Rapid Non-Destructive Headspace Oxygen Analysis of 1.0 ml Pre-filled Syringes

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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 pre-filled 1.0 ml syringe samples. The recently launched LIGHTHOUSE Syringe Headspace Analyzer was used for the measurements in this study. This system is configured and optimized for the headspace analysis of pre-filled syringes. Product filled syringes with headspace heights down to approximately 2mm can be analyzed with this platform.

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.

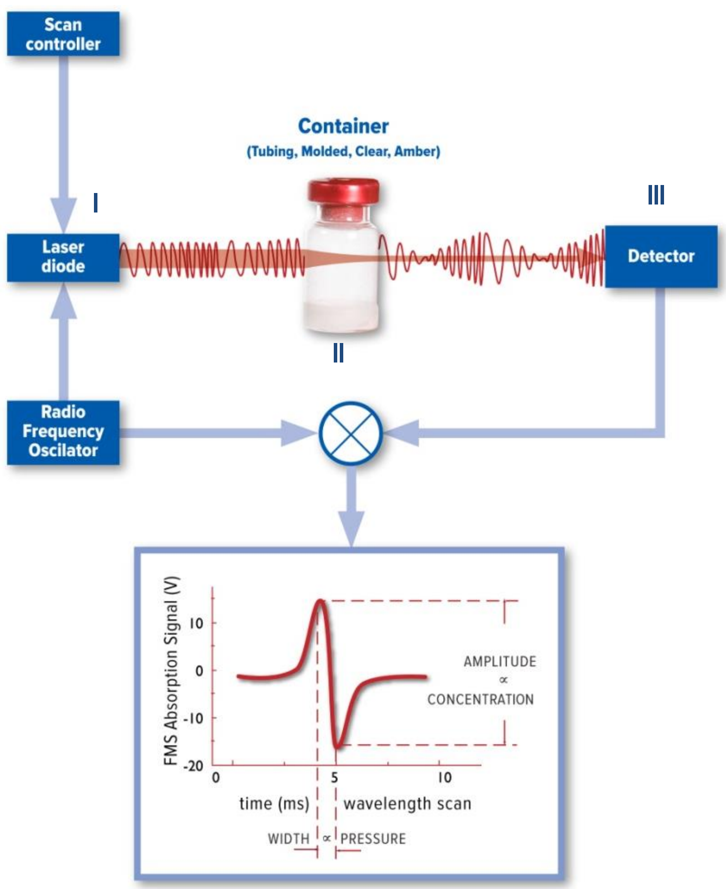

Figure 1. Schematic of frequency modulation spectroscopy for laser-based headspace analysis.

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.

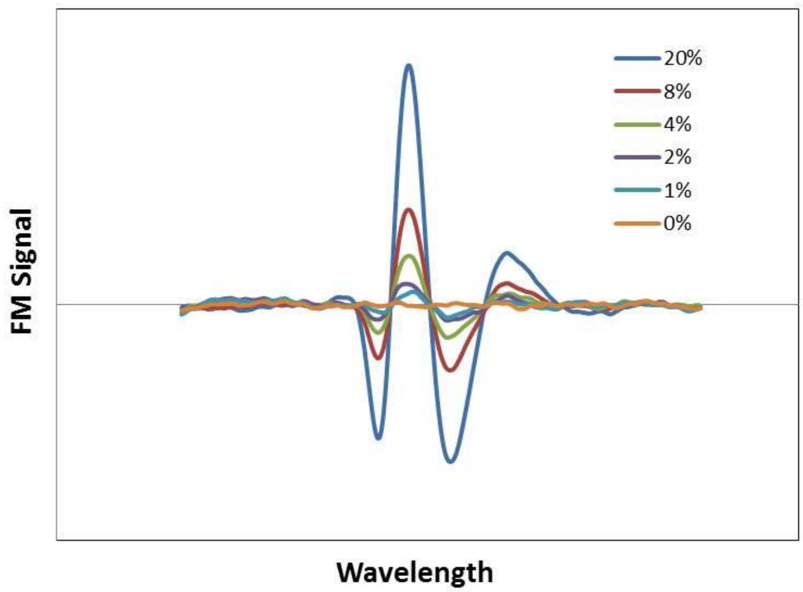

Figure 2. Frequency modulation signals from oxygen absorption in 10mL ampoules filled with certified gas mixtures of oxygen in nitrogen. The peak-to-peak amplitude of each spectrum is proportional to the oxygen concentration.

Sample Set

A total of one hundred and eighty (180) pre-filled 1.0ml syringes were provided for analysis. The syringes contained a 0.6ml product fill and had an empty headspace height of approximately 3mm. The syringes were divided into nine groups of twenty samples each: All of the samples were filled and stoppered under a nitrogen overlay so that headspace oxygen levels should be reduced compared to atmospheric oxygen levels. The samples came from three different batches. Within a batch, samples were collected from different time points in the filling run (start, middle, end of filling) as can be seen in the sample overview in Table 1.

Table 1: Overview of measured sample set

Batch nr.	Filling time point	Nr. of samples
1	Start of batch	20
	Middle of batch	20
	End of batch	20
2	Start of batch	20
	Middle of batch	20
	End of batch	20
3	Start of batch	20
	Middle of batch	20
	End of batch	20

Measurement Protocol

Headspace Oxygen measurements

Headspace oxygen measurements were performed using a validated LIGHTHOUSE FMS-760 Syringe 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.

Each product filled syringe was measured three consecutive times. The measurement results were stored electronically and a mean value and standard deviation were calculated for each vial.

Results

A set of known oxygen standards was measured to determine the performance of the headspace analyzer prior to sample measurement. The standards of known oxygen concentration were each measured three consecutive times. The mean measured headspace levels and the corresponding standard deviations are listed in Table 2.

Table 2: Mean measured headspace oxygen levels in known standards.

Label	Headspace oxygen Mean (% atm)	St. Dev. (% atm)
20% standard	20.20	0.22
8% standard	8.20	0.40
4% standard	3.98	0.35
2% standard	2.03	0.44
1% standard	1.02	0.16
0% standard	0.31	0.18

Figure 3 below graphically summarizes the measurements on all 180 pre-filled syringe product samples. The dotted lines in the graph separate the three different batches. The results for the sample subsets are plotted in the order 'Start', 'middle', and 'end'.

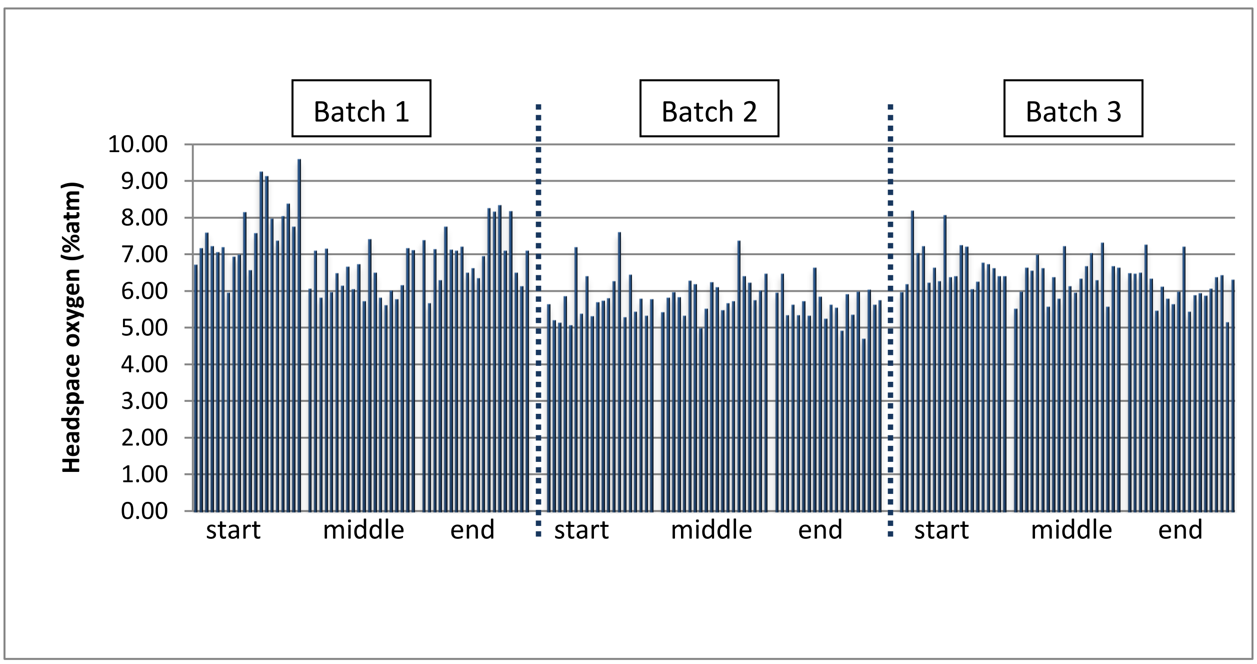

Figure 3: Values plotted are the mean results of three consecutive headspace oxygen measurements of syringe samples from Batches 1, 2 and 3. Each batch contained three subsets of samples taken from either the beginning, middle or end of the batch.

The figures below graphically summarize the measurements on the 60 pre-filled syringe product samples in each of the three batches 1, 2, and 3. The red bars are measurements taken of a certified 8% oxygen standard for comparison. The corresponding data for the samples in Batch 1 can be found in the Appendix of this report.

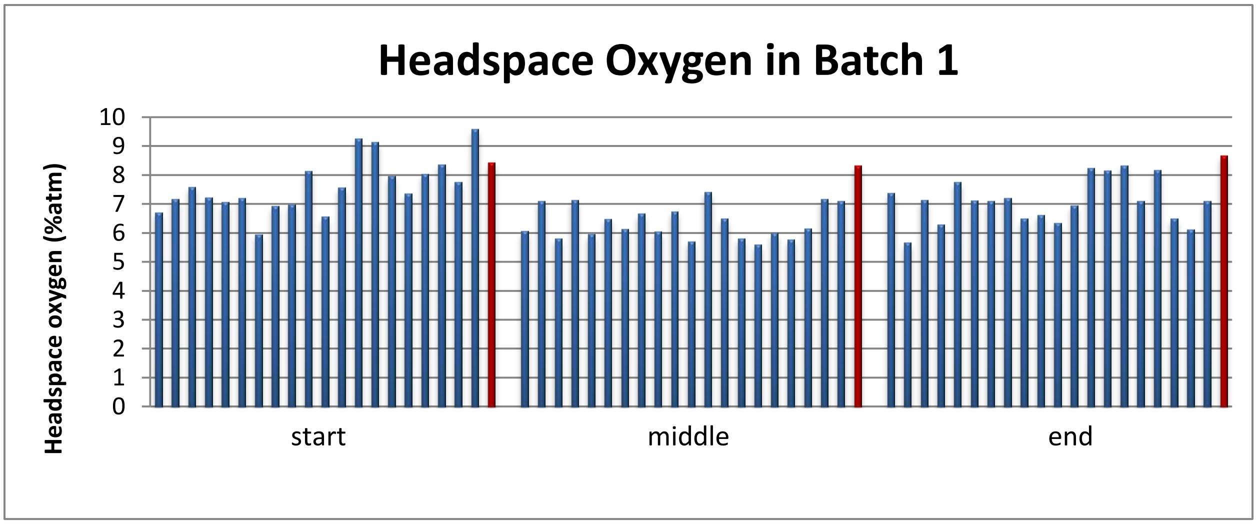

Figure 4: Values plotted are means of three consecutive headspace oxygen measurements. Labels below each group of samples indicate what part of the batch the samples are from. The red data points are measurements on a certified 8% reference standard that were made for comparison.

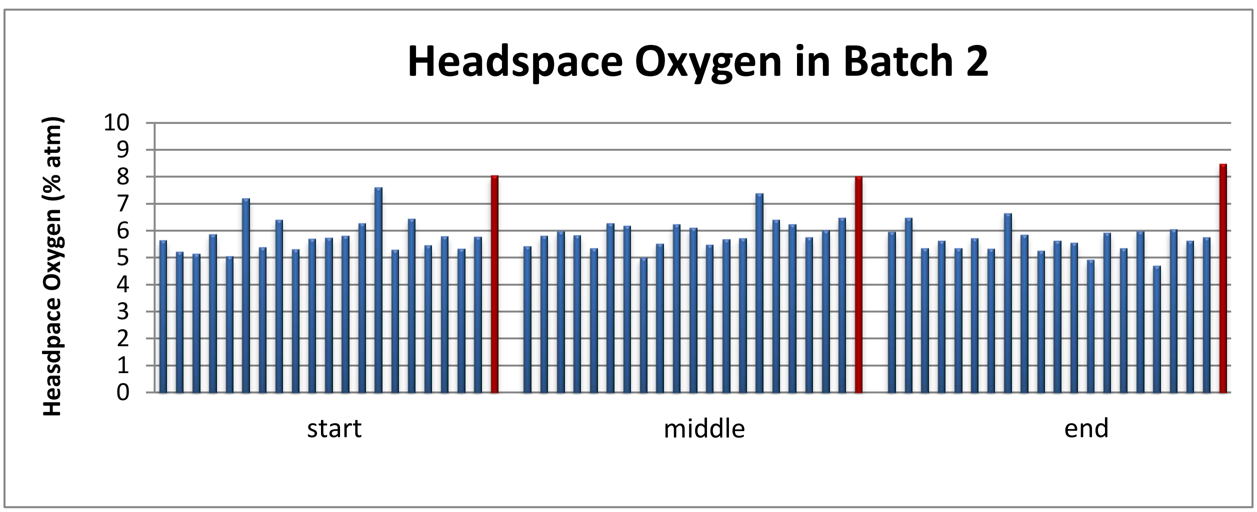

Figure 5. Values plotted are means of three consecutive headspace oxygen measurements. Labels below each group of samples indicate what part of the batch the samples are from. The red data points are measurements on a certified 8% reference standard that were made for comparison.

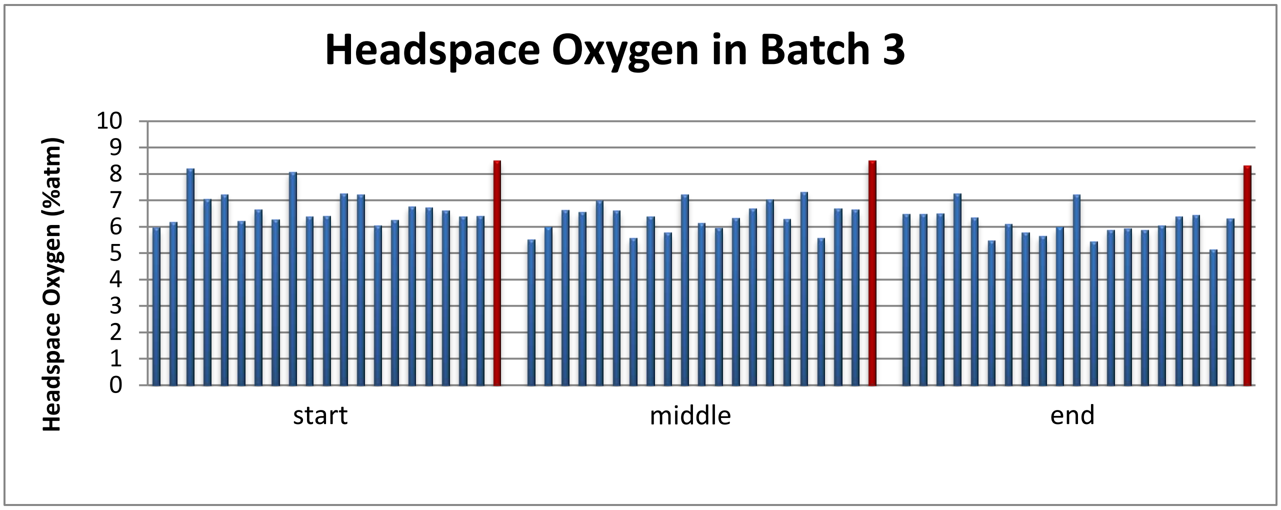

Figure 6: Values plotted are means of three consecutive headspace oxygen measurements. Labels below each group of samples indicate what part of the batch the samples are from. The red data points are measurements on a certified 8% reference standard that were made for comparison.

Discussion

The data plotted in Figure 3 and summarized in Table 3 below gives insight into the ability of the filling, purging, and stoppering process. Finished syringe samples were produced with a slight variation of the headspace oxygen levels, batch to batch.

As can be seen from Table 3, low headspace oxygen levels were measured in the three batches with a mean value of 7.03%, 5.80%, and 6.42% for Batches 1, 2, and 3, respectively. The measured standard deviation was below 1% for all three batches with a few individual syringe samples having outlying headspace oxygen levels (up to 9.6% atm).

Table 3: Mean measured headspace oxygen levels in prefilled pre-filled syringe samples.

	Headspace oxygen	
Batch nr.	Mean (% atm)	St.Dev. (% atm)
1	7.03	0.92
2	5.80	0.56
3	6.42	0.61

The data plotted in Figures 4, 5, and 6 gives insight into the ability to produce finished syringe samples with consistent, low headspace oxygen levels within a single batch. The results for Batches 1, 2, and 3 show fairly consistent headspace oxygen levels across the samples labeled 'start', 'middle', and 'end'. Individual samples that did not seem to be purged as efficiently as the rest of the batch could be easily identified.

Conclusions

The results presented here show that the LIGHTHOUSE FMS-760 Syringe Headspace Analyzer was able to measure oxygen levels in the provided 1.0 ml pre-filled syringe samples having headspace heights of approximately 3mm. The ability to rapidly and nondestructively to measure oxygen levels in pre-filled syringes is a significant advancement as standard destructive headspace oxygen analysis methods do not work with small syringe headspace volumes.

The rapid nature of the laser-based headspace method can enable efficient characterization, optimization, and validation of the filling and purging process. The nondestructive nature of the measurement can save product in development activities allowing for efficient & accurate stability and end-of-shelf life studies. In this particular case, insight was gained into the efficiency and consistency of the process for producing syringe samples with low headspace oxygen levels.

For Further Reading

1) LIGHTHOUSE Appliction Note 102: "In-process monitoring of headspace oxygen levels in parenteral containers"
Downloadable at:
http://www.lighthouseinstruments.com/uploads/documents/LIGHTHOUSE_Syringe_AppNote_104.pdf

2) Veale, J. "New Inspection Techniques for Aseptic Processing" Chapter 11 of Practical Aseptic Processing, Fill and Finish, Vol. 1, edited by Jack Lysfjord
Can be ordered at
https://store.pda.org/bookstore/PlaceboDetails.aspx?placeboabbreviation=17283

3) LIGHTHOUSE Application Note 104: "Headspace oxygen monitoring and container closure integrity testing of pre-filled syringes"
Downloadable at:
http://www.lighthouseinstruments.com/uploads/documents/LIGHTHOUSE_Raised_Stopper_Detection_App_Note_103.pdf

Appendix

Listed below are the mean measured headspace oxygen levels in the product filled syringes.

Batch 1: Start		
Label	Mean O2	STDEV
sample 1	6.72	0.47
sample 2	7.17	0.30
sample 3	7.59	0.28
sample 4	7.23	0.39
sample 5	7.06	0.38
sample 6	7.20	0.29
sample 7	5.96	0.40
sample 8	6.93	0.07
sample 9	7.00	0.27
sample 10	8.14	0.28
sample 11	6.57	0.49
sample 12	7.58	0.09
sample 13	9.26	0.41
sample 14	9.14	0.40
sample 15	7.98	0.29
sample 16	7.37	0.22
sample 17	8.04	0.30
sample 18	8.38	0.27
sample 19	7.76	0.20
sample 20	9.60	0.30
8% std	8.43	0.26

Batch 1: Middle		
Label	Mean O2	STDEV
sample 1	6.06	0.19
sample 2	7.11	0.16
sample 3	5.81	0.26
sample 4	7.15	0.31
sample 5	5.96	0.10
sample 6	6.49	0.30
sample 7	6.14	0.34
sample 8	6.67	0.30
sample 9	6.05	0.32
sample 10	6.74	0.34
sample 11	5.71	0.14
sample 12	7.42	0.15
sample 13	6.49	0.38
sample 14	5.81	0.18
sample 15	5.61	0.04
sample 16	6.01	0.35
sample 17	5.78	0.10
sample 18	6.15	0.16
sample 19	7.17	0.09
sample 20	7.11	0.14
8% std	8.33	0.23

Batch 1: End		
Label	Mean O2	STDEV
sample 1	7.39	0.22
sample 2	5.66	0.39
sample 3	7.15	0.33
sample 4	6.29	0.05
sample 5	7.75	0.24
sample 6	7.12	0.26
sample 7	7.10	0.10
sample 8	7.21	0.27
sample 9	6.49	0.21
sample 10	6.63	0.39
sample 11	6.36	0.18
sample 12	6.96	0.33
sample 13	8.26	0.32
sample 14	8.16	0.41
sample 15	8.34	0.11
sample 16	7.11	0.13
sample 17	8.18	0.27
sample 18	6.50	0.40
sample 19	6.13	0.10
sample 20	7.11	0.29
8% std	8.68	0.05

Batch 2: Start		
Label	Mean O2	STDEV
sample 1	5.65	0.42
sample 2	5.21	0.07
sample 3	5.14	0.16
sample 4	5.86	0.14
sample 5	5.06	0.27
sample 6	7.19	0.18
sample 7	5.39	0.27
sample 8	6.40	0.28
sample 9	5.31	0.34
sample 10	5.69	0.37
sample 11	5.73	0.20
sample 12	5.81	0.34
sample 13	6.27	0.61
sample 14	7.60	0.26
sample 15	5.28	0.17
sample 16	6.44	0.34
sample 17	5.44	0.13
sample 18	5.79	0.27
sample 19	5.32	0.45
sample 20	5.78	0.13
8% std	8.05	0.31

Batch 2: Middle		
Label	Mean O2	STDEV
sample 1	5.43	0.34
sample 2	5.82	0.15
sample 3	5.97	0.15
sample 4	5.83	0.18
sample 5	5.33	0.19
sample 6	6.27	0.07
sample 7	6.19	0.15
sample 8	4.99	0.06
sample 9	5.52	0.11
sample 10	6.24	0.31
sample 11	6.10	0.26
sample 12	5.48	0.35
sample 13	5.67	0.10
sample 14	5.72	0.24
sample 15	7.38	0.11
sample 16	6.40	0.32
sample 17	6.23	0.46
sample 18	5.75	0.28
sample 19	6.01	0.26
sample 20	6.47	0.39
8% std	8.02	0.27

Batch 2: End		
Label	Mean O2	STDEV
sample 1	5.95	0.25
sample 2	6.48	0.38
sample 3	5.34	0.31
sample 4	5.62	0.23
sample 5	5.34	0.05
sample 6	5.72	0.18
sample 7	5.33	0.15
sample 8	6.64	0.19
sample 9	5.84	0.23
sample 10	5.24	0.03
sample 11	5.63	0.18
sample 12	5.54	0.29
sample 13	4.91	0.20
sample 14	5.91	0.13
sample 15	5.35	0.25
sample 16	5.98	0.06
sample 17	4.70	0.04
sample 18	6.04	0.45
sample 19	5.62	0.09
sample 20	5.75	0.32
8% std	8.49	0.09

Batch 3: Start		
Label	Mean O2	STDEV
sample 1	5.96	0.16
sample 2	6.18	0.24
sample 3	8.20	0.68
sample 4	7.04	0.24
sample 5	7.23	0.25
sample 6	6.22	0.31
sample 7	6.64	0.23
sample 8	6.27	0.12
sample 9	8.07	0.16
sample 10	6.37	0.14
sample 11	6.41	0.29
sample 12	7.26	0.28
sample 13	7.22	0.11
sample 14	6.05	0.23
sample 15	6.26	0.17
sample 16	6.77	0.32
sample 17	6.73	0.02
sample 18	6.61	0.16
sample 19	6.40	0.30
sample 20	6.41	0.16
8% std	8.51	0.43

Batch 3: Middle		
Label	Mean O2	STDEV
sample 1	5.51	0.67
sample 2	5.98	0.22
sample 3	6.63	0.38
sample 4	6.55	0.27
sample 5	6.99	0.23
sample 6	6.63	0.09
sample 7	5.58	0.16
sample 8	6.38	0.26
sample 9	5.78	0.30
sample 10	7.23	0.29
sample 11	6.13	0.41
sample 12	5.96	0.22
sample 13	6.33	0.09
sample 14	6.68	0.17
sample 15	7.03	0.26
sample 16	6.30	0.38
sample 17	7.33	0.25
sample 18	5.58	0.22
sample 19	6.68	0.30
sample 20	6.64	0.23
8% std	8.50	0.13

Batch 3: End		
Label	Mean O2	STDEV
sample 1	6.49	0.17
sample 2	6.47	0.18
sample 3	6.50	0.08
sample 4	7.26	0.02
sample 5	6.34	0.14
sample 6	5.47	0.25
sample 7	6.12	0.29
sample 8	5.78	0.14
sample 9	5.65	0.17
sample 10	5.99	0.20
sample 11	7.21	0.32
sample 12	5.44	0.28
sample 13	5.89	0.25
sample 14	5.93	0.12
sample 15	5.87	0.32
sample 16	6.06	0.47
sample 17	6.38	0.21
sample 18	6.43	0.16
sample 19	5.15	0.11
sample 20	6.31	0.19
8% std	8.32	0.25