

WHITE PAPER

Introduction to Laser-Based Headspace Inspection and the Application to 100% Container Closure Inspection of Sterile Pharmaceutical Containers ●

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Abstract:

Laser-based headspace inspection is a method used for the inspection of finished sterile 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. Container closure integrity in particular can be monitored rapidly and non-destructively by headspace gas analysis. Changes in the headspace gas pressure or gas composition are leak indicators for sterile product packaged under modified atmosphere conditions. For containers stoppered under vacuum, a leak causes a rise in headspace pressure towards atmospheric levels. For containers stoppered at or near atmospheric pressure of an inert gas and exposed to air, a leak causes oxygen ingress into the headspace.

The leak rates that result in pressure rise or oxygen ingress are dependent on container volume and pressure differential for a given hole size.

In general the headspace pressure and oxygen concentration of small volume parenterals (e.g. 2-10mL) packaged under vacuum rise more quickly than the headspace pressure and oxygen concentration of large volume parenterals packaged near atmosphere. Detectable changes in the headspace conditions of a gross leaker occur within minutes. A micro-leak (< 1 micron) will exhibit detectable changes in the headspace after a few hours to a few days depending on the initial headspace conditions.

Automated laser-based headspace inspections systems are now implemented and validated for 100% container closure inspection of sterile pharmaceutical containers at production speeds. Such implementations give insight into the process, ensure the maintenance of sterility for finished product after capping, and can be seen as a tool for meeting current regulatory guidance.

General Introduction

Aseptic manufacturing processes have evolved over a long period of time and are capable of producing high quality parenteral products. The current growth in sterile biopharmaceutical products brings challenges as well as opportunities to the industry in terms of developing in-process monitoring and control strategies that keep processes in a state of control and minimize the risk of product defects. Contributing to our understanding of aseptic manufacturing processes are new advanced test and measurement technologies. The objective of this white paper is to describe one relatively new method in detail and discuss one of its important applications. The method is laser-based non-destructive headspace analysis and the application is 100% container closure inspection of sterile vials after capping to ensure the maintenance of sterility and stability.

Non-Destructive Headspace Analysis

The ability to measure headspace gas composition and headspace pressure rapidly and non-destructively allows manufacturers to monitor a number of quality parameters such as container closure integrity, oxygen content, and moisture content simultaneously. Historically these quality parameters were monitored using off-line destructive technologies or older in-line technologies that have weaknesses such as limited dynamic range and high false reject rates. Common individual product defects include leaks caused by cracks in the glass or improperly seated stoppers, and common process issues include insufficient drying of individual products during lyophilization cycles and insufficient removal

of oxygen from oxygen-sensitive liquid products during the fill and finish stage of production.

As stated in the introduction, we will focus on the container closure application in this white paper. Leak detection can be performed by measuring headspace gas composition and/or headspace pressure and comparing measured values to product specifications. Lack of container closure integrity allows for gas exchange to and from the headspace and possibly (if the leak rate is high enough) can allow microorganisms from outside the container to enter into the container. If a container lacks closure integrity then no assurances can be made concerning the stability or sterility of the product.

For example if a lyophilized product is specified to be stoppered at a headspace pressure of 0 atm and an in-process test measures 0.3 atm then the vial was either stoppered at an incorrect pressure or has leaked. The ability to test an entire batch would reveal if the problem is a process upset (i.e. incorrect setpoint for pressure in which case all vials would read 0.3 atm) or a random leaker. Another example is during liquid filling of oxygen sensitive products. If a product is specified to have less than 2% headspace oxygen and an in-process test measures 5% then the vial was either improperly purged or has leaked since processing ingressing some amount of oxygen.

A last example relates to moisture testing of lyophilized product. If a product is specified

to have less than 2% moisture and an in-process or release test measures 5% then the vial was either improperly processed or has leaked since processing ingressing some amount of water vapor.

The ability to test all product in a batch and rapidly quantify multiple headspace parameters (oxygen, moisture and pressure) will reveal if the issue is a process upset or a random package defect related to a container closure failure.

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

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. One such technique is known as frequency modulation spectroscopy (FMS). This technique was developed in academic and

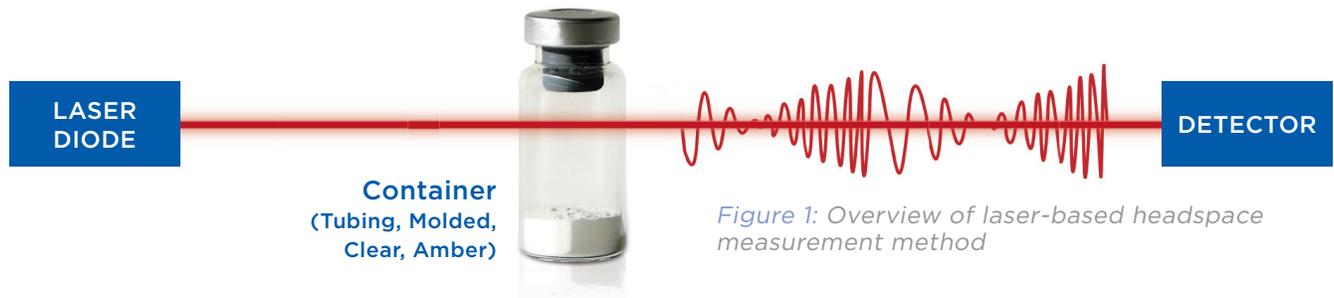


Figure 1: Overview of laser-based headspace measurement method

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

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.

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. Two basic configurations are benchtop systems for use off- or atline, and fully automated systems for 100% monitoring, control and inspection

Benchtop 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 single laser source for either oxygen monitoring at 760 nm or pressure/moisture monitoring at 1400nm. 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 an oxygen monitoring instrument would utilize standards of known oxygen 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.

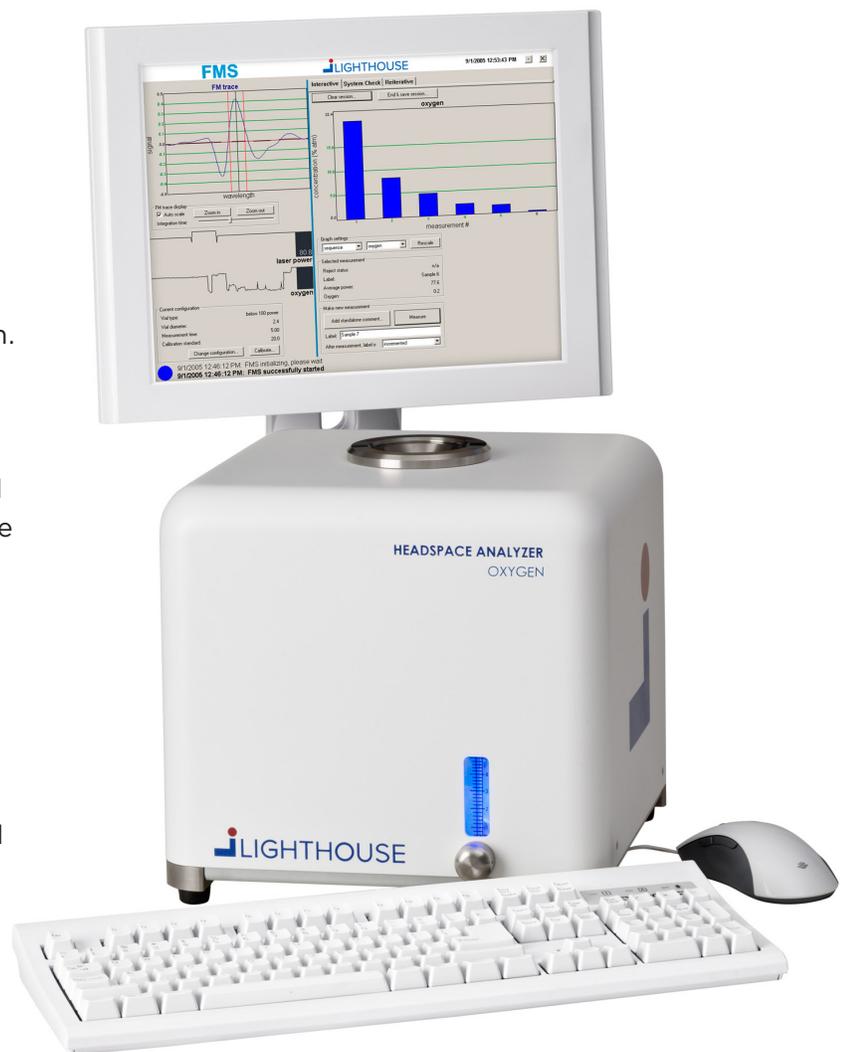


Figure 2: Benchtop headspace analyzer



*Figure 3:
Automated headspace
inspection machine*

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 leak detection and 100% container closure inspection.

The automation platform can be configured with single or multiple measurement heads to allow for oxygen monitoring and/or simultaneous pressure/moisture 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.

Container Closure Inspection Using Headspace Analysis

Introduction

Container closure integrity plays an important role in maintaining the stability and sterility of parenteral products and can generally be compromised due to component defects (e.g. cracks in glass, out of specification stopper dimensions or improper vial/ stopper combinations) or process defects (e.g. stopper pop-up prior to capping). Headspace gas analysis is useful for in-process leak detection by monitoring changes in headspace gas composition or changes in total headspace pressure. Cracks in glass, displaced stoppers and dimensional issues with vial/stopper combinations are defects that allow gas flow from outside a container to the inside and result in a loss of container closure integrity.

For containers initially processed with a modified atmosphere in the headspace (e.g. purged with an inert gas or evacuated to a reduced pressure), the practical implications of lost seal integrity are threefold. First, a headspace pressure rise or the ingress of oxygen will occur and can be correlated to a leak rate. Second, sterility can no longer be assured - studies have experimentally demonstrated a correlation between the leak rate and the probability of microbial ingress. Third, gas ingress (particularly oxygen and

container headspace has anything but air at one atmosphere. Examples of products packaged under modified atmospheres are oxygen sensitive liquids that are flame sealed or stoppered under inert atmospheres of nitrogen or argon and lyophilized products that are stoppered under nitrogen, usually at reduced pressure (either full or partial vacuum). In these cases a vial that leaks will exchange gas with the environment outside the container resulting in a total pressure rise (in the case of a vial stoppered under vacuum) or a partial pressure rise in oxygen (in the case of vials and ampoules closed under inert gas blankets).

Total pressure changes and oxygen partial pressure changes can be detected using headspace gas analysis as described in earlier sections. Two key questions are: 1) how long does it take for the headspace gas composition or pressure in a leaking vial to measurably change? And, 2) What level of risk does the measured leak rate pose to product stability and product sterility? A model has been developed to simulate the two situations most common for gas flow into leaking sterile pharmaceutical containers, effusive flow and diffusive flow. Experiments were set up and performed to verify the model.

General theory

Headspace gas analysis can be used for non-destructive leak detection. The discussion herein pertains to sterile product containers closed under modified atmosphere process (MAP) conditions. That is to say the

Diffusive flow

Defects in containers packaged at atmospheric pressure will exchange gas with the surrounding atmosphere through diffusion. A diffusion model was developed to show the time required for

oxygen ingress as a function of hole diameter, hole length and container volume. The model was then tested on vials with well defined defects.

To test the model a 20cc vial was purged with nitrogen to achieve 0% headspace oxygen. The vial was stoppered and capped. The stopper was punctured with a 5 cm long 21 G needle with a diameter of 318 micron ($A_0=8 \times 10^{-4} \text{cm}^2$). Note that this is equivalent to a ~5 micron defect through a 1 mm thick glass wall. Oxygen diffusion into the vial was measured using an FMS-Oxygen Headspace Analyzer over a 14 day period (Figure 4). The partial pressure of oxygen rose from 0 atm to 0.2 atm over the 14 day period. The model predicts the experimental results very well indicating that it could be used in a predictive manner for different hole sizes and headspace conditions.

Effusive flow

Effusive flow occurs when there is a pressure difference between the outside and inside of the container. This situation is typical for freeze-dried product. Most freeze-dried product is stoppered under vacuum or partial vacuum.

To test the leaking pharmaceutical container model in the Effusive flow regime, a certified 5 micron hole was laser-drilled in a 10 ml glass vial. The vial was evacuated to a pressure of 100 mbar and the subsequent rise in pressure due to the 5 micron leak was monitored with a FMS-Moisture/Pressure Headspace Analyzer. The results are shown in Figure 5. After a few minutes the headspace pressure has increased by 35 torr (50 mbar) as a result of the 5 micron hole. After approximately four hours the vial has leaked up to one atmosphere.

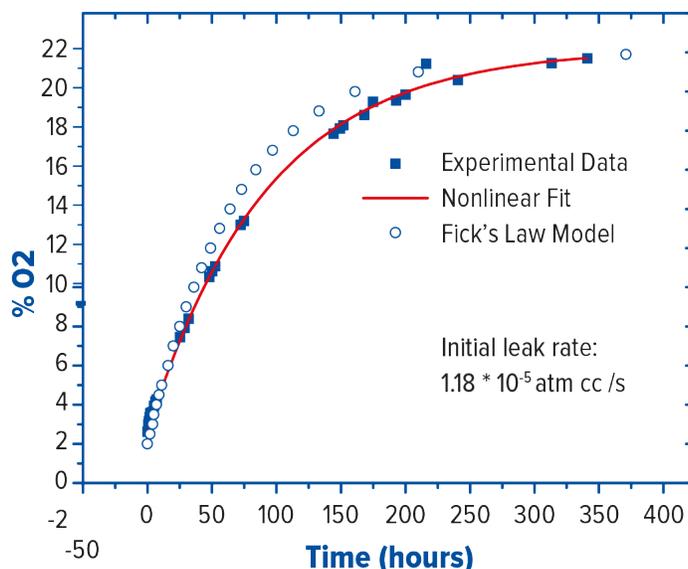


Figure 4: Oxygen ingress through a container defect equivalent to a 5 micron hole in a glass vial having an initial headspace condition of 1 atm of nitrogen.

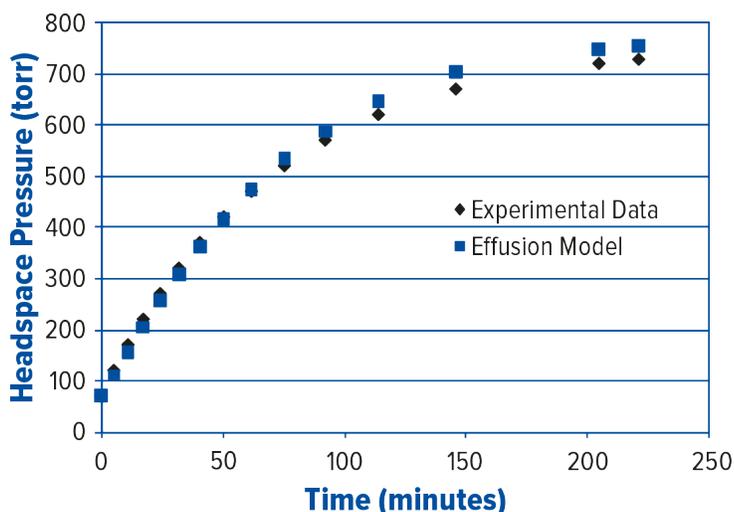


Figure 5: Rise in headspace pressure in a vial initially at 100 mbar of nitrogen and leaking through a 5 micron hole. The model matches the experimental results very well.

Industry Leak Detection Case Studies

The following case studies describe how non-destructive headspace inspection systems were used to monitor container closure integrity for commercial batches of lyophilized product and help solve process issues.

Industry Case Study 1

The first case study involves an investigation of container closure integrity for an oxygen sensitive lyophilized product stoppered with a partial vacuum of nitrogen at 800 mbar. A number of vials from a commercial batch showed elevated levels of oxygen during routine QC analysis using a destructive oxygen analysis method. It was decided to test the entire batch using a non-destructive headspace oxygen analyzer. The test criteria was to reject any vial with greater than 1% oxygen. Figure 6 shows the headspace oxygen concentration in vials of freeze dried product that have maintained seal integrity. The headspace oxygen levels are all below 1%. Figure 7 shows a very different situation

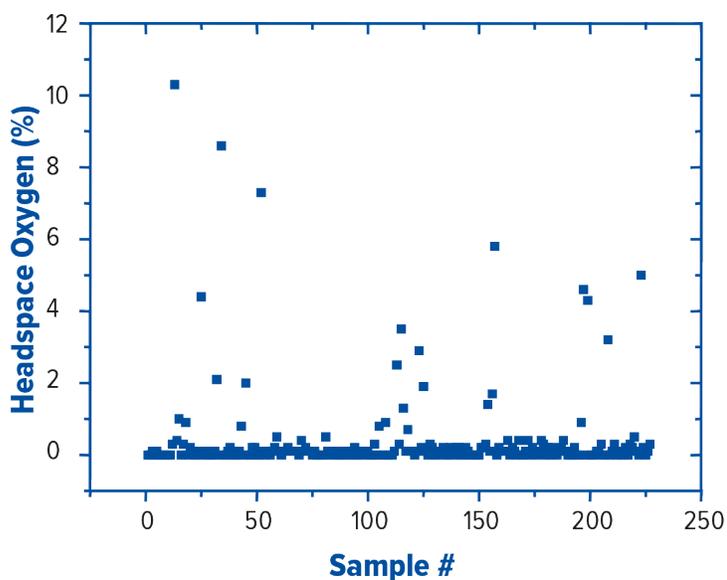


Figure 7: Headspace oxygen content of vials located in zones 1-3. Vials in these locations show air ingress due to stopper pop-up.

for a set of vials from the same batch of product. In figure 7 over 10% of the vials have lost seal integrity as evidenced by headspace oxygen levels from 1.5% to 10%. One difference between the two sample sets was their physical location inside the lyophilizer - the 'bad' vials all came from trays located at the shelf edge. These results from 100% headspace oxygen inspection led the manufacturer to investigate the root cause of the container closure integrity failures as a mechanical issue in the stoppering process resulting in stoppers not being properly seated at the shelf edges.

Industry Case Study 2

The second case study demonstrates a process development effort aimed at evaluating the container closure integrity of two different vial stopper combinations under actual in-process conditions. The same vial was evaluated with a grey butyl and a coated stopper. The lyophilized product was specified to be stoppered at 400 torr of nitrogen. The study evaluated each vial stopper combination for its ability to hold vacuum. The study used 1000 product filled vials (500 with each type of closure) distributed over 8 shelves (4 shelves with each type of closure). Figure 8 shows the headspace pressure in vials of freeze dried product stoppered with a grey butyl elastomeric closure at 400 torr. All of these vials have maintained seal integrity. The headspace pressure levels are uniform and match the pressure set in the lyophilization chamber prior to lowering the shelves. Figure 9 shows the headspace pressure in vials from the same batch that were stoppered using a coated closure. Over 15% of these vials did not maintain seal integrity after removal from the lyophilization chamber.

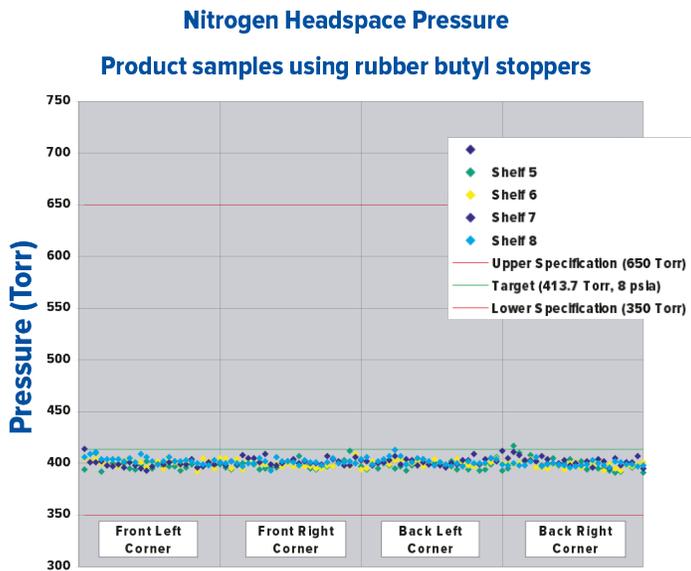


Figure 8: Headspace pressure in vials sealed with a grey butyl stopper. There is no evidence of stopper pop up on any shelf or in any location on a given shelf.

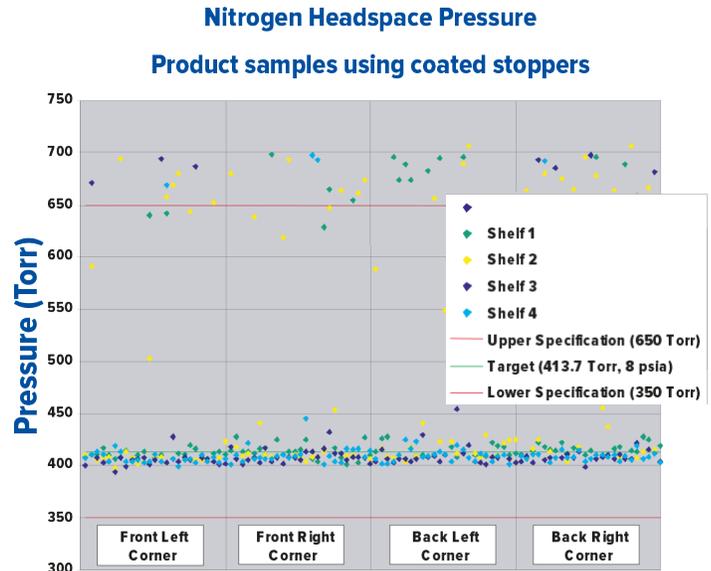


Figure 9: Headspace pressure in vials sealed with a teflon coated stopper. There is widespread evidence of stopper pop up on all shelves and in all locations on any given shelf.

The gas ingress to these vials resulted in headspace pressures from 30 to 300 torr above the target level. The fact that bad seal integrity was not dependent on location in the freeze dryer pointed to an issue with the coated stopper/vial combination, and/or an overall process issue. Further container closure studies using non-destructive headspace analysis allowed the manufacturer to optimize the stoppering process. Closure failures with the coated stopper were lowered from > 15% of the batch to < 1% with the optimized process. An automated headspace inspection machine was implemented for 100% final product inspection guaranteeing the detection and rejection of any residual vials that had lost container closure integrity.

Regulatory Perspective

Aseptic processing where sterilization is accomplished through filtration requires a greater level of monitoring and control compared to other pharmaceutical manufacturing processes. Several guidance documents from regulatory bodies around the world specifically address issues related to the finishing of sterile products where new inspection technologies, like laser-based headspace analysis, can be useful for gaining deeper insight to a manufacturing process and complying with regulations. Below we will briefly review those sections of the European Union (EU) and Food and Drug Administration (FDA) guidelines pertaining to the manufacture of sterile products where headspace gas analysis is applicable. In each section below the guidance is italicized and followed by a comment of how the implementation of nondestructive headspace analysis can help comply with the guidance.

The EU Guidelines—Annex 1 Manufacture of Sterile Medicinal Products

117. Containers should be closed by appropriately validated methods. Containers closed by fusion, e.g. glass or plastic ampoules should be subject to 100% integrity testing. Samples of other containers should be checked for integrity according to appropriate procedures.

As described in this white paper, laser headspace inspection can perform leak detection by monitoring changes in headspace gas composition. If a glass or plastic ampoule is sealed with a modified atmosphere (e.g. under an inert gas) then oxygen ingress is a leak indicator.



120. Vial capping can be undertaken as an aseptic process using sterilised caps or as a clean process outside the aseptic core. Where this latter approach is adopted, vials should be protected by Grade A conditions up to the point of leaving the aseptic processing area, and thereafter stoppered vials should be protected with a Grade A air supply until the cap has been crimped.

This provision related to capping of freeze-dried vials has caused a significant level of debate and discussion. The underlying intent of the guidance is to reduce the risk of microbial ingress in the event of a loss of container closure integrity during the time between a vial being stoppered and capped. Compliance involves installing the Grade A air. However, performing headspace analysis on individual vials after capping would provide a direct measure that seal integrity has been maintained.

121. Vials with missing or displaced stoppers should be rejected prior to capping. *Where human intervention is required at the capping station, appropriate technology should be used to prevent direct contact with the vials and to minimise microbial contamination.*

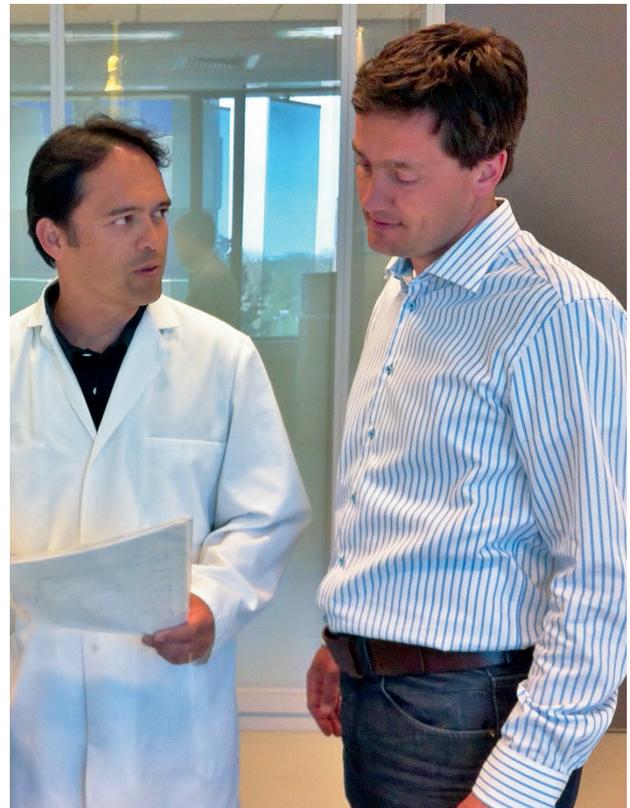
This guidance addresses container closure integrity and the risk of microbial ingress. For freeze dried products, which are almost always packaged with a nitrogen headspace at reduced pressure, headspace analysis of the gas composition and/or pressure can determine if container closure is lost due to a missing or displaced stopper. Liquid products that are purged with nitrogen or another inert gas prior to stoppering can be analyzed by headspace analysis to determine if stoppers are properly seated. If elevated oxygen levels are detected then the vial has leaked.

123. Containers sealed under vacuum should be tested for maintenance of that vacuum after an appropriate, pre-determined period.

For freeze dried products or liquid products that are packaged under vacuum, headspace analysis of the gas composition or pressure can determine if the vacuum is maintained.

124. Filled containers of parenteral products should be inspected individually for extraneous contamination or other defects. *When inspection is done visually, it should be done under suitable and controlled conditions of illumination and background. Operators doing the inspection should pass regular eye-sight checks, with spectacles if worn, and be allowed frequent breaks from inspection. Where other methods of inspection are used, the process should be validated and the performance of the equipment checked at intervals. Results should be recorded.*

There are microscopic defects, such as hairline cracks under the overseal in the neck of a vial, which will not be detected by visual inspection. These defects can cause gas ingress to the vial that is detectable via headspace gas analysis.



**FDA Guidance for Industry
Sterile Drug Products Produced by Aseptic
Processing — Current Good Manufacturing
Practice**

The sections below describe situations where headspace gas analysis can be used to comply with the FDA aseptic guidance.

**Paragraph IV. Buildings and Facilities
Section E Design pg 11**

*If stoppered vials exit an aseptic processing zone or room prior to capping, appropriate assurances should be in place to safeguard the product, such as local protection until completion of the crimping step. **Use of devices for on-line detection of improperly seated stoppers can provide additional assurance.***

The use of on-line headspace analysis systems can detect improperly seated stoppers when liquid filled or freeze dried vials are packaged under vacuum or with a nitrogen blanket.

**Paragraph VI. Components and
Containers/Closures, Section B-2
Containers/Closures pg 18 Inspection of
Container Closure System**

*A container closure system that permits penetration of microorganisms is unsuitable for a sterile product. **Any damaged or defective units should be detected, and removed, during inspection of the final sealed product.** Safeguards should be implemented to strictly preclude shipment of product that may lack container closure integrity and lead to nonsterility. Equipment suitability problems or incoming container or closure deficiencies can cause loss of container closure system integrity. For example, failure to detect vials fractured by faulty machinery as well as by mishandling of bulk finished*

stock has led to drug recalls. If damage that is not readily detected leads to loss of container closure integrity, improved procedures should be rapidly implemented to prevent and detect such defects.

Defective units that have lost container closure integrity will ingress gas from outside the container and result in a measurable change in oxygen content or total pressure if the headspace was modified during processing. Container closure integrity can be assessed using headspace gas analysis by measuring any change in the headspace gas composition or change in the vacuum level.

Most of the guidance referenced above relates to container closure integrity which can be monitored using non-destructive headspace gas analysis. The growth in sterile manufacturing capacity, the use of contract fill and finish facilities, and the use of new components have resulted in an increase in the number of defective containers and a subsequent increase in the number of product recalls. Table 1 shows the top 5 reasons for product recall in 2006 with defective containers being the number 2 reason.

Conclusions

Rapid non-destructive headspace analysis has been enabled by FMS, a high-sensitivity laser absorption technique. The ability to rapidly and non-destructively measure headspace oxygen, pressure, and moisture levels has led to new applications in both the manufacturing and development environments. Headspace inspection platforms based on FMS and used for 100% inspection of sterile product packaged under a modified atmosphere help assure the quality of the finished sterile product. In particular, it is now possible to quantify the headspace conditions in every sample at filling and/or on the packaging line. As described in this white paper, characterizing the headspace conditions is a direct measure of the seal integrity of the container. Containers that have lost closure are detected through changes in the

headspace conditions and can be rejected from the line. This means that defective samples posing a sterility risk (leak rate large enough to allow for microbial ingress) and/or a stability risk (elevated oxygen or moisture levels) are identified and removed from the batch.

In addition to a higher percentage of manufactured drug product being inspected to ensure quality, the products tested during production with rapid nondestructive headspace inspection remain in-line. This increases product yields and reduces waste compared to using off-line destructive testing. Finally, the quantitative insight that headspace inspection gives into the process enables sterile pharmaceutical manufacturers to streamline processes and reduce costs in the long run.

Table 1 (Source: J. Famulare, 2007 PDA/FDA Joint Regulatory Conference)

Top 5 Reasons for Recalls - 2006

1. Subpotent product
2. Defective container
3. Lack of sterility assurance
4. Impurity / degradation products
5. cGMP deviations (failure to perform or document required activities)

Table 1: Top 5 reasons for product recall in 2006 with defective containers being the number 2 reason.

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