White Paper:

Using Laser-Based Headspace Moisture Analysis for Rapid Nondestructive Moisture Determination of Sterile Freeze-Dried Product

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D.I. Duncan[1], J.R. Veale[2], I. Cook[3], K. Ward[3]

ABSTRACT: Residual product moisture content is a critical parameter when considering the stability and shelf life of lyophilized pharmaceutical product. Consequently, moisture analysis is performed in product and process development, as well as in commercial manufacturing to specify and control the maximum allowable moisture content. This is traditionally performed using Karl Fischer titration or thermo-gravimetric analysis (TGA) methods, which are destructive and labor & time intensive. Replacing these slow traditional methods with a rapid non-destructive method would streamline moisture analysis efforts and help improve the quality of finished product. Laser-based headspace inspection utilizing frequency modulation spectroscopy (FMS) is a rapid analytical method used for the analysis and inspection of finished sterile drug product. Quantifying the physical conditions in the headspace of sterile containers enables the monitoring of critical quality parameters and gives detailed insight into the process. In particular, quantifying the amount of water vapor in the headspace of freeze dried vials with an optical method enables rapid non-destructive moisture determination. Experiments have demonstrated that the amount of headspace water vapor directly correlates to the lyo cake moisture content. Stability studies have shown that the degradation of the active pharmaceutical ingredient correlates to the initial water vapor concentration present in the freeze-dried vial. These results indicate that rapid water vapor determination with an optical method could replace the slow destructive traditional methods for the moisture analysis of freeze-dried product. Industry applications of headspace moisture analysis described in this white paper include freeze drying cycle optimization, lyo chamber moisture distribution mapping, and 100% moisture inspection of commercial freeze-dried product.

General Introduction

Headspace moisture analysis

This section describes the application of headspace gas analysis, specifically frequency modulation spectroscopy (FMS), to non-destructive measurement of moisture content in freeze dried product. Water is present in a lyophilized cake in a variety of forms, including free, adsorbed, chemically bound, and water of crystallization. Not all of these water forms are linked to product stability through degradation of excipients and active ingredients. Generally, free or active water is available for chemical reaction and has the greatest impact on product stability. Traditional moisture analysis methods for pharmaceutical products such as Karl Fischer (KF) titration and thermo-gravimetric analysis (TGA) do not distinguish between active water and bound water. For example, KF measures the total water in the freeze dried cake if the sample is wholly soluble in the KF medium. From a practical point of view, the KF and TGA methods are time consuming, involve chemical reagents, and destroy the sample. Aseptic processing would benefit from non-

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destructive measurement technologies that provide information on each individual vial not just a small statistical sampling. Testing entire batches can provide insight into the efficiency of the drying process and the dynamics of freeze drying in different locations within the freeze dryer. In addition, quality control groups would benefit from being able to non-destructively sample a statistically significant number of finished product vials as part of a release test as well as monitoring individual vials repeatedly over the product shelf life. Finally, 100% moisture inspection of finished vials in manufacturing would guarantee the quality of finished product with respect to stability against moisture.

This white paper describes the correlation between the headspace moisture, as measured by FMS, and the cake moisture as measured by Karl Fischer titration, as well as the direct correlation between headspace moisture and degradation of the active pharmaceutical ingredient as measured by high performance liquid chromatography (HPLC) methods. A case study is presented showing how freeze-drying cycles can be developed and moisture content optimized through measurement of headspace moisture. The data show that even for an apparently well developed lyophilization cycle a number of random vials are produced with out-of-specification moisture content. The only way to identify those random out-of-specification vials is to perform 100% moisture inspection. The results suggest that laser measurement technologies provide useful insight into monitoring and controlling freeze drying processes and inspecting individual finished samples. Data is also presented demonstrating how the laser-based headspace technique enables moisture distribution mapping across the shelves of a freeze dryer. Insight is then gained not only into the lyophilization cycle but also into the performance of individual freeze dryers.

Laser Technology for Rapid Non-destructive Headspace Analysis

Tunable diode laser absorption spectroscopy (TDLAS) is a rapid and non-destructive analytical method suitable for monitoring gas concentrations and vacuum levels in the headspace of sterile product containers. Diode lasers are compact and robust solid state electro-optic devices that are the critical components in many industrial applications such as sensors for combustion control and fiber optic telecommunications. Devices can be fabricated to emit narrow bandwidth wavelengths in the red and near infrared (NIR) regions of the electromagnetic spectrum where molecules of interest to the pharmaceutical industry such as oxygen and moisture absorb light. In addition, diode lasers are well suited to frequency modulation signal processing techniques that increase detection sensitivity and compensate for the relatively weak absorption strengths of near infrared overtone and combination transitions. These device characteristics make diode laser based measurement systems ideal for monitoring and controlling the gas concentration and pressure of common atmospheric molecules, such as oxygen and moisture, during pharmaceutical manufacturing of sterile products.

Pharmaceutical applications where trace gas levels are monitored in small volume parenteral containers require the use of a high sensitivity laser absorption technique such as frequency modulation spectroscopy (FMS). This technique was developed in academic and industrial laboratories in the 1980's and 1990's. Systems for rapid non-destructive headspace analysis, based on modulated diode laser spectroscopy, were first introduced to the pharmaceutical industry in 2000 and are now routinely used in product development, process development and commercial manufacturing.

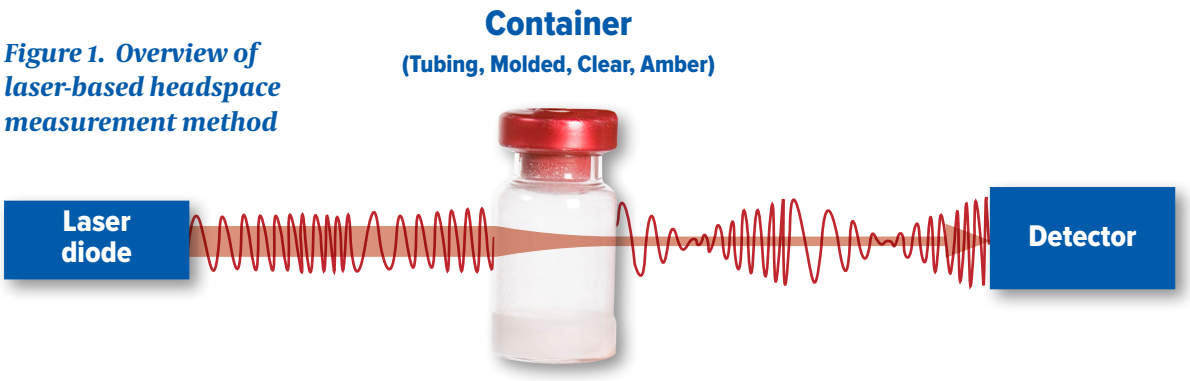


Figure 1. Overview of laser-based headspace measurement method

Headspace Inspection Systems

Diode laser based systems can be configured in a variety of ways to monitor and control processes and/or inspect individual containers for headspace oxygen, moisture or vacuum. The two basic configurations include bench-top Systems for use off- or at-line, and fully automated Systems for 100% monitoring, control and inspection

Bench-top Systems

Benchtop systems are used for at-line and laboratory applications. Systems can be mounted on carts and wheeled from line to line for in-process monitoring or permanently situated in laboratories for product development, release testing and investigations.

The Systems are generally configured with a laser source for either oxygen monitoring at 760 nm or moisture/pressure monitoring at 1400 nm. The Systems are microprocessor controlled through personal or industrial computers and measurement results are displayed on graphical user interfaces. Change parts and calibration standards allow the System to be used with a range of different container diameters.

Systems are calibrated using National Institutes of Standards and Technology (NIST) traceable standards of known gas concentration or pressure. Standards are constructed from the same containers used to package the pharmaceutical product. In this way calibration is done with containers that are identical to the sample containers under test. For example a moisture monitoring instrument would utilize standards of known water vapor concentration in containers of the same type and diameter as sample containers. In absorption spectroscopy the path length is the critical parameter when measuring gas concentration and using standards that have the same optical path length as the samples guarantees calibration.

Automated Systems

Automated systems are configured either as standalone machines or integrated into filling and packaging lines for 100% real-time control or inspection applications. Typical applications include oxygen monitoring on liquid filling lines, moisture monitoring on packaging lines, and leak detection on filling and/or packaging lines. Again, this white paper will focus on moisture determination of lyo product both in the laboratory and in production.

The automation platform can be configured with single or multiple measurement heads

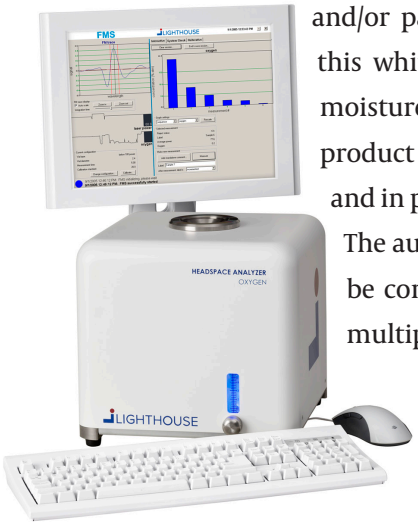


Figure 2. Benchtop headspace analyzer

to allow for oxygen monitoring and/or simultaneous moisture/pressure monitoring. The vial handling system (conveyors, motors, pneumatics, etc.) is plc controlled and the laser measurement systems are microprocessor controlled. Machine change parts (rails and starwheels) and calibration standards are customized for each different container diameter and typically 20 minutes are required for a no-tool change over between vial sizes.

Calibration is performed using National Institutes of Standards and Technology (NIST) traceable standards of known gas concentration or pressure. In automated systems these calibration vials can be permanently fixed on the main starwheel and used to automatically calibrate the system during use.

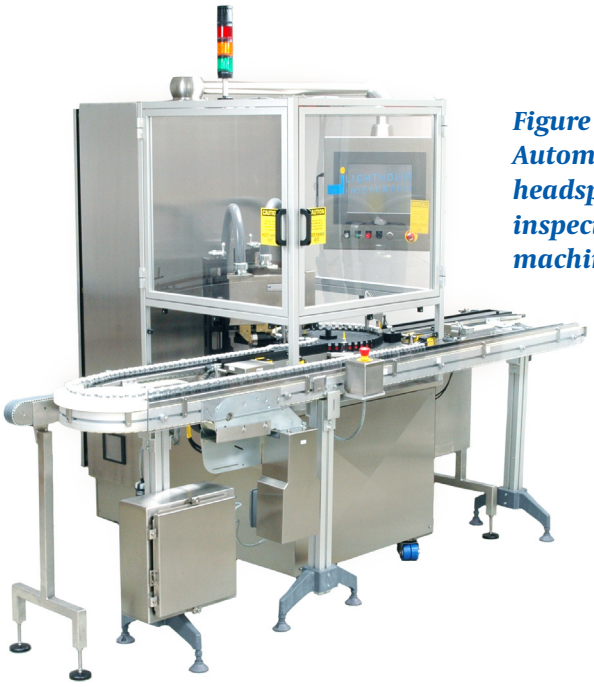


Figure 3. Automated headspace inspecion machine

Correlation of Headspace Moisture to Cake Moisture and Product Stability

Correlation to cake moisture as measured by Karl Fischer titration

As described in previous sections, headspace moisture is measured by shining laser light through the vial headspace, tuning the laser to an absorption wavelength of the water molecule, and analyzing the absorption signal to determine the headspace water vapor pressure. The vial headspace water vapor pressure can determine the moisture content of a lyophilized cake by performing a correlation between FMS and Karl Fischer using a set of samples with moisture ranging from below the target moisture level to 150% above the specification.

The data (Peeters, 2006) presented in Figure 4 shows this correlation between the headspace moisture as measured by FMS and the total moisture content as measured by KF for a set of lyophilized samples of a biological pharmaceutical product with a range of moisture from 0.5% by weight to 3% by weight. The samples were prepared with a range of moistures by drying five sets of samples to five different end points. Vial headspace moisture was then measured using the FMS method and total product moisture content was then destructively measured using KF. The samples measuring 0.5% moisture by weight through KF measured approximately 3 mbar of headspace moisture and the vials measuring 3% moisture by weight correlated to approximately 13 mbar of headspace moisture. The plot in Figure 4 shows 180 data points with a linear correlation between the two methods of $R^2 = 0.9656$.

Correlation to degradation of the active pharmaceutical ingredient

In addition to the moisture correlation between FMS and KF, there is also a direct correlation between the headspace moisture and the amount of active ingredient as measured by high performance liquid chromatography (HPLC) (Peeters 2006). Samples from the same set, with amounts of moisture in the cake/headspace ranging from 0.5%/3mbar to 3%/13mbar, were stored at 60°C for 12 weeks. At the end of twelve weeks

the amount of active ingredient in each sample was measured using HPLC. Figure 5 shows a plot of the log of the degradation rate, (ln(k)), versus the headspace moisture content, where the degradation rate constant k is given by

$$k = -(1/t)\ln(At/Ao),$$

where t is the time in weeks, At is the amount of active ingredient at time t and Ao is the initial amount of active ingredient. The data show how a given amount of headspace moisture correlates to a degradation of the active ingredient as a function of time. The correlation between the FMS headspace water vapor measurements and the degradation of the active pharmaceutical ingredient has an $R^2 = 0.9906$.

The fact that the stability of freeze-dried product can be predicted so well with a headspace water vapor measurement means that there is a possibility to develop a new primary method for lyo moisture determination that is both rapid and non-destructive. More specifically, the stability of a freeze-dried formulation could be specified in terms of headspace water vapor rather than cake moisture content. Finished product would then be inspected and controlled with a rapid headspace measurement because stability of the product has been defined as a function of headspace moisture content. The advantages of this approach over others employing the traditional destructive and time-consuming methods, KF and TGA, would be significant in terms of time and resource saved.

The correlation between the amount of headspace moisture as measured by FMS and the total water in a lyophilized formulation as measured by KF will vary depending on the structural characteristics of the formulation. Cook et al (2008) demonstrated that the crystalline structures of mannitol and sodium chloride gave a higher ratio of headspace moisture (FMS) to 'total water' (KF) than did amorphous sucrose or a predominantly amorphous mixture of mannitol and glucose. The data in Table 1 show how the relationship

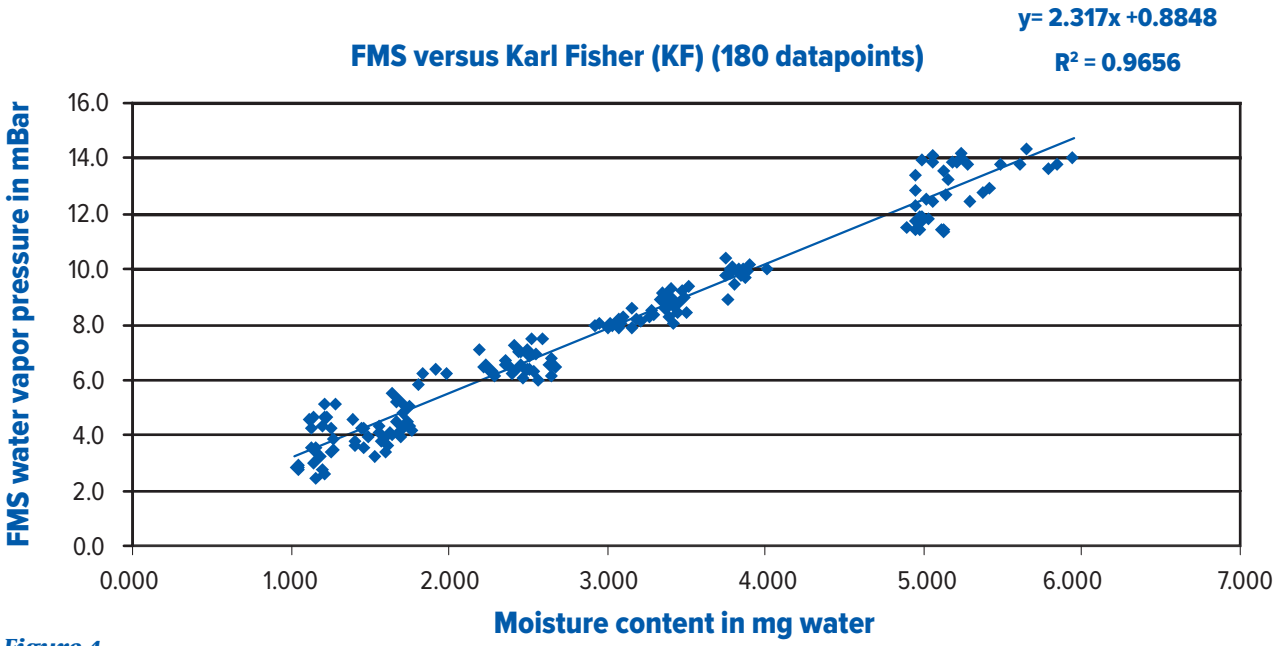


Figure 4

Correlation of headspace moisture levels to residual product moisture content as measured by Karl Fischer is plotted here. The product samples consisted of a 200 mg cake packaged in a clear tubing 10cc vial under one atmosphere of nitrogen. Similar correlations were seen using NIR moisture analysis methods. (Results courtesy of Organon Schering-Plough)

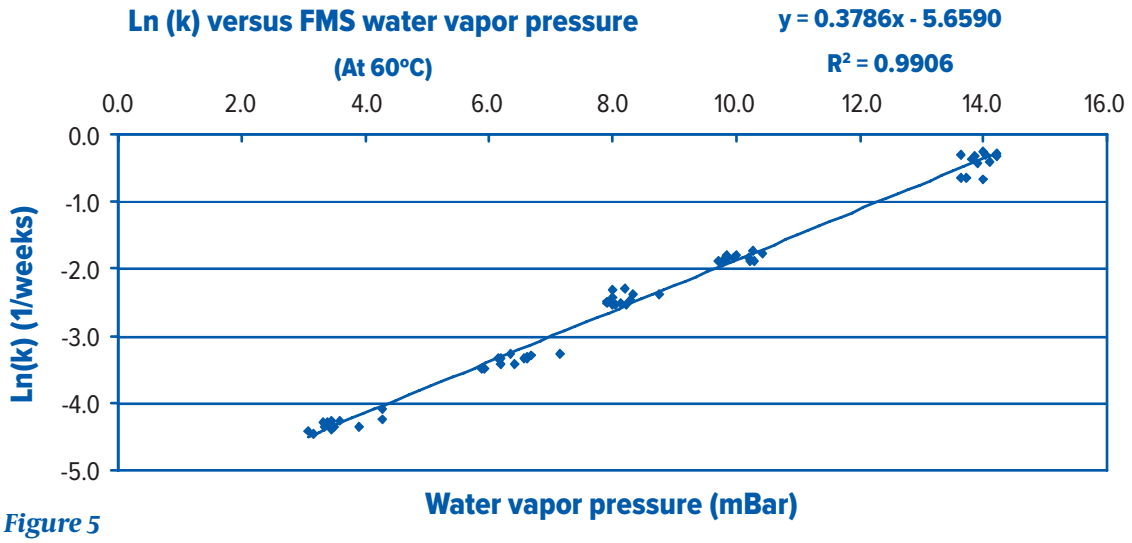


Figure 5
FMS vs HPLC for a pharmaceutical formulation

varies between total water by percentage weight in the freeze dried cake and the amount of moisture in the headspace above the cake as a function of the cake material. Row 3 of the table shows the ratio of headspace moisture as measured by FMS to total water as measured by KF. The ratios show roughly the vapor pressure of water above the cake for given KF moisture values for these excipient formulations resulting from the cycle and processing conditions employed.

The rapid and non-destructive nature of the FMS method makes it an ideal tool to characterize lyophilization cycle efficiency and freeze dryer performance across shelves, between shelves and as a function of drying cycle parameters. The method is also suitable for performing 100% moisture inspection of finished product and monitoring moisture content of individual vials over the shelf-life. The following sections describe some practical implementations of the method.

Industry Case Study

In the case study described here, two batches of freeze dried product were manufactured using two different lyophilization cycles. The first batch was manufactured with an initially defined lyophilization cycle. The second batch of product was manufactured with an optimized version of the initial lyophilization cycle. Each batch contained 1600 clear tubing 10cc vials (a total of 3200 lyophilized samples). At the end of secondary drying each vial was stoppered under 800 mbar of nitrogen. The chamber was vented to atmosphere, and the vials were removed and crimped.

Industry case study results

The headspace moisture in all samples from each batch was measured using the FMS laser absorption method described in this white paper. Results of the

	Sucrose 4%	Mannitol 2% + Glucose 1%	Mannitol 2%	NaCl 5%
Karl Fischer (% w/w)	2.87	3.9	5.04	1.03
FMS Headspace Moisture (Torr)	2.03	3.3	6.32	6.13
FMS:KF Ratio	0.71	0.85	1.25	5.95

Table 1 Comparison of Karl Fischer measurements to headspace moisture measurements for amorphous, crystalline and combination structures.

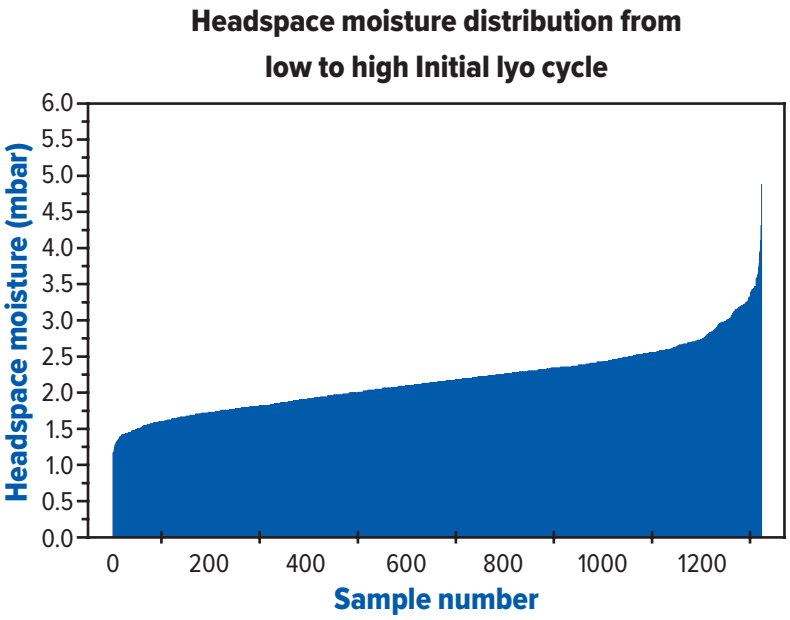
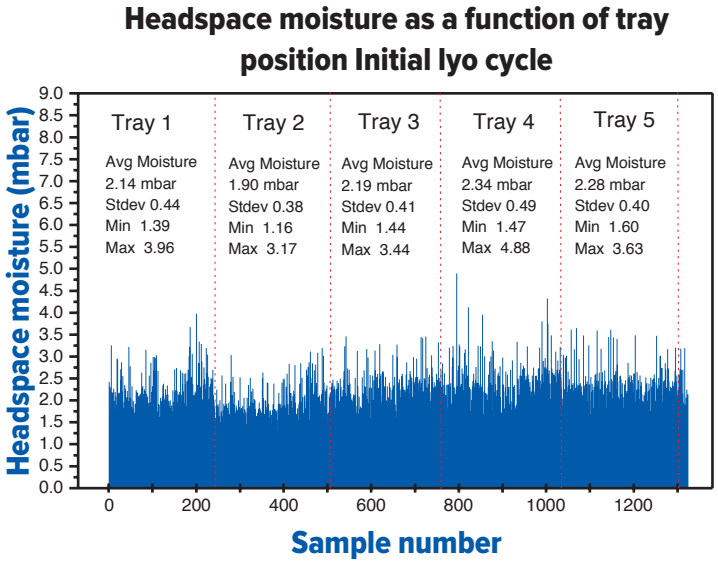


Figure 6
The headspace moisture distribution for all samples in the initial lyo cycle is plotted from low to high values. The high moisture tail in this distribution indicates a significant portion of samples that have not dried efficiently and contain elevated levels of water. Visual inspection verified that some samples in the high moisture tail also had a defective cake appearance. The moisture distribution has a significant slope from low to high values indicating non-homogenous drying.

headspace moisture analysis of product manufactured using the initial lyo cycle are shown in Figures 6 & 7. The results are plotted in two ways. Figure 6 displays the headspace moisture values of all samples from all trays plotted from low to high values. This moisture distribution gives insight into the efficiency of the lyophilization cycle as a whole. The high moisture tail in this distribution indicates a significant portion of samples did not dry efficiently and contain elevated levels of water, signifying non-homogenous drying across the shelves.

Figure 7 displays the headspace moisture value as a function of tray position. For each tray the average, standard deviation, maximum and minimum moisture values are reported. It is clear from this graph and the statistics that the drying efficiency for this lyo cycle is dependent on location within the freeze dryer. For example, average headspace moisture values and the standard deviation across tray 2 were much lower than samples in tray 4.

Figure 7
Headspace moisture values as a function of tray position are plotted for samples from the initial lyo cycle. It is clear that the drying efficiency for this lyo cycle depends on location within the freeze dryer. For example, average headspace moisture values and standard deviations across the tray show that samples in Tray 2 dried more efficiently than samples in Tray 4.



The lyophlization cycle was modified and a second set of 1600 vials was produced.

Results of the headspace moisture analysis on product manufactured using the modified lyo cycle are shown in Figures 8 & 9. The overall headspace moisture values are lower indicating dryer product on average. Headspace moisture as a function of tray position (Figure 9) shows more consistent drying across the freeze dryer shelf. The overall moisture distribution plotted in Figure 8 clearly shows that the modified freeze drying cycle has produced more consistent, homogenous and dryer product. This distribution is now much flatter than the moisture distribution in Figure 6. However, there is still a small high moisture tail indicating that a number of samples have not dried efficiently during the lyophilization cycle.

The high headspace moisture samples produced in each cycle offer some interesting insight. The sample with the highest moisture content in the first cycle (Figure 6) was in tray 4 and had a moisture value of 4.88 mbar. In the second cycle where average moisture values dropped by 44% compared to the first cycle, the sample with the highest moisture content (Figure 8) was in tray 6 and had a moisture value of 7.96 mbar, a factor of 1.6 times higher than the highest moisture sample in the first batch. This seems to indicate that cycles which appear to be optimized may still need further study; for example, the level of exposure to radiative heat can vary depending on vial location and packing density, while the nucleation of the sample could be non-uniform leading to a different frozen structure that may affect the drying time in certain vials, which may require freezing rate studies. Additionally, the primary drying cycle temperature may not be based on the critical temperature of the formulation, which may lead to the collapse/partial collapse of a sample; there are a number of reasons for the above observation, of which only a few are mentioned here. Only 100% mois-

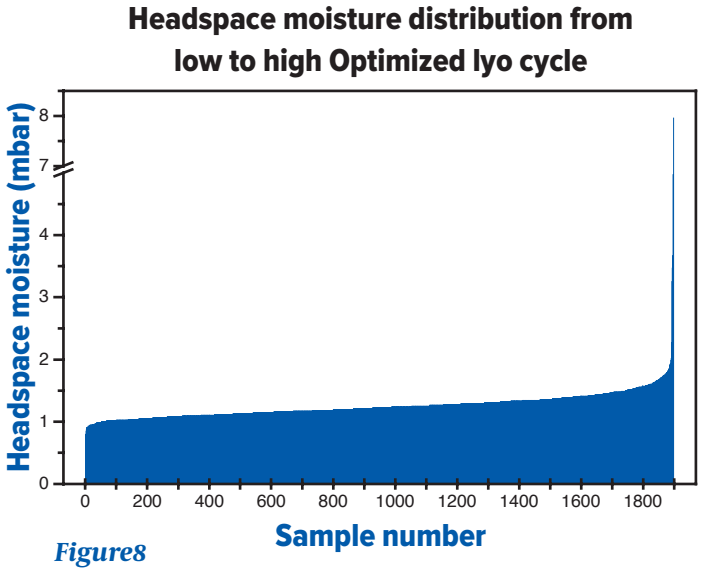


Figure8

The headspace moisture distribution plotted from low to high values clearly shows that the optimized freeze drying cycle has produced more consistent, homogenous drying. This distribution is now much flatter than the moisture distribution of the initial lyo cycle.

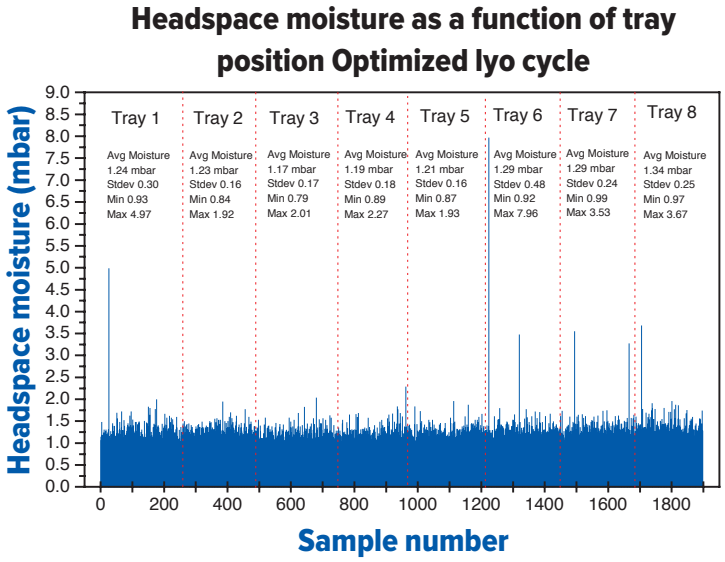


Figure 9

Headspace moisture as a function of tray position for the optimized lyo cycle shows more consistent drying across the freeze dryer shelf. Average headspace moisture values are also lower indicating better drying. It should be noted that even for this improved lyo cycle there are a number of vials (6 out of 1898) that have elevated levels of moisture content. The trays containing these 'wet' samples can easily be identified from the higher standard deviations measured across those trays (Trays 1, 6, 7, and 8).

ture inspection of the finished product samples could find these high moisture samples and keep them from entering the market. Even in the apparently optimized lyophilization cycle there were still a significant number of vials (6 out of 1898) that have moisture content that is a factor of x2 or more above the average. The potency of these out-of-specification product samples will certainly degrade over the product shelf-life.

Industry case study conclusions

Headspace gas analysis can characterize freeze drying cycles and provide insight to freeze dryer dependent drying effects. The technique can also provide 100% inspection capabilities for identifying out of specification product. The total time for the moisture analysis of the two batches described above (~3200 samples) was approximately nine hours using a manual benchtop system. Automated systems could inspect this number of vials in minutes. The results of this industry case study demonstrate that laser-based headspace moisture analysis has application to lyo cycle development, characterization of freeze dryer performance, and quality control of finished lyo product.

Moisture mapping in a freeze dryer

Regulatory authorities require proof that lyophilisation cycles have been developed logically and demonstrate uniformity. One measure of uniformity can be consistency of residual water content throughout a batch. In primary drying heat transfer is affected by gaseous convection and conduction as well as the degree of shelf contact and evenness of heat applied. Therefore cycle length can be affected by vial location, degree of vial/(tray)/shelf contact, radiative heating, packing density, product formulation and the cycle conditions themselves.

Once a KF/FMS correlation is established for the product in question the moisture variation can be mapped for an entire shelf. The correlation between KF and FMS headspace water vapor measurements for the 4% freeze dried sucrose was $R^2 = 0.9894$. The following graphs in figures 10 & 11 show a headspace moisture map for an entire shelf of vials of 4% sucrose, which were processed using the same freeze drying cycle in a small research scale freeze dryer. However, Figure 10 shows data for vials contained in a steel bottomed tray and Figure 11 shows data for vials that were in direct contact with the freeze dryer shelf.

It can be clearly seen in Figure 10 that partial vapour pressure values are relatively uniform at the far left and right of the graph (front and back rows of the tray), whereas for the centre vials which represent vials closer to the middle of the tray, the partial vapour pres-

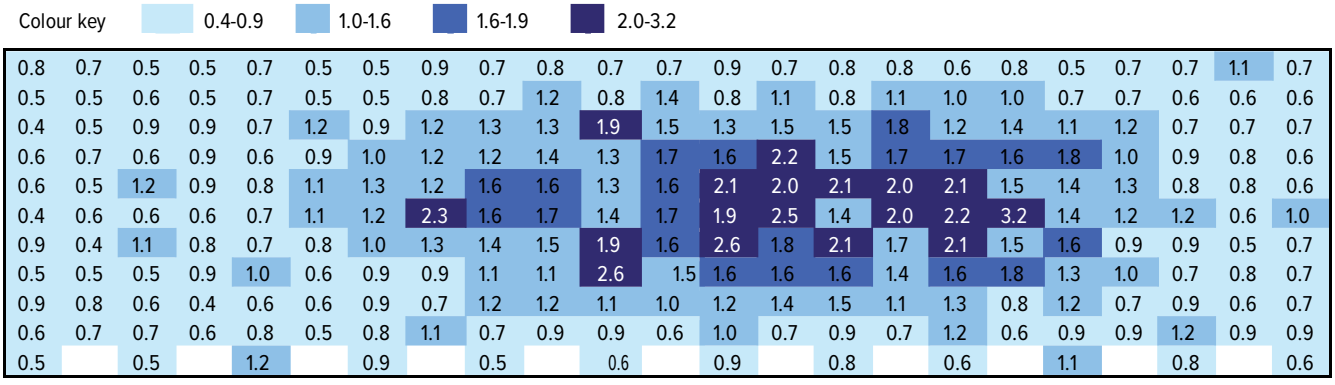


Figure 10 – Moisture map for samples with a steel bottomed tray (units given in torr)

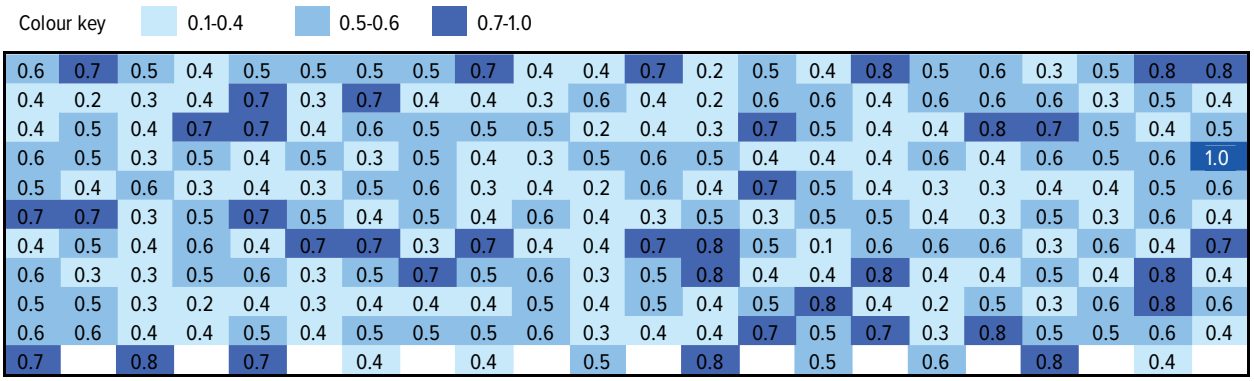


Figure 11 – Moisture map for samples with direct shelf contact (units given in torr)

sures are much higher and less uniform. This moisture variation may be attributable to a reduced level of radiative heat that the central vials can experience, as a result of which, the shelf contact plays a more significant role. This is reflected by the fact that the central vials in the middle rows furthest from the edge exhibit the highest moisture values, with the highest value of 3.2 torr corresponding to 4% water w/w by KF.

In Figure 11, the partial vapour pressures are much more uniform and the absolute values lower as the vials were in direct shelf contact. In addition to this, the sample vials would have been less reliant on radiative heat input due to more efficient shelf heat transfer from direct contact. Based on the KF/FMS correlation the lowest value of 0.2 torr corresponds to 1% water w/w by KF and the highest value of 1.0 torr equates to 1.8% water w/w by KF.

The above is just one example of the type of information that can be yielded from moisture mapping. There are many factors involved in order to develop an optimized cycle in order to achieve uniform drying, not all of which can be covered here. Factors such as primary and secondary drying temperature and time, thermal properties of the material, container used, loading conditions, freeze dryer capacity all play an important role.

Conclusions

Rapid non-destructive headspace moisture analysis has been enabled by FMS, a high-sensitivity laser absorption technique. The ability to rapidly and non-destructively determine the water vapor content in freeze-dried vials has opened up the possibility for new practical applications in lyo product moisture determination, lyo cycle development, and moisture mapping of freeze dryers. From a scientific point-of-view, there are a number of interesting problems involved in understanding the nature of the water present in lyophilized products (Cook et al, 2008). Using headspace moisture analysis as a tool while building a thorough understanding of the excipients, the various process conditions, temperature, storage, and stopper properties, may enable a deeper understanding of the location and dynamics of water present in lyophilized cakes. The current industry implementations described in this white paper demonstrate that the technique already provides practical insight into the lyophilization process and can be used to control the quality of finished product.

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About Us

LIGHTHOUSE is the leading manufacturer and provider of optical, non-destructive headspace inspection systems for in-line, at-line, and R&D applications specific to the pharmaceutical industry. LIGHTHOUSE developed the non-destructive headspace inspection systems with funding from the Food and Drug Administration. We have close to 200 laser based systems installed around the world at some of the world's leading pharmaceutical, biopharmaceutical and contracting manufacturing companies including: Amgen, Baxter, Bayer, Boehringer Ingelheim, BMS, DSM, Eli Lilly, Genentech, GlaxoSmithKline, Helvoet Pharma, Hospira, Johnson & Johnson, Merck, Novartis, Patheon, Pfizer, Roche, Serum Institute of India, Sankyo, Sanofi-Aventis, Schering-Plough, West Pharmaceutical Services, and Wyeth.

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