occur from the atmosphere into the vessel itself. Other sources are media employed within the system to perform the lyophilizing task. These would be the thermal fluid circulated through the shelves for product heating and cooling, the refrigerant employed inside the vapor condenser cooling surface and oil vapors that may migrate back from the vacuum pumping system.*

*Anyone, or a combination of all, can contribute to the leakage of gases and vapors into the system. It is necessary to monitor the leak rate periodically to maintain the integrity of the system.”*

In the next section, we briefly describe the advances in the technology since its early adoption.

#### 1.3.1. Advances in the technology

Some key advances in the technology allow for quantitative analysis today:

1. Since the early 2000's, advances in design and manufacturing techniques allow small form-factor integrated system and sensor design. Fig. 4 shows the comparison in size from 1994 to 2017.



Fig. 4. Comparison of the size and form factor in mass spectrometers from 1994 (Fisons) installed on an Edwards freeze-dryer to 2017 (AMS mini mass spectrometer-Atonarp Inc.)



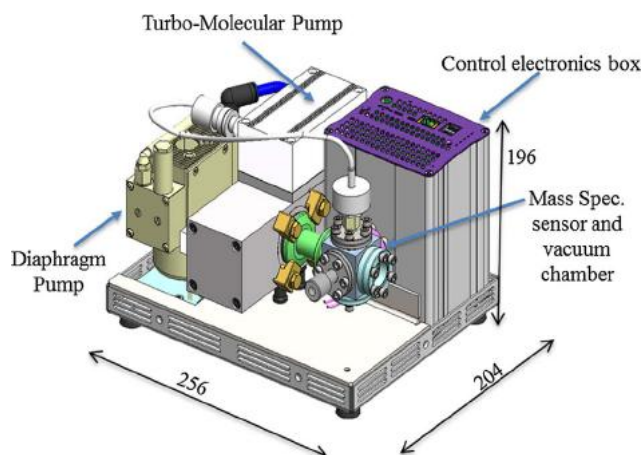


Fig. 5. Components in the AMS Mini Mass Spectrometer (Atonarp Inc.)

2. Traditional RGAs needed external pumps to operate at low pressure (about  $1.33 \times 10^{-4}$  Pa or  $10^{-6}$  Torr) in addition to their bulky design which has now been eliminated thanks to continuous miniaturization of mass spectrometry technology. Fig. 5 shows the integrated pumping system and gas analysis chamber on the AMS mini mass spectrometer.
3. With design improvements, systems can now operate at high pressure, above the mTorr range (0.133 Pa)
  - a) Higher pressure leads to larger signal from low-abundance ionic species.
  - b) Some RGAs use an Electron Multiplier (EM) that requires frequent calibration and suffer from reproducibility issues in quantitative analysis. Replacing an EM with a simple Faraday plate detector allows better quantitation and system-to-system comparison and quantification/control.
4. Ability to filter silicone oil contamination of the instrument prolongs its life and improves accuracy of the detector/analysis system (more details discussed in the section “Challenges with Silicone Oil Detection and Detector Contamination”).

## 2. Materials and methods

Mannitol used for the primary and secondary drying analysis was obtained from Acros Organics (New Jersey, NY), lot A0381892. Crystalline sucrose (EP/BP/NF) was obtained from Fisher Scientific (Hampton, NH). Vials used for freeze-drying included 10 mL and 20 mL tubing vials with 20 mm finish from SCHOTT (Lebanon, PA). Stoppers were 20 mm finish Flurotec® stoppers from West Pharmaceutical Services (Exton, PA) designed for lyophilization. Compressed helium used for the leak detection analysis was obtained from Haun companies (Syracuse, NY) and the 1.6 cST silicone oil heat transfer oil was obtained from DOW Chemicals (Midland, MI).

A capacitance manometer (MKS Baratron, Model# 690), Pirani gauge (Granville Phillips, Helix Technology Corporation, Model# 275), Atonarp AMS Smart Spectrometer (Atonarp Inc., Japan), and a second commercially available residual gas analyzer (RGA2, vendor not disclosed) were mounted on the ports provided on top of the product chamber. A TDLAS system (Physical Sciences Inc. Model# Lyoflux 200) was mounted in the duct connecting the product chamber and condenser above the isolation valve.

### 2.1. Freeze-drying

For the following experiments 5% (w/v) Mannitol and 5% (w/v) sucrose solution was utilized. Prior to filling vials, the solution was

filtered through a 0.22- $\mu$ m membrane filter. Each freeze-drying cycle with mannitol utilized 220, 10 mL vials with 3 mL fill loaded on a single shelf of a 2.3 m<sup>2</sup> shelf area LyoFast freeze dryer (IMA Life, Tonawanda NY).

Freeze-drying cycle for 5% (w/v) Mannitol was as follows (Fig. 2):

- Freezing: room temperature to a final temperature of  $-50^{\circ}\text{C}$  at a rate of  $1^{\circ}\text{C}/\text{min}$ . Freezing hold of 60 min.
- An annealing ramp of  $1^{\circ}\text{C}/\text{min}$  with a hold at  $-10^{\circ}\text{C}$  was used with refreeze at  $-50^{\circ}\text{C}$ .
- Primary drying: ramping at  $1^{\circ}\text{C}/\text{min}$  to a shelf temperature of  $15^{\circ}\text{C}$  and chamber pressure of 50 mTorr (6.7 Pa). Hold until Pirani and capacitance manometer converge.
- Secondary drying: ramping at  $1^{\circ}\text{C}/\text{min}$  up to a final shelf setpoint of  $45^{\circ}\text{C}$  and chamber pressure of 50 mTorr (6.7 Pa). Hold for 2520 min.

Freeze-drying cycles with sucrose utilized 162, 20 mL vials filled with 2.7 mL of solution loaded on to a single shelf of a Lyostar II (SP Industries, Stone Ridge, NY). The resulting cake height was approximately of 0.5 cm. All cycles for sucrose were performed using a chamber door with sample thief attachment. For the cycles #1 and #3 (Figs. 7, 11, respectively) a one shelf configuration was utilized to facilitate ease of sample extraction. Cycle 2 (Fig. 9a and b) had vials loaded on the center shelf of a three-shelf configuration. Product temperature was monitored using 36-gauge type T thermocouples (Omega Engineering, Inc., Stamford, CT) with a resolution of  $\pm 0.1^{\circ}\text{C}$ . Thermocouples were placed in the vials so the tips were barely in contact with the bottom-center of the vials. Two thermocouples were arranged in the center of the tray and one was located at the edge.

Freeze-drying cycle for 5% (w/v) sucrose was as follows:

- Freezing: room temperature to a final temperature of  $-45^{\circ}\text{C}$  at a rate of  $0.5^{\circ}\text{C}/\text{min}$ . Freezing hold of 60 min.
- Primary drying: ramping at  $0.5^{\circ}\text{C}/\text{min}$  to a shelf temperature of  $-25^{\circ}\text{C}$  and chamber pressure of 50 mTorr (6.7 Pa). Hold for 2100 min.
- Secondary drying: ramping at  $0.2^{\circ}\text{C}/\text{min}$  up to a final shelf setpoint of  $35^{\circ}\text{C}$  and chamber pressure of 50 mTorr (6.7 Pa). Hold for 300 min.
  - o Cycle 3 (Fig. 11) utilized a ramp rate of  $0.3^{\circ}\text{C}/\text{min}$ .
- Storage: Upon completion of the secondary drying hold, the product chamber remained at 50 mTorr (6.7 Pa) and shelves were set to  $5^{\circ}\text{C}$  for storage of the product until unloading could take place.

### 2.2. Karl Fischer analysis

The freeze-dried cakes were reconstituted with “dry” methanol, and the residual water content was determined by coulometric Karl Fischer (KF) titrimetry (Photovolt Aquatest 2010, Minneapolis, MN).

### 2.3. Silicone oil detection

Tests designed to detect the presence of silicone oil were performed on the 2.3 m<sup>2</sup> shelf area Pilot LyoFast freeze-dryer (IMA Life, Tonawanda NY). Varying quantities from (0.1 mg to 0.8 mg) of 1.6 cSt silicone heat transfer fluid was placed on a glass Petri dish obtained from Eisco Labs Fisher Scientific (Hampton, NH). The Petri dish was placed in the center of shelf 2 of the 4 shelf freeze-dryer. The pressure in the chamber was reduced to 22 mTorr (3 Pa) and sampling started at or below 112 mTorr (15 Pa). Finally, the shelf temperature was set to  $+40^{\circ}\text{C}$ . A mass-to-charge ( $m/z$ ) of 73 amu is used to detect the dimethylsiloxane  $\text{C}_2\text{H}_6\text{OSi}$  monomer unit. Other fragments (e.g.  $m/z = 71, 74$  and  $75$  amu are potentially available).

## 2.4. Helium detection

Tests designed to detect the presence of system leaks using compressed Helium were performed on the 23 m<sup>2</sup> shelf area Production LyoMax freeze-dryer (IMA Life, Tonawanda NY). A calibrated leak of the compressed Helium gas was introduced close to a simulated leak on the port of the freeze-dryer and sampling of the composition was performed using the mass spectrometer placed also on the freeze-dryer chamber. Once the Helium was introduced, the mass spectrometer takes a few seconds to detect the gas and provide an alarm.

## 3. Results and discussion

### 3.1. Primary and secondary drying monitoring

In this section, we test two different mass spectrometers for their ability to detect the primary analytes of interest for freeze-drying applications. In particular, we check for ability to detect water vapor and nitrogen during primary and secondary drying, and silicone oils, using the instrument as a heat transfer fluid leak analyzer and finally, helium for detecting system leaks.

In Fig. 2 we see different process analytical tools available to monitor primary drying end point detection, including the Pirani gauge and capacitance manometer, TDLAS [8] and thermocouples from a freeze-drying cycle on a 2.3 m<sup>2</sup> IMA Life LyoFast 2 Freeze dryer (Tonawanda, NY). 220 10 mL vials with 3 mL fill of 5% (w/v) mannitol were used for the freeze-drying cycle. An extended secondary drying hold was maintained to compare the response of the different PAT tools during the cycle.

While thermocouples are useful in tracking freezing and primary drying, they are known to create a bias in probed vials under study. A probed vial will nucleate at higher temperatures and hence dry faster than the rest of the batch [4]. On the other hand, a Pirani gauge being pressure dependent allows for comparative pressure measurement against a pressure independent capacitance manometer [2]. The comparative pressure measurement technique is a useful means to detect the end of primary drying and early secondary drying. TDLAS similarly allows for reliable determination of the end of primary drying and early secondary drying. However, late in secondary drying when water vapor concentration is significantly lower and average residual moisture in the cake is < 3%, it can be challenging to use a TDLAS for reliable quantitative process characterization.

In Fig. 6, we compare normalized signals (against max signal at the

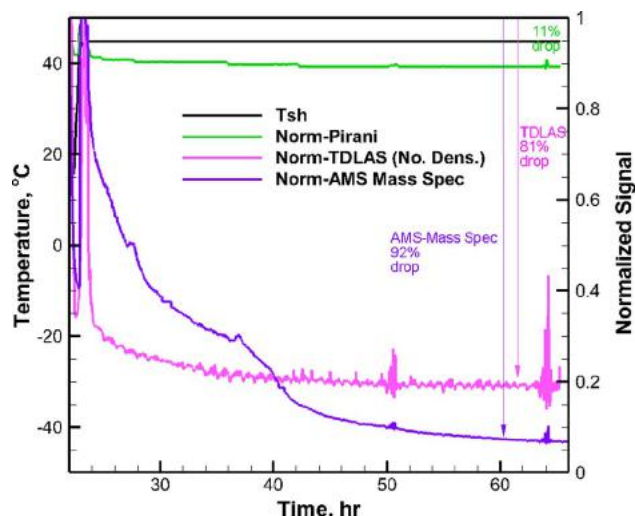


Fig. 6. Normalized signal (against max signal at the start of secondary drying) for the Pirani gauge compared to signal from the TDLAS and that of the AMS mini mass spectrometer; Tsh is the shelf temperature.

start of secondary drying) for the Pirani gauge compared to the TDLAS LyoFlux 100 and that of the AMS mass spectrometer for the cycle described in Fig. 2. By time  $t = 55$  hr, while there is merely a 11% drop in the signal from the Pirani gauge, there is a 81% drop in the signal from the TDLAS and a 92% drop in the signal from the mass spectrometer. This response is often an indicator of instrument sensitivity to real-time process changes. The enhanced sensitivity to drop in water concentration in the chamber offered by the mass spectrometer can be used in predicting batch average residual moisture in the cake based on the response from the mass spectrometer.

#### 3.1.1. Free-Drying cycles with sucrose

The entire drying process was monitored by an Atonarp AMS Smart Spectrometer, a Pirani pressure gauge, product thermocouples and an RGA. A plot of the cycle including Capacitance manometer chamber pressure, Pirani gauge reading, shelf temperature setpoint, product thermocouple temperatures, and AMS signals for the mass-to-charge ratio = 18 amu and a second RGA (RGA2) are summarized in Fig. 7.

Use of the comparative pressure measurement between a Pirani pressure gauge and a capacitance manometer has been widely accepted as a reliable method of determining the end of primary and secondary drying during lyophilization [33,4]. Pirani gauges are sensitive to the thermal conductivity of the measured gas. When calibrated in dry nitrogen, the gauge will read similarly to a capacitance manometer. However, during primary drying, the gas composition of the product chamber is predominantly water. This causes the Pirani gauge pressure to read 1.6x higher than the capacitance manometer while ice is sublimating from the product. At the end of primary drying, when all ice has sublimated, the apparent pressure signal from the Pirani gauge drops to equilibrate with the signal from the capacitance manometer. An additional smaller burst of moisture can typically be seen when the shelf temperature is ramped to secondary drying as more water is driven off.

Note: The hold at 5 °C after completion of secondary drying was included as a product hold until the lyophilizer could be back filled with nitrogen stoppered and unloaded.

Throughout the drying process, the AMS and RGA2 were monitoring gas species below a mass-to-charge ratio of 50 amu. The water signal ( $m/z = 18$  amu) was analyzed after the cycle was complete and is plotted alongside other process data in Fig. 7. The water signal on the AMS was subjected to a smoothing algorithm with a forward and backward pass using a 3-point moving average. It is also expected that the data on the RGA2 is subjected to a smoothing algorithm though the authors are not aware of the exact procedure used by the manufacturer. For both mass spectrometers, the water signals correspond well with the behavior seen in the Pirani gauge. The saturation of water during sublimation and drop-off in signal indicating the endpoint of primary drying once all remaining ice has been removed, is detected by both technologies. The small amount of moisture removed during secondary drying desorption is apparent as well. We note that the signal from the AMS mass spectrometer is about 3 times higher in comparison to RGA2's output throughout the cycle. This is due to an operating pressure 1000 times higher than that of RGA2, which allows for greater number of ions to be detected and hence a larger signal from the AMS.

To get a clearer comparison of the mass spectrometer to the Pirani gauge, the signals from each instrument were normalized against the max signal during the burst of secondary drying. This is displayed in Fig. 8. A 98% decrease in signal was observed in both mass spectrometers from the peak water signal during primary drying to the end of the hold time at the conclusion of secondary drying compared to a drop of only 40% seen in the Pirani gauge. Additionally, the increase in the water signal during secondary drying was 4.5 times the signal at the end of the cycle for the mass spectrometers compared to 19% for the Pirani gauge. The additional sensitivity compared to a Pirani gauge may yield important process information especially at the end of secondary drying, where achieving target residual moisture in the cake can be critical for product stability.

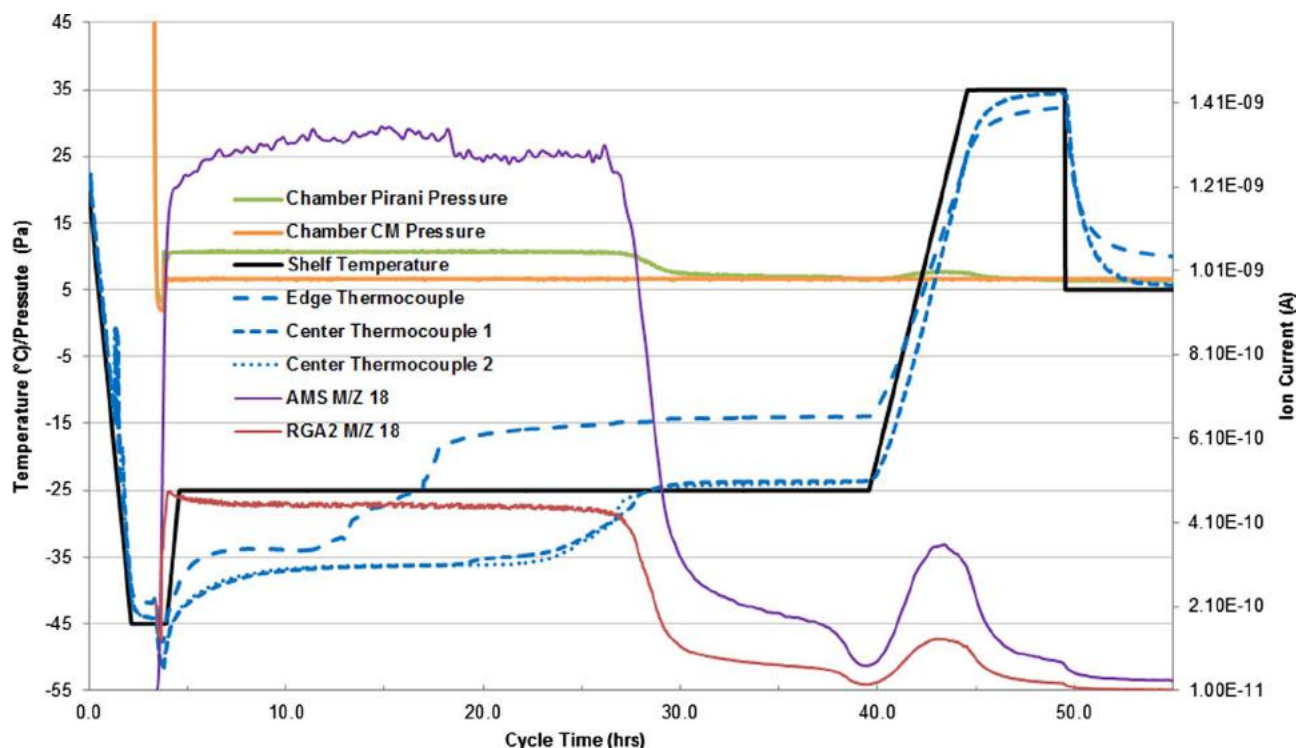


Fig. 7. Plot of cycle data including different PATs for the 5% w/v sucrose solution.

An additional lyophilization cycle with 5% w/v sucrose was performed to compare the signal from the mass spectrometer with that of the TDLAS based LyoFlux 200 (Physical Sciences Inc, Andover, MA) on the Lyostar II used in the previous cycle. Cycle data and water signals from the LyoFlux 200 and AMS are plotted in Fig. 9a-b.

As seen previously, the Pirani gauge and AMS water signals are in agreement for the endpoints of primary and secondary drying. The additional water concentration data collected by the LyoFlux 200 aligns well with the data from the AMS mass spectrometer as well. Some differences in the shape of primary drying portion of the cycle are observable for the LyoFlux 200 and AMS which is possibly an effect of measurement location on the observed water signal. The AMS is

mounted to a sanitary port on the top of the product chamber while the LyoFlux 200, mounted in the duct between the drying chamber and the condenser, measures vapor flow between the product chamber and condenser. The effect of mounting position is an area of interest to be explored in upcoming work. While the impact of the location on each instrument's signal warrants further investigation, it is interesting to note the difference in the profile from the mass spectrometer (Fig. 9a) compared to that of the TDLAS (Fig. 9b) during the hold in primary drying (between 5 and 30 h). While for the AMS, we see a steady decline in the water response during the hold in primary drying, presumably from the decrease in average sublimation rate, as the product mass transfer resistance increases. However, the data from the TDLAS and Pirani gauge indicate that there is no change, presumably from saturation of the signal in the TDLAS and the Pirani gauge. This may be an interesting artefact of the dynamic range of each of these instruments. Furthermore, here, the scanning rate was reduced for each  $m/z$  over the course of the spectrum to enhance the signal-to-noise ratio. While this increases the individual scan time, the system was still capable of multiple scans in under a minute allowing for sufficient resolution compared to the total process time.

Data for the AMS ion current and LyoFlux 200 water concentration from the end of primary drying (30 h cycle time) to end of cycle were plotted against each other below in Fig. 10. The response between the two measurements is linear with a  $R^2 = 0.98$  suggesting that mass spectrometry may be used as a quantitative measurement of water concentration in the chamber.

To gain a better understanding of how cake moisture during secondary drying and the water signal from a mass spectrometer are related, an identical cycle where vials were stoppered using a sample thief during the ramp from the end of primary drying to secondary drying as well as during secondary drying was performed. At the end of primary drying, shelf temperature was ramped to 35 °C at a rate of 0.3 °C/min. Vials in the center of the tray were stoppered at five points during the ramp and hold of secondary drying. All remaining vials were stoppered at the end of the cycle.

The residual water content, water signal from the mass

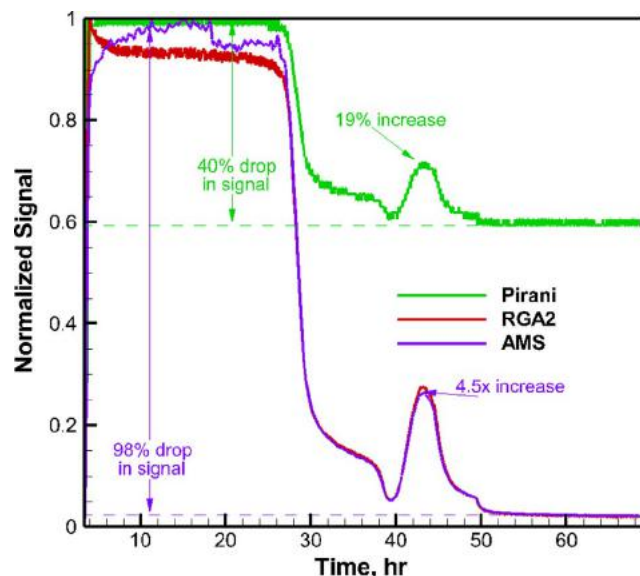


Fig. 8. Comparison of the normalized mass spectrometer and Pirani gauge signal during the lyo cycle.



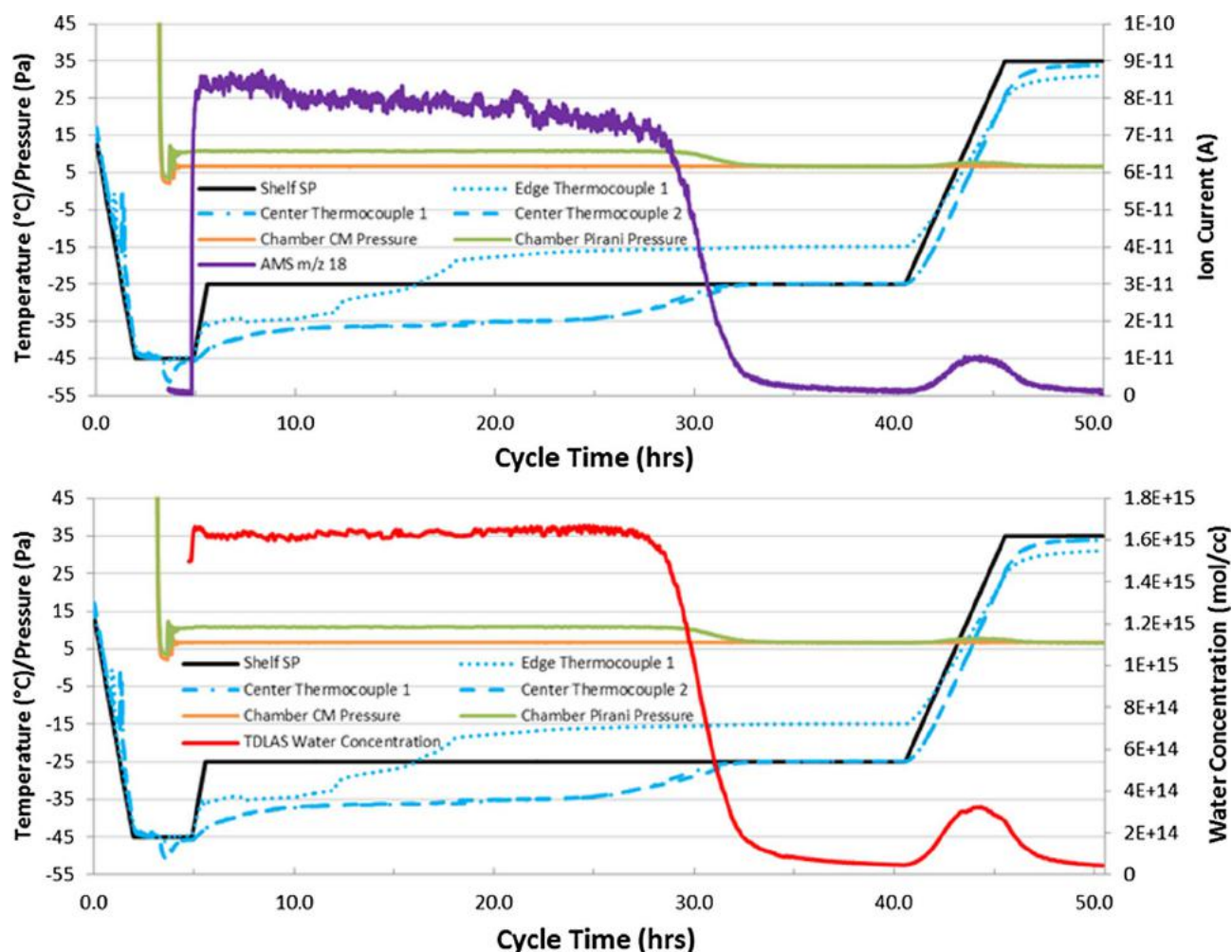


Fig. 9. a (top): Plot of cycle data including AMS water signal for 5% w/v sucrose solution Figure 9b (bottom): Plot of cycle data including the TDLAS LyoFlux 200 water concentration signal for 5% w/v sucrose solution.

spectrometer, and Pirani gauge readings vs. secondary drying time are summarized in Fig. 11. While the Pirani gauge reading appeared to plateau at approx. 42 h, both the water content determined by Karl Fischer analysis and the water signal measured by MS, continued to decrease. The data suggest higher sensitivity of mass spectrometer based measurement when compared to the Pirani gauge, and of the potential to utilize the data to predict batch average water content. Next, we investigate the capability of the mass spectrometer to detect trace concentrations of silicone oil heat transfer fluids and helium gas.

### 3.2. Detection of silicone oil heat transfer fluids

#### 3.2.1. Challenges with silicone oil detection and detector contamination

One of the inherent challenges with detection of silicone oil as a contaminant to the freeze drying process is that the contaminant also degrades the mass spectrometer hardware, specifically its ion optics. Fig. 12a shows a degradation example of an AMS that was exposed to vapors of silicone oil. Here, the AMS was used to sample from a constant pressure environment inside a freeze dryer. It can be seen that

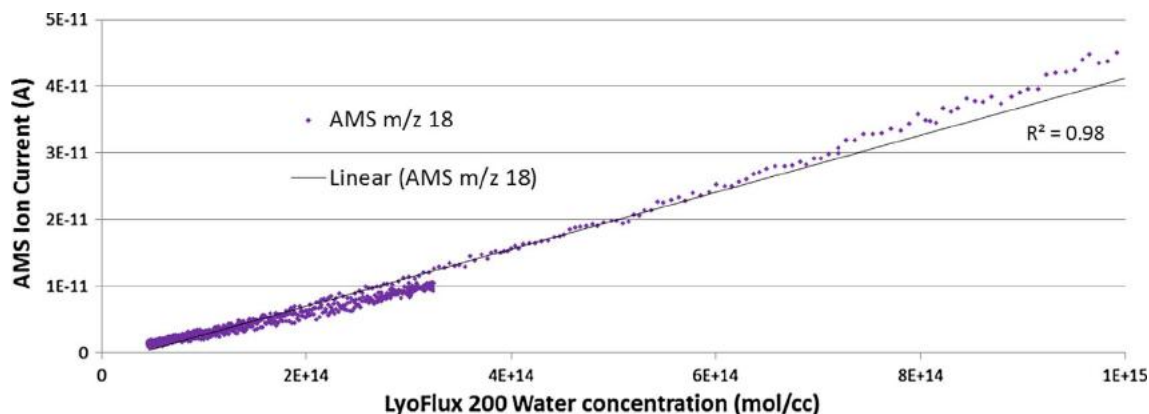


Fig. 10. Plot of AMS water signal vs. LyoFlux 200 water concentration for 5% w/v sucrose solution. Note: What seems to be two sets of data (up to a concentration of  $3 \times 10^{14}$  mol/cc) is in fact due to the same magnitude of mass spectrometer signal from two different time points in the cycle (reducing concentration from primary drying and secondary drying).



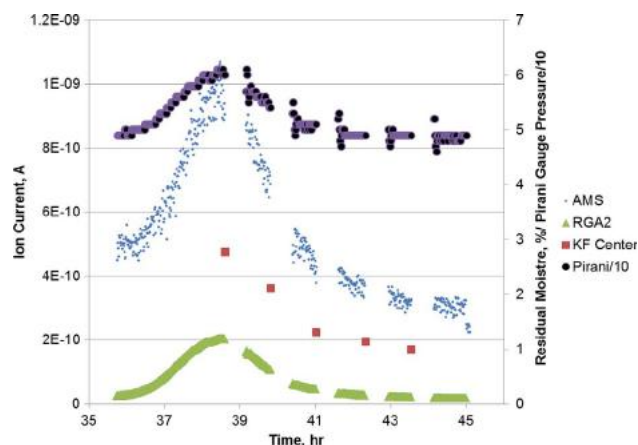


Fig. 11. Comparison of data from the AMS mass spectrometer, data from the Pirani gauge and RGA2 compared to residual moisture in the cake obtained from Karl Fischer analysis.

there is a drop in the total response from the detector after  $t = 7$  hr. This seems to be due to the loss in the AMS sensitivity. A subsequent verification/calibration run unfortunately did not improve the sensitivity but merely shifts the instrument's response. The inner surface of the exposed AMS was later subjected to a destructive test and subsequent scanning electron microscopy was performed. In Fig. 12b, it can be seen that the entrance lens and shield (in green) were coated by the silicone oil. To overcome silicone oil contamination issues, a species-specific filtration system was developed and implemented on the AMS mass spectrometer. It will be discussed in the following section.

### 3.2.2. The silicone oil filtration system

In order to minimize exposure of the AMS instrument's ion optics and other internal parts to the silicone oil vapor, a filtration system was developed as shown in Fig. 13 in the AMS mass spectrometer. This filtration system was designed to allow for preferential analyte filtration. The module uses a configurable duty cycle between (a) a fully open sampling pathway, which allows detection of all species present and thus can be used for silicone oil check, and (b) a filtered pathway that effectively removes the silicone oil from sampled gas. As a result, the overall exposure by the silicone oil is significantly reduced. The dual sampling path of the filtration system offers several unique features:

1. The filtered sampling pathway is used to monitor the freeze-drying

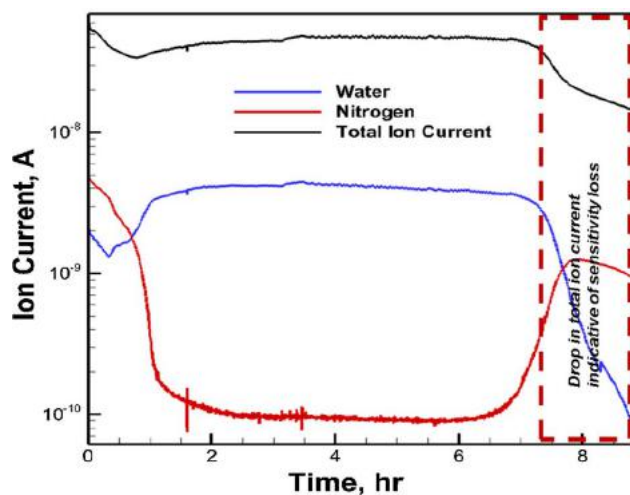


Fig. 12. a (Left): Degradation of mass spectrometer signal whose electronics were exposed to vapors of silicone oil; 12b (Right): Entrance lens and shield (in green) have been coated on exposure to the silicone oil.

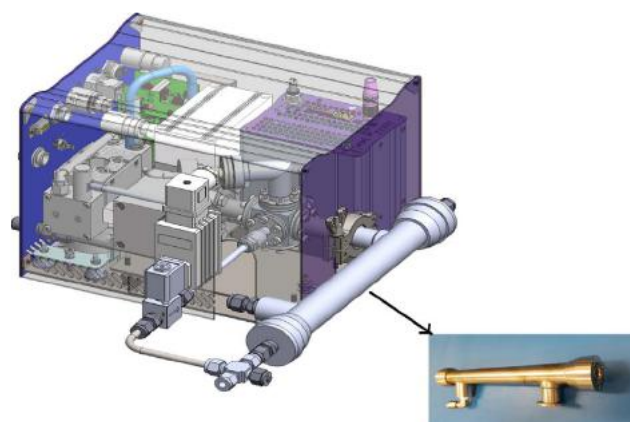
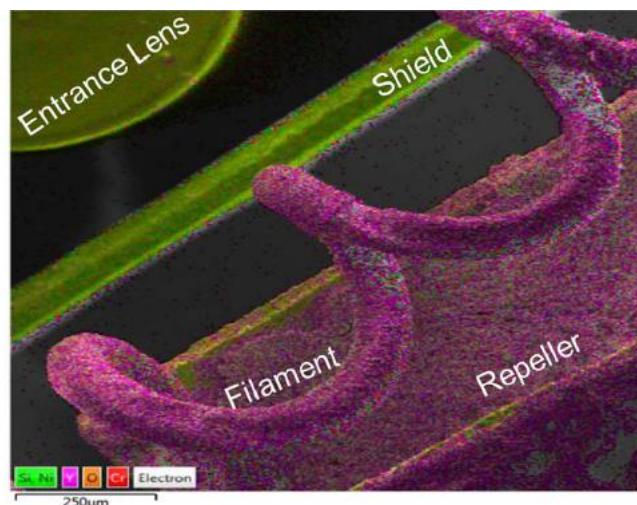


Fig. 13. Silicone oil filtration module used on the AMS mini mass spectrometer.

process.

2. The inclusion of the filtration system prevents the detrimental accumulation of the oil vapors over time, thereby extending the life-time and reliability of the instrument.
3. The direct non-filtered pathway is used only for a limited time to check for silicone oil or other higher mass contaminants.
4. The opening frequency and overall duty cycle based on specific requirements are configurable.
5. The system can be calibrated for quantitative measurement, which allows for quantitative estimation of the size of the leaks as threshold indicators.

The AMS mini mass spectrometer was used to detect trace concentrations of 1.6 cSt heat transfer silicone oil introduced into the chamber of the freeze dryer. Incremental quantities of 0.1, 0.2 and 0.8 mg of oil were placed on a Petri dish at the center of shelf 2 of a 2.3 m<sup>2</sup> LyoFast (IMA Life, Tonawanda, NY) pilot freeze-dryer. The mass spectrometer was connected to a validation port on the side of the product chamber. The pressure in the chamber was reduced to 22 mTorr (3 Pa) and sampling started at or below 112 mTorr (15 Pa). The shelf temperature was set to + 40 °C. A typical scan from introducing 0.8 mg of the oil is shown in Fig. 14. The spectrometer was made to cycle between sampling through the filter (to protect the instrument electronics) vs. sampling directly by-passing the filter (un-filtered path). There is 3-fold reduction in the signal for  $m/z = 73$  amu corresponding to the 1.6 cSt oil when sampling through the filter. The spikes in the graph correspond to points at which samples are taken by-passing the



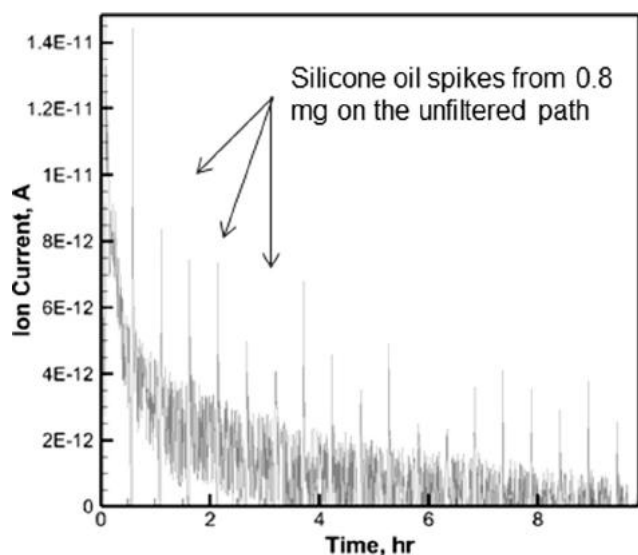


Fig. 14. Scan data from the AMS mass spectrometer after introducing 0.8 mg of 1.6 cSt silicone oil placed on the shelf of a 2.3 m<sup>2</sup> freeze dryer.

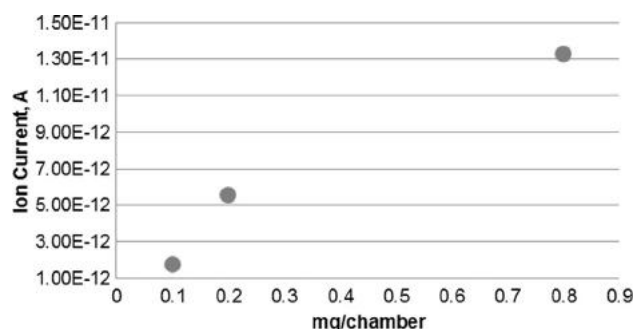


Fig. 15. Maximum signal response obtained from the AMS mass spectrometer for increasing quantities of 1.6 cSt silicone oil placed on the shelf of a 2.3 m<sup>2</sup> freeze dryer.

filter. The decay in the overall signal is representative of the contaminant oil in the detector system being evacuated through the vacuum system.

The maximum signal response obtained from the mass spectrometer for increasing quantities of oil (described as mg of silicone oil/unit chamber volume) is shown in Fig. 15. The procedure is used to generate a calibration curve for a given oil viscosity and fixed sampling location on the freeze-dryer. An investigation of silicone oils of other viscosities as well as other sampling locations was performed but is not within the scope of the current study. A detailed investigation is required to understand the impact of sampling location as identified in the “Future Research” section below.

### 3.3. Detection of low $m/z$ analytes for maintenance operations

Leaks into a freeze-dryer from an unclassified area can occur for several reasons. It is essential to isolate the location of the leak before it can be fixed. Helium is commonly introduced as a sampling gas during such leak check and maintenance operations. To isolate the location of the leak, one typically systematically isolates different sections of the freeze-dryer, introducing helium gas at potential leak sites. For example, leaks could occur around the chamber or condenser ports whose gaskets may have worn with routine operation. Helium is typically introduced in trace concentrations using a pressurized calibrated leak through a purging nozzle near the potential leak site. With the freeze-dryer at low pressure, the gas entering the vacuum pump through the condenser is sampled using a helium leak detector. The helium purge is moved from one potential leak site to the next until the leak detector

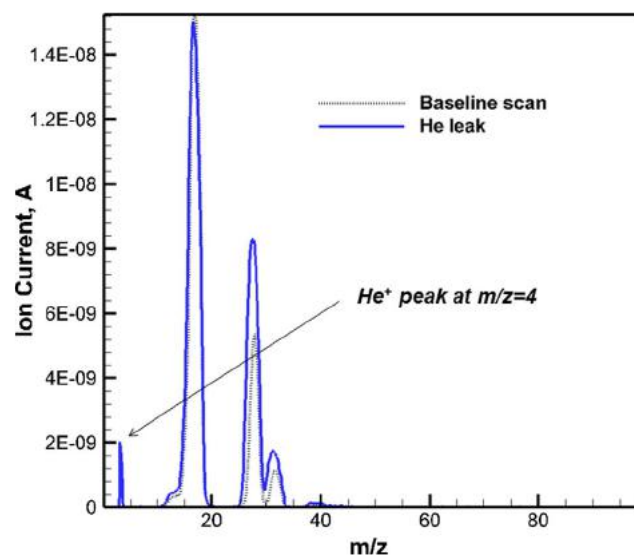


Fig. 16. Signal response obtained from the AMS mass spectrometer on simulating a leak and detection of helium gas on a 23 m<sup>2</sup> production freeze-dryer.

picks up helium. Such detectors are often cumbersome to move around and maintain. Here we investigated the option of using the mass spectrometer connected to the freeze-dryer in locating the presence of a simulated leak in one of the validation ports of a 23 m<sup>2</sup> production freeze-dryer. The resulting scan is shown in Fig. 16. The grey line shows the background scan with the superimposed blue spectrum representing the scan after helium is introduced through the calibrated leak at the same process conditions. The spike corresponding to  $m/z = 4$  amu represents the signature for Helium. It is evident that the mass spectrometer because of its high sensitivity can function as a replacement for a traditional dedicated helium leak detector often used for leak checks on a freeze-dryer.

## 4. Future research

One of the biggest barriers to the deployment of new PAT tools in production scale environment has been the ease of implementation, or the lack of it thereof, and the need for equipment revalidation. The Mass Spectrometry technology implementation in comparison, for the production environment merely requires access to a standard validation port on the freeze-dryer, typically on the chamber or the duct between chamber and condenser. This simplifies installation not just on new equipment but also for retrofit on existing freeze-dryer systems. The electronics on any mass spectrometer do not permit steam sterilization. Thus, real-time monitoring and control in the production environment can be made possible by installing an in-line 0.22  $\mu$ m filter. This creates a sterile boundary at the filter allowing in-process implementation.

We hope that this current effort will trigger additional interest within the community in using miniature mass spectrometers for process analysis. The analyzer serves as an effective multi-purpose quantitative real-time process monitoring/control tool. Hence, we would like to see continued research in several areas that could benefit the freeze-drying community. Some areas of interest are identified below:

- (1) Molecular models for the heat transfer fluids used in freeze-drying are unknown due to the proprietary nature of the chemicals. It would be useful to use computational fluid dynamics in understating the nature of the vaporization process when silicone oil leaks into a freeze-dryer. This would help understanding the optimum location for sampling through a mass spectrometer for different process gases. Some known possible locations are directly mounting on the chamber, sampling from the top vs side; the chamber-to-condenser pathway; mounting on the condenser for helium detection.

- (2) It is known that mass spectrometers can be used for the detection of non-aqueous solvents. It would be interesting to further investigate this topic. Some on-going research in this area for detection of non-aqueous solvents was presented by Liechty *et al.* [34].
- (3) The authors were encouraged to find sensitivity of the AMS mini mass spectrometer system capable of predicting reliably, quantitative real-time water vapor concentration, and relate it to batch average residual moisture data in the cake for primary drying and secondary drying end point determination. This topic is worth further investigation as a possible means for both, development studies, scale-up and aseptic production operation. It can be extremely useful to predict the water vapor concentration as a means for process control during primary and secondary drying non-invasively. It would be useful to gain further confidence in this, as a community, for implementation at production scale.
- (4) Finally, it is imperative to understand the critical threshold levels of silicone oil and the impact of these levels on the product. It would be useful to introduce this as a “best-practice” topic requiring a community-wide consensus.

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