Rapid Non-Destructive Headspace Oxygen Analysis of Product Ampoules

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Introduction

Laser-based headspace analysis from LIGHTHOUSE allows for the rapid non-destructive analysis of headspace oxygen levels in parenteral containers (vials, ampoules, syringes, bottles, pouches).
Here we describe measurements of headspace oxygen levels in different types of product ampoule samples.

Instrumentation and Method

Laser Absorption Spectroscopy: General Background Information

Laser absorption spectroscopy is an optical measurement method for rapid and non-invasive headspace gas analysis of sealed containers. The technique can measure a number of physical parameters within the headspace of a container, including gas concentrations and total headspace pressures.

The LIGHTHOUSE systems incorporate a high sensitivity detection method known as *frequency modulation spectroscopy* (FMS). A description of frequency modulation spectroscopy for laser-based headspace analysis is given below and schematically depicted in Figure 1.

Light from a near-infrared laser diode is tuned to match the internal absorption frequency of the target molecule (Figure 1, step I). The light is then passed through the headspace region of a container (Figure 1, step II), scanned in frequency and detected by a photodetector (Figure 1, step III). The amount of light absorbed is proportional to the target molecule concentration as can be seen in the graphical insert in Figure 1.

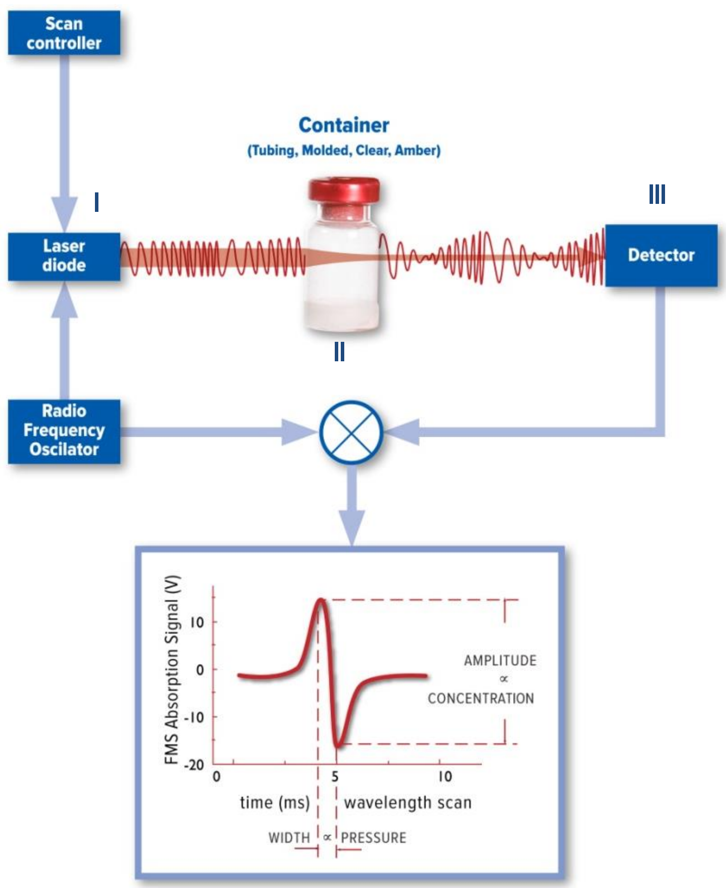

Figure 1. Schematic of frequency modulation spectroscopy for laser-based headspace analysis.

Headspace Oxygen Measurements

Measurement Principle

The LIGHTHOUSE FMS-760 Headspace Oxygen Analyzer operates on the principles of frequency modulation spectroscopy (FMS) as described earlier. Light from a near infrared diode laser is directed through the headspace region of a sealed (parenteral) container. Since oxygen absorbs near infrared light in a band of transitions centered at 762 nm, the LIGHTHOUSE FMS-760 diode laser operates at this wavelength.

The amount of laser light absorbed by an individual transition in the oxygen A-band is proportional to the oxygen concentration in the headspace of a container. During a measurement, the laser frequency is repeatedly scanned over the absorption feature and successive scans are averaged to improve the signal to noise ratio. As can be seen from the graph depicted in Figure 2, the averaged light absorption signal is proportional to the headspace oxygen concentration.

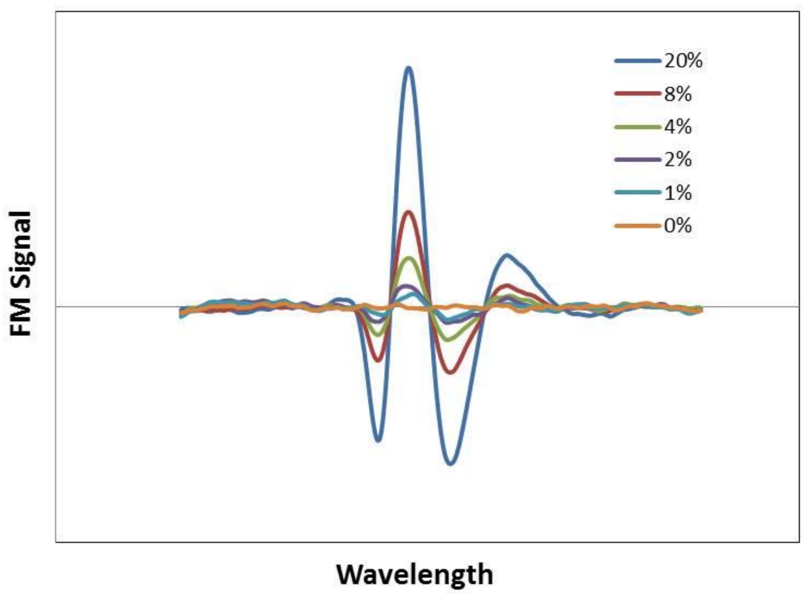

Figure 2. Frequency modulation signals from oxygen absorption in 10mL ampoules filled with certified gas mixtures of oxygen in nitrogen. The peak-to-peak amplitude of each spectrum is proportional to the oxygen concentration.

Sample Set

Five different sets of product ampoules each containing three samples per set, were provided for analysis. The characteristics of each sample set are listed in Table 1 below. The objective of the measurements was to investigate the feasibility of measuring headspace oxygen levels in these product ampoules.

Table 1: Overview of customer sample set

Sample set #	Ampoules	Type	Approximate Diameter
Set 1	25 mL	Amber tubing	22.5 mm
Set 2	10 mL	Clear tubing	17.8 mm
Set 3	5 mL	Clear tubing	14.8 mm
Set 4	2 mL	Amber tubing	10.8 mm
Set 5	1 mL	Clear tubing	10.7 mm

Measurement Protocol

Headspace Oxygen measurements

Headspace oxygen measurements were performed using a validated LIGHTHOUSE FMS-760 Headspace Oxygen Analyzer. The instrument was turned on and allowed to warm up for thirty minutes. Calibration was performed using certified NIST-traceable oxygen standards manufactured by LIGHTHOUSE.

Prior to sample analysis, six oxygen standards (at 20, 8, 4, 2, 1, and 0% oxygen) were each measured three consecutive times to verify performance of the analyzer. The results are presented and discussed in the following sections of this report.

Results

Prior to analysis of the sample vials, a set of known standards was measured to determine the performance of the headspace analyzer. Standards with known oxygen levels were each measured three consecutive times. The mean measured headspace oxygen levels and corresponding standard deviations are listed in Table 2.

Table 2: Headspace oxygen measurement of known standards

	Headspace oxygen	
Label	Mean (% atm)	St. Dev. (% atm)
20% standard	20.13	0.12
8% standard	7.93	0.09
4% standard	4.02	0.07
2% standard	2.02	0.12
1% standard	0.96	0.05
0% standard	0.05	0.03

The sample ampoules provided by the customer were each measured for headspace oxygen levels three consecutive times. The mean measured headspace oxygen levels and corresponding standard deviations are listed in Table 3. The larger 25ml and 10 ml had a reject limit set by the customer of 5% atm. The customer set the reject limit for the smaller ampoules at 2% atm oxygen. The mean measured headspace oxygen levels are also plotted per ampoule configuration in Figure 3.

Table 3: Mean measured headspace oxygen levels in customer samples

	25ml ampoule		10ml ampoule		5ml ampoule		2ml ampoule		1ml ampoule	
Label	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.
Sample 1	1.78	0.12	0.70	0.16	0.50	0.17	0.26	0.19	0.17	0.05
Sample 2	1.49	0.09	0.93	0.09	0.49	0.05	0.26	0.10	0.24	0.13
Sample 3	1.13	0.07	0.88	0.21	0.75	0.10	0.26	0.15	0.22	0.18

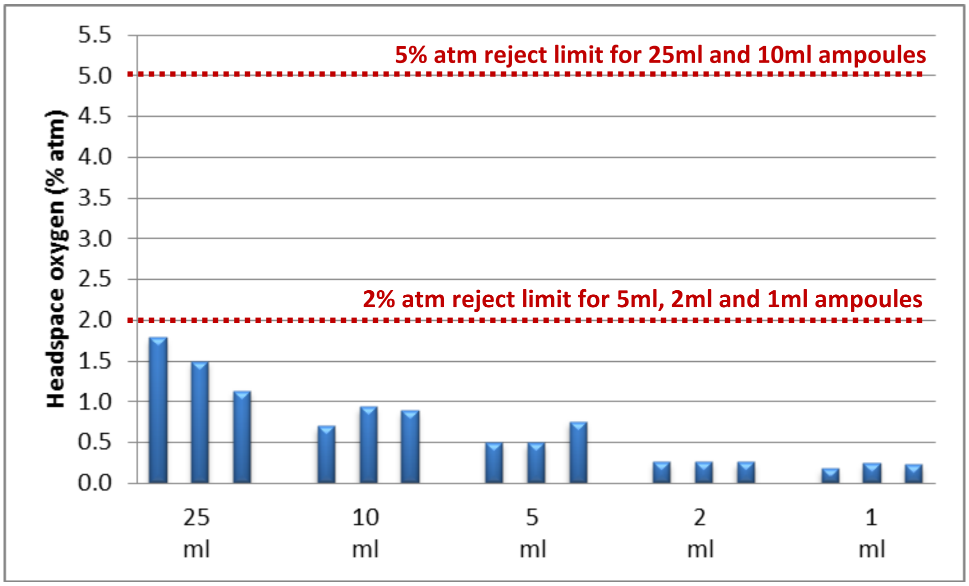

Figure 3: Mean measured headspace oxygen levels in the different ampoules. The red dotted lines indicate the two reject limits set by the customer.
For the 25ml and 10 ml ampoules a 5% atm oxygen reject limit was set. For the smaller ampoules a reject limit of 2% atm oxygen was set.

Discussion

The results presented in this report show that measurement of the headspace oxygen levels was possible within the product ampoules provided by the customer.
As can be seen from the graph in Figure 3, the headspace oxygen levels in the 25 ml amber and 10 ml clear ampoules were well below the set 5% oxygen limit of the process. The headspace oxygen levels of the smaller ampules also fell well below the set 2% oxygen limit.

Conclusions

The results presented here show that the LIGHTHOUSE FMS-760 Headspace Oxygen Analyzer was able to measure oxygen levels in all the product ampoules that were provided by the customer. The amber glassware of the 25ml and 2ml ampoules did not interfere with the measurements.

The ability to rapidly and non-destructively measure oxygen levels in product ampoules enables insight into the overall quality of a batch with respect to the purging process. In addition, the nondestructive nature of the measurement can save product in development activities allowing for efficient & accurate stability and end-of-shelf life studies.

For Further Reading

1) LIGHTHOUSE Application Note 102: "In-process monitoring of headspace oxygen levels in parenteral containers"
Downloadable at:
http://www.lighthouseinstruments.com/uploads/documents/LIGHTHOUSE_Oxygen_Monitoring_AppNote102_cover.pdf

2) A complete overview of the headspace analysis method can be found in:
Veale, J. "New Inspection Developments" Chapter 15 of Practical Aseptic Processing, Fill and Finish, Vol. 1, edited by Jack Lysfjord.