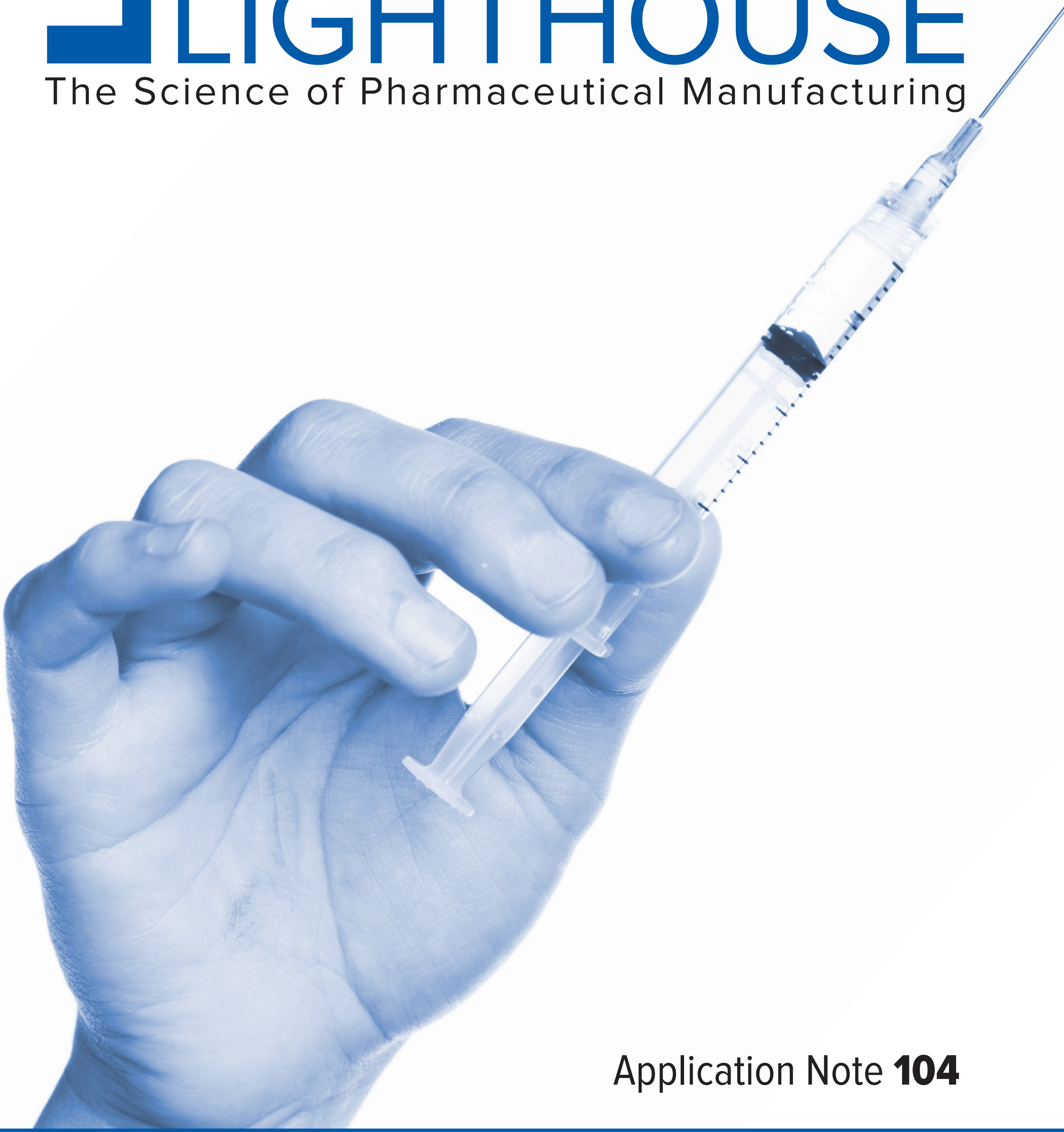
LIGHTHOUSE

The Science of Pharmaceutical Manufacturing



Application Note **104**

Headspace oxygen monitoring and container closure integrity testing of pre-filled syringes

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Introduction

The need to monitor headspace oxygen levels in pre-filled syringes arises from the requirement to ensure the stability and potency of oxygen-sensitive product. Besides a loss of efficacy and reduction in shelf life, exposure of such products to oxygen can result in product discoloration, changes in dissolution rate and profile, and even toxicity or other pharmacological properties associated with negative side effects.

The inspection of headspace oxygen in syringes having a purged nitrogen headspace can also be used to determine whether the container closure integrity of the syringe has been maintained. A pre-filled syringe that has suffered from a container closure integrity failure will immediately begin to diffuse air into the headspace. This ingress of air results in headspace oxygen levels that are elevated compared to syringes that have maintained closure integrity. Defective syringes with high levels of headspace oxygen can then be detected and removed in an inspection process.

This Application Note describes how laser-based headspace analysis is used for the rapid non-destructive determination of headspace oxygen levels in pre-filled syringes. Data is presented demonstrating two major applications of this technique: 1) headspace oxygen monitoring on a pre-filled syringe line filling oxygen-sensitive product, and 2) container closure testing of pre-filled syringes.

Headspace Oxygen Determination in Pre-filled Syringes

Traditional analytical methods for determining headspace oxygen levels in parenteral containers are slow and/or destructive. This results in headspace oxygen analysis which is both time and resource intensive. Conventional destructive techniques, such as electrochemical methods or gas chromatography, are difficult to implement at- or in-line for immediate feedback about the filling process.

Figure 1. The LIGHTHOUSE Headspace Oxygen Inspection systems.

The destructive nature of the measurement also means that these conventional methods cannot be utilized for 100% inspection of product. Once the septum of the container is pierced to take a headspace sample, container integrity is compromised and the sample must be disposed of. Pre-filled syringes provide additional challenges for traditional headspace analysis techniques because of the small headspace volumes and the difficulty in accurately extracting the headspace gas for analysis.

LIGHTHOUSE laser-based headspace systems enable rapid non-destructive headspace oxygen analysis in pre-filled syringes. Benchtop systems are used in formulation, packaging, and process development as well as for QC activities. The robustness and easy operation of the platforms allow for at-line or automated in-line implementation in the Production environment. The rapid non-destructive nature of the measurement allows for immediate feedback to the filling process, 100% inspection of containers, and there is no disposal of destroyed product.

The patented LIGHTHOUSE platforms utilize a high sensitivity detection technique known as Frequency Modulation Spectroscopy (FMS). Light from a near-infrared semiconductor laser is tuned to match the internal vibrational frequency of the oxygen molecule. Measuring the absorption of the laser light after it passes through the syringe headspace allows for the determination of the headspace oxygen concentration. Sophisticated modulation techniques are applied to the output of the diode laser and to the signal processing, making the method ten thousand times more sensitive than methods based on first order radiation absorption such as NIR.

A key parameter for making good headspace oxygen measurements in pre-filled syringes is the size of available headspace. Because of the difficulties in controlling and measuring headspace oxygen levels in syringes with older filling line technology and analytical methods, a standard approach for filling oxygen-sensitive product has been to minimize the syringe headspace as much as possible. Vacuum methods for stoppering syringes minimize the headspace volume to a small bubble of air, therefore minimizing the amount of oxygen in contact with the product. The disadvantage of this approach is that it is impossible to actually measure the oxygen levels to prove and validate that the process is being controlled to specification for stability of the product. In the past few years, filling line technology has progressed to the point that lines for pre-filled syringes are delivered with purging systems that can consistently purge the syringe headspace to oxy-

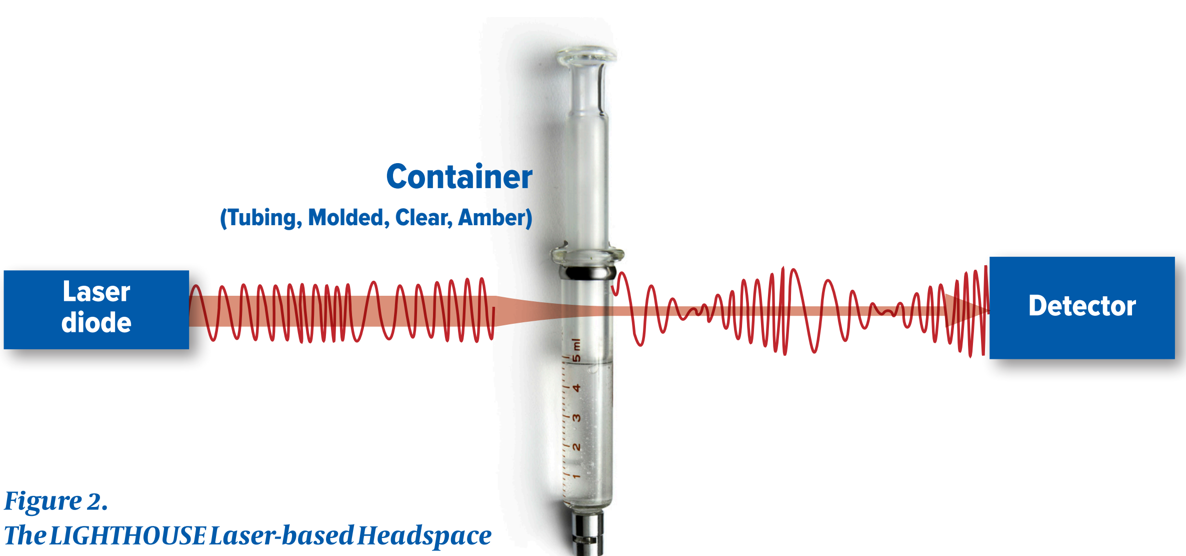

Figure 2.
The LIGHTHOUSE Laser-based Headspace Inspection Method

12mm headspace

STND	AVG	STD DEV
20%	19.91	0.26
8%	8.66	0.37
4%	4.42	0.41
2%	2.35	0.29
1%	1.37	0.41
0%	0.60	0.36

Table 1.
Measurements on syringe oxygen standards having a headspace of 12mm

2mm headspace

STND	AVG	STD DEV
20%	20.07	0.82
8%	8.99	0.55
4%	4.19	0.79
2%	2.49	0.66
1%	1.43	0.71
0%	0.59	0.51

Table 2.
Measurements on syringe oxygen standards having a headspace of 2mm

gen levels < 5%. Nitrogen purging needles are used to purge the syringe before and during filling as well as at stoppering to achieve low levels of oxygen. Filling in an isolator that is also overlayed with nitrogen allows the filling and purging process to reach headspace oxygen levels down to 2%. To achieve consistent, controllable purging, these filling lines usually stopper the syringes with headspace volumes having heights of 3 to 10 mm between the stopper and the liquid fill.

A study was done to investigate the performance of the LIGHTHOUSE FMS-Oxygen Headspace Analyzer as a function of syringe headspace volume. A standard 1 ml glass syringe with a diameter of 8 mm was used for the experiment. To manufacture certified oxygen standards, empty syringes were evacuated and then backfilled with certified gas mixtures having oxygen contents of 0, 1, 2, 4, 8, and 20% oxygen. The syringes were then flame-sealed to make NIST traceable syringe standards at known oxygen levels. Masks were made having slits of different sizes ranging from 2 to 12 mm. The masks were placed over the oxygen standards to simulate headspace volumes having heights of 2 to 12 mm. Each standard was then measured ten times with each mask to characterize the performance of the oxygen analyzer as a function of headspace size at the different levels of oxygen. The results for the 12 mm and 2 mm masks are show in Tables 1 & 2 and show that oxygen analysis can be performed even for a syringe headspace as small as 2 mm.

In conclusion, recent advances enable the possibility to quantitatively define and control the oxygen levels for product being filled in syringes. Key advantages to the laser-based headspace oxygen analysis method are the fact that the measurement is both rapid and non-destructive. A single sample can be non-destructively measured multiple times saving valuable product. The method itself is rapid and straightforward – the headspace system operator needs no special expertise to run the equipment and perform headspace oxygen measurements.

Applications of rapid non-destructive headspace oxygen analysis in pre-filled syringes

The ability to make headspace oxygen measurements in pre-filled syringes means that the stability of oxygen-sensitive product in syringes, and the quality of the syringe filling process with respect to adequate purging, can be efficiently characterized and validated. It is also possible to use the headspace oxygen measurement to perform container closure integrity testing of pre-filled syringes that have a purged headspace.

Headspace oxygen monitoring

Making multiple measurements on the same sample is especially advantageous when performing stability studies to determine the oxygen-sensitivity of a formulation. Figure 3 shows the results of a simple experiment performed with two common antioxidant formulations, sodium ascorbate and ascorbic acid. Three samples of each formulation were sealed under a headspace of air. The headspace oxygen levels were then measured and monitored over a period of ten days. The results show how the laser-based headspace method enables the determination of the full oxidation curve for these two formulations. The rapid non-destructive nature of the method required a total of only six samples (three of each formulation). The total time required for the sixty measurements (one measurement per sample over ten days) was less than ten minutes. These results demonstrate the efficiency of stability studies using laser-based headspace analysis as the savings in material and time are significant when com-

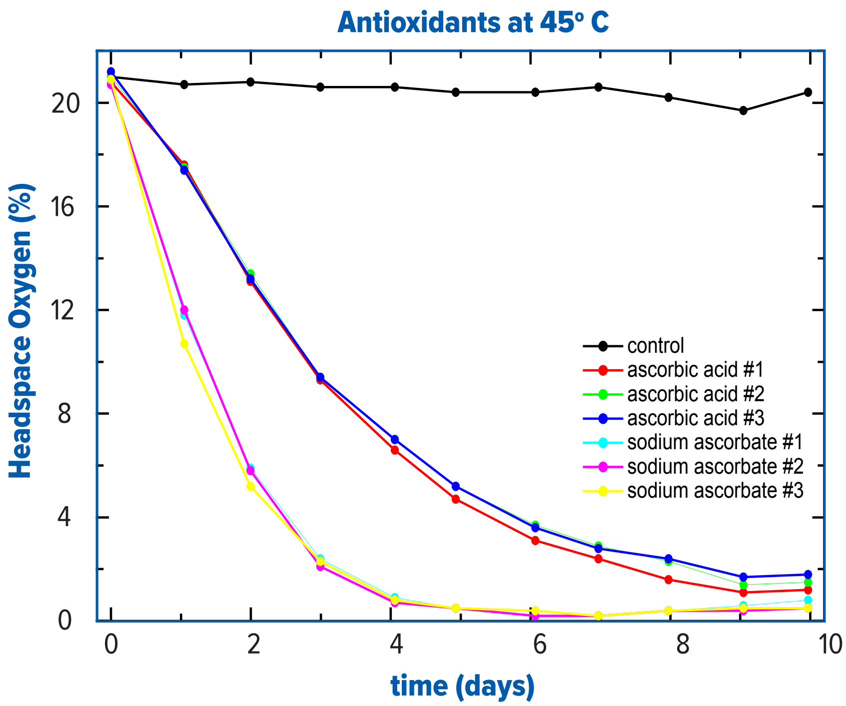

Figure 3. Results of a stability experiment using antioxidants. A full mapping of the oxidation curves is made over ten days using only three samples of each formulation.

pared to doing the same study using traditional destructive methods for oxygen analysis.

Once the oxygen specification of a formulation has been determined, a filling process must be designed and optimized to ensure that headspace oxygen levels are kept below specification. Optimizing and validating the filling and purging process with respect to achieved headspace oxygen levels can be a time-consuming exercise when using traditional headspace oxygen analysis methods. These are slow and require being set up in an analytical laboratory instead of at-line in the filling area. The portability and ease-of-use of the FMS-760 Oxygen Analyzer allows for set up on a rolling cart that can be placed next to the filling line for optimization and validation studies. In this way, immediate feedback is given with respect to the headspace oxygen

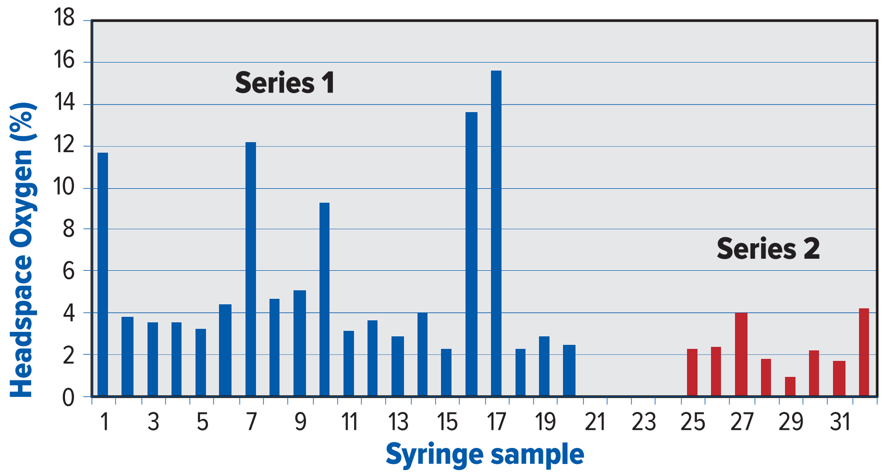

Figure 4. Measured headspace oxygen in syringes filled on a line being validated in a new parenteral manufacturing facility. An initial series of syringes showed a number of syringes having headspace oxygen levels above the 5% specification. A later series showed the optimized process producing syringes with average headspace oxygen levels of 3%.

levels in the syringe headspace. Parameters can be quickly optimized and validation data collected to demonstrate that the filling and purging process is working to specification. Figure 4 shows some headspace oxygen data collected from syringes filled on a new pre-filled syringe line. This line is located in a new state-of-the-art parenteral facility and was in the process of being validated. The syringe was a standard 1 ml glass syringe that was purged with nitrogen during filling as well as at stoppering. The stopper was inserted into the syringe to leave a headspace of approximately 6mm. Data from an initial series shows some syringes having elevated oxygen levels above the specification of 5%. Data collected from a later series shows how the optimized process produced syringes having average headspace oxygen levels of 3%.

Container closure integrity testing

Finally, the headspace oxygen measurement can be used to perform container closure integrity testing of a syringe with a purged headspace. If the syringe has lost closure integrity, there will be a diffusion of air into the headspace. Since the purged syringe initially has low levels of oxygen, the diffusion of air into the headspace will result in increasing headspace oxygen levels over time. Elevated headspace oxygen levels will then identify a syringe that has lost closure integrity. The increase of headspace oxygen levels through a leak happens relatively fast and has been verified by experiment as well as by a headspace leak rate model.

Figure 5 shows the results of a container closure experiment. The experiment used 1 ml ampoules that are comparable in size to 1 ml syringes. Three ampoules were prepared with laser-drilled holes having diameters of 5, 10, and 15 microns. These ampoules were then flushed with nitrogen and sealed. The headspace oxygen levels were measured over time to determine the rate of oxygen increase by diffusion through the hole into the headspace. The results for all three ampoules show the oxygen levels increasing rapidly, with each ampoule reaching atmospheric levels of oxygen within 24 hours.

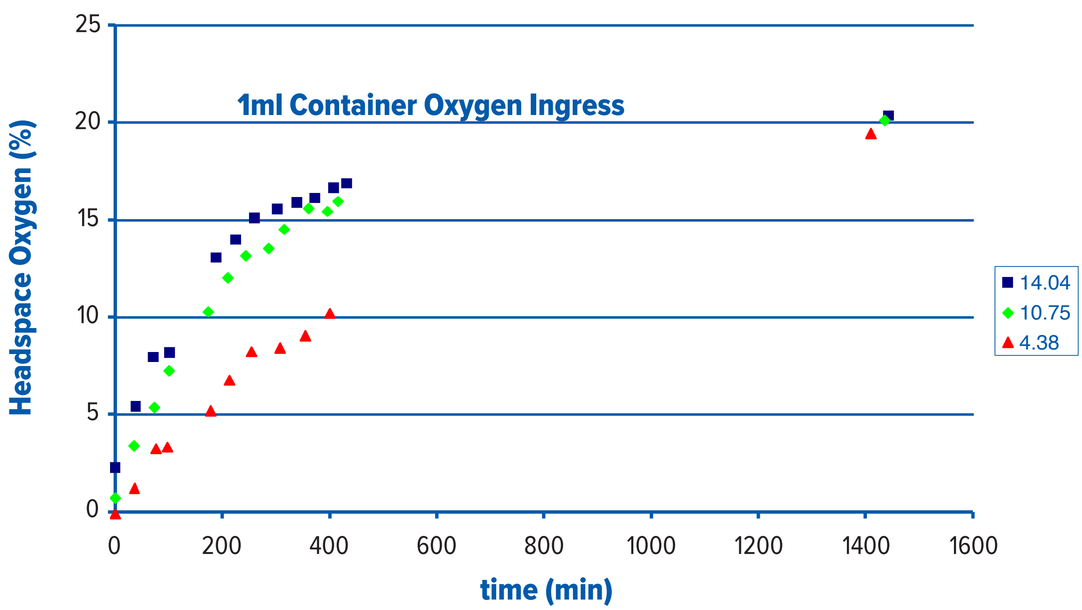

Figure 5. Oxygen ingress as a function of time into a 1ml container through laser-drilled holes of 4.38, 10.75, and 14.04 microns.

		Measured vs Theoretical Oxygen Concentration					
VOLUME	HOLE SIZE	INITIAL (% ATM)		8 HOURS (% ATM)		24 HOURS (% ATM)	
(ML)	(MICRONS)	MEASURED	THEORY	MEASURED	THEORY	MEASURED	THEORY
1	4.38	0	0	10.19	10	19.44	18.2
	10.75	0.69	0.69	15.93	17.4	20.12	20.9
	14.04	2.25	2.25	16.85	18.47	20.33	20.9

Table 3. Comparison of theoretical headspace oxygen ingress modelling with some of the experimental results plotted in Figure 5.

A headspace leak rate model was used to compare theoretical modelling results to the results of the experiment (see Reference 1 for details of the model and for more about pharmaceutical applications of laser-based headspace inspection). Table 3 shows that the headspace leak rate model is able to predict the experimental headspace oxygen results quite well.

This is advantageous for guarding the stability and extending the shelf life of large molecule formulations that are oxygen-sensitive. Headspace analysis systems can help streamline stability studies, process development, & equipment validation, and also enable real time process monitoring and 100% inspection of headspace oxygen levels in the finished product.

Conclusions

Laser-based headspace analysis enables rapid non-destructive oxygen determination in the headspace of pre-filled syringes. The increasing use of filling lines with advanced purging systems means that increasing numbers of pre-filled syringes will be delivered with headspaces purged with nitrogen.

References

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About Us

LIGHTHOUSE is the leading manufacturer and provider of laser-based headspace inspection systems for finished sterile product applications specific to the pharmaceutical industry. These applications include leak detection, container closure studies, headspace oxygen monitoring, product moisture determination, and lyo cycle development in both production and R&D environments. LIGHTHOUSE developed the laser-based headspace technology with funding from the Food and Drug Administration. We have hundreds of headspace inspection 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, Covidien, DSM, Eli Lilly, Genentech, GlaxoSmithKline, Helvoet Pharma, Johnson & Johnson, Merck, Novartis, Patheon, Pfizer, Roche, Schering-Plough, Serum Institute of India, Sankyo, Sanofi-Aventis, Talecris Biotherapeutics, TEVA, West Pharmaceutical Services, and Wyeth.

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