Laser-Based Headspace Analysis

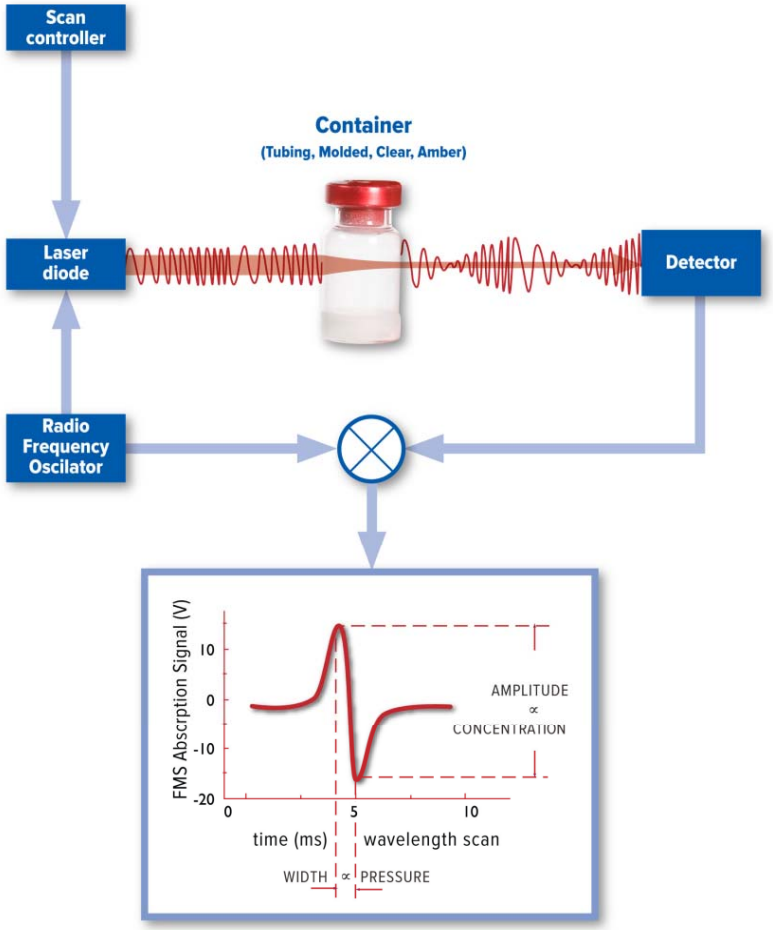
Container Closure Applications

PDA Alternatives to the Dye Test Workshop
29th of April, 2010
Dr. Derek Duncan
Product Line Manager, Europe
LIGHTHOUSE

Agenda

- **Headspace Method Principles**
  - Physical test to determine container closure integrity
- **Headspace Leak Rate Model**
  - Modeling and understanding headspace dynamics of a leaking container
- **Container Closure Studies**
  - Optimising packaging components and processes
- **Scale up to Manufacturing Inspection Applications**
  - Designing an appropriate 100% inspection process in manufacturing

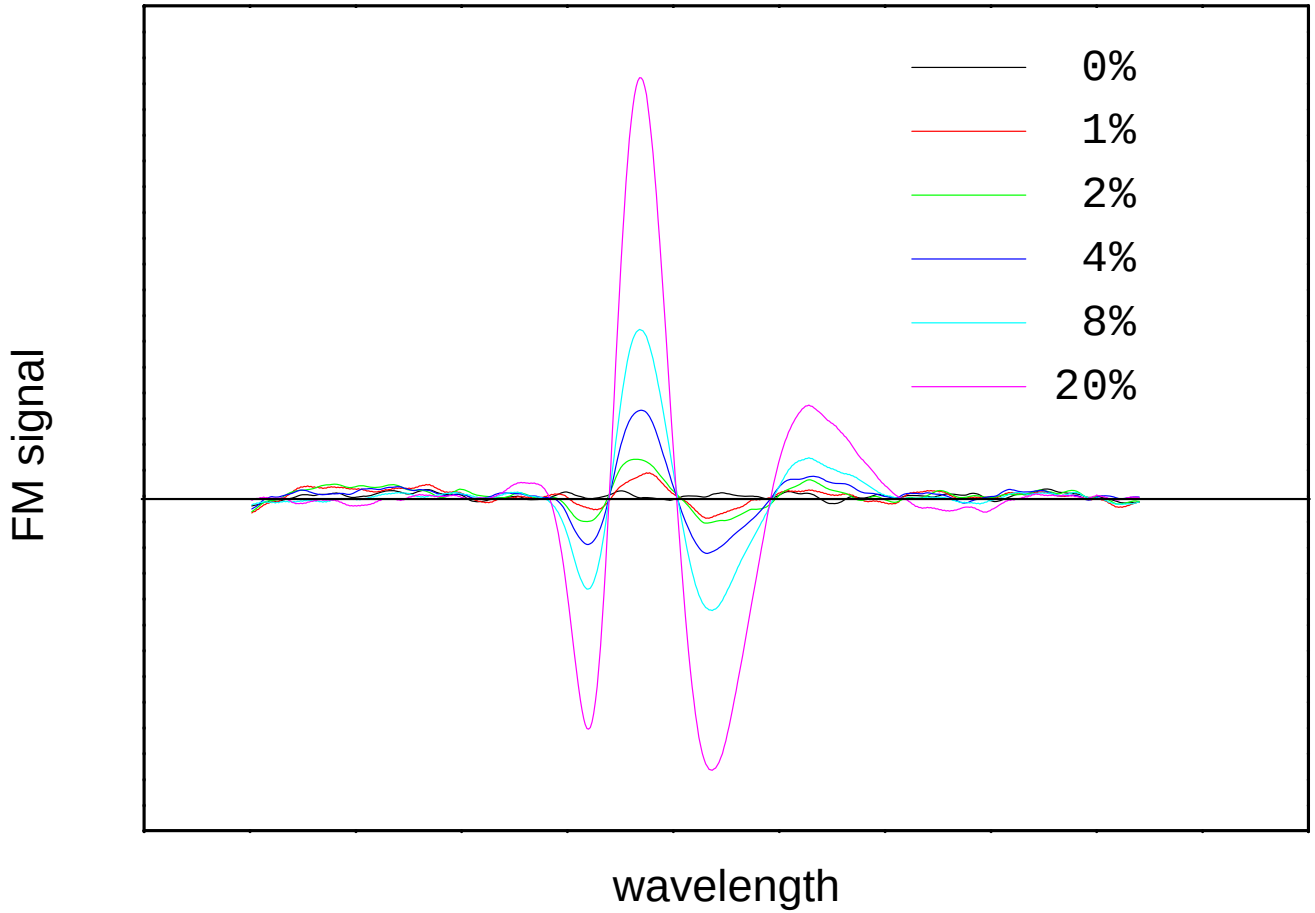
Frequency Modulation Spectroscopy



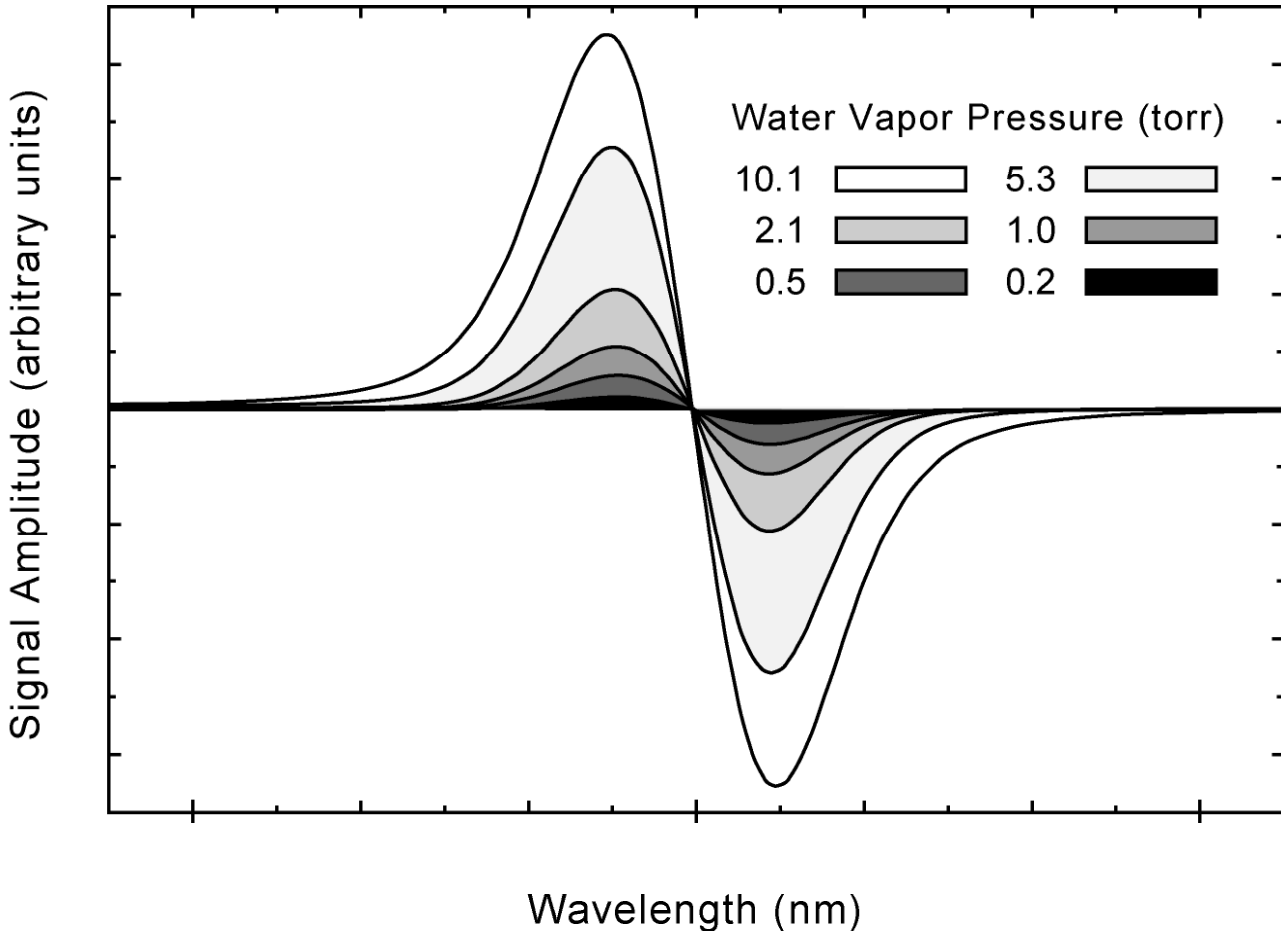
Headspace Method

Modulation techniques result in 10,000x increase in sensitivity compared to first order absorption techniques such as NIR

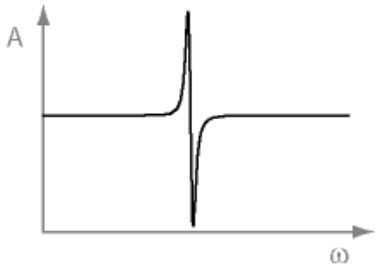
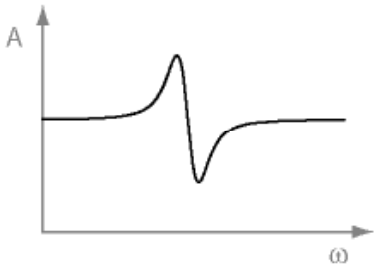
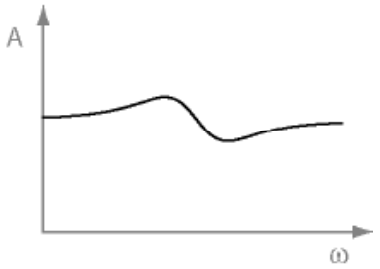
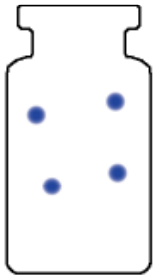
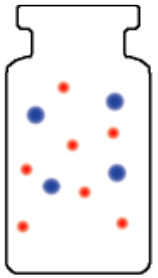
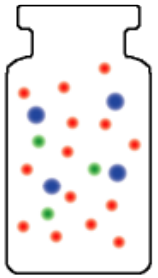
Headspace Oxygen Signal



Headspace Moisture Signal



Headspace Pressure Signal

	Full Vacuum	Partial Vacuum	Partial Vacuum Leaked To Atmosphere
Absorption Signal			
Signal Width $\Gamma_i = \sum \gamma_{i-j} P_j$	$\Gamma_{H_2O} = \gamma_{H_2O-H_2O} P_{H_2O}$	$\Gamma_{H_2O} = \gamma_{H_2O-H_2O} P_{H_2O} + \gamma_{H_2O-N_2} P_{N_2}$	$\Gamma_{H_2O} = \gamma_{H_2O-H_2O} P_{H_2O} + \gamma_{H_2O-N_2} P_{N_2} + \gamma_{H_2O-O_2} P_{O_2}$
Headspace Pressure	 0.5 mbar H_2O 0 mbar N_2 0% O_2	 0.5 mbar H_2O 500 mbar N_2 0% O_2	 0.5 mbar H_2O 900 mbar N_2 10% O_2

Headspace Inspection Platforms

Initially developed with FDA funding

Automated systems:

VISTA/THC: Oxygen, pressure, moisture

VISTA/O: Oxygen

VISTA/P: Pressure, moisture

At-/Off-line systems:

FMS-760: Oxygen

FMS-1400: Pressure/Moisture

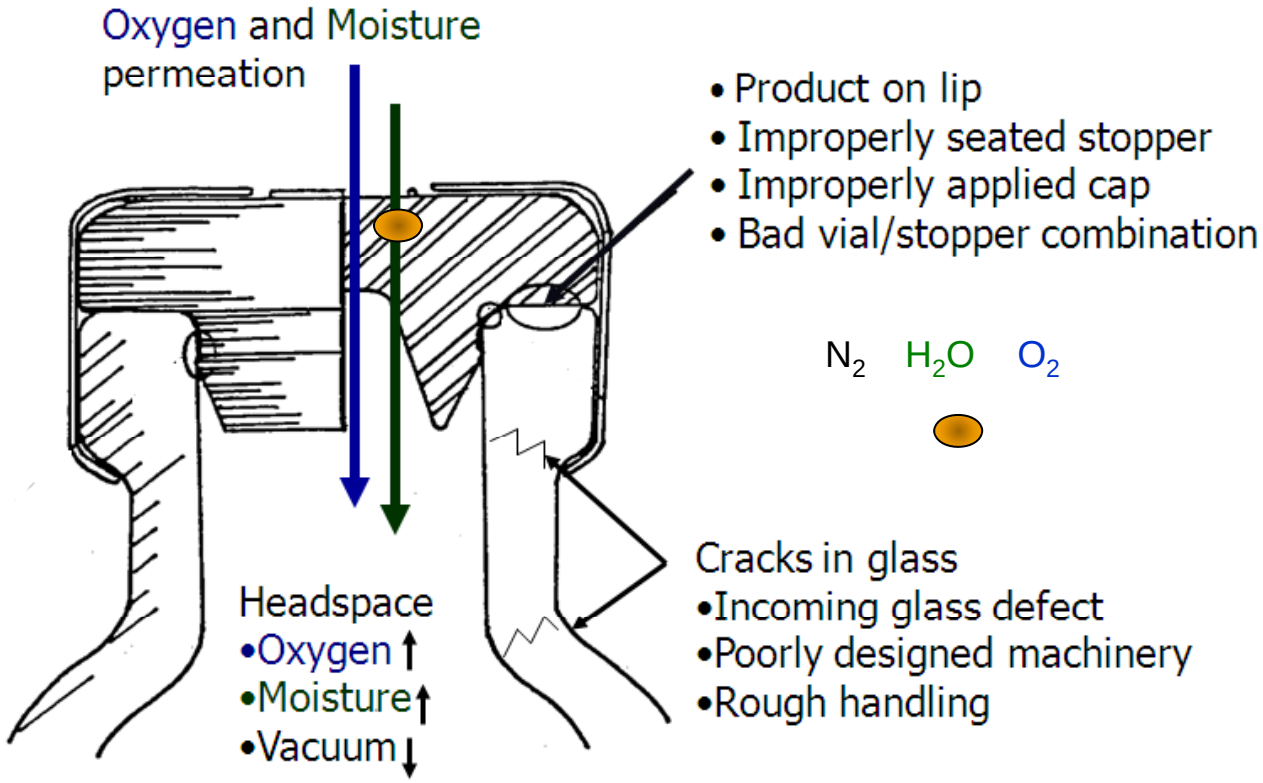
Calibration with traceable standards

- Certified gas mixtures of oxygen and nitrogen
- Certified vacuum levels
- Certified moisture levels
- Patented configuration for continuous machine calibration

Headspace Leak Rate Model

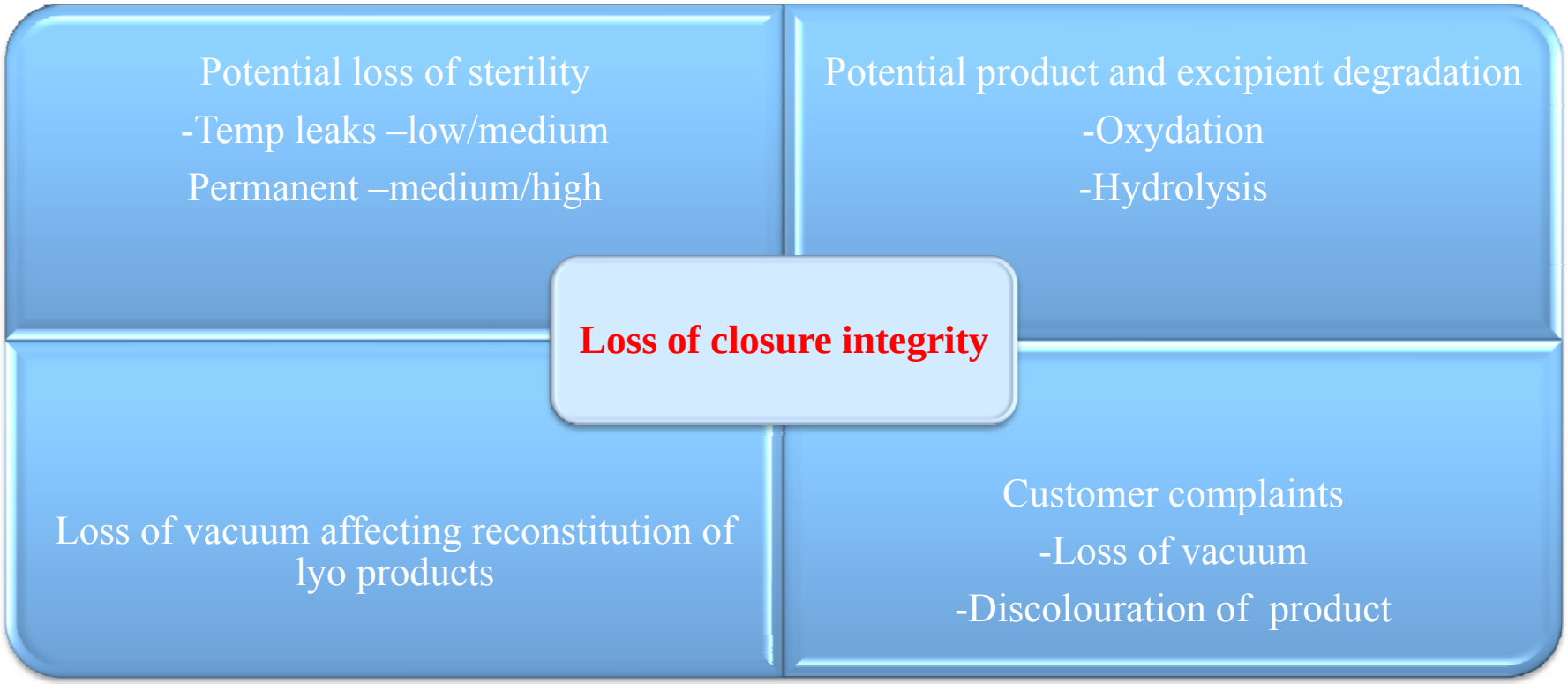
Calculating and Validating Headspace Dynamics for a Leaking Container

CCI failures result in gas exchange for modified headspace conditions



Risks associated with CCI failure

Increased attention from the regulators



Recalls

Top 5 Reasons for FDA Reported 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)

Source: Famulare, J (2007) "CDER Compliance Update," PDA/FDA Joint Regulatory Conference

Headspace Leak Rate Model

- **Allows you to model headspace dynamics due to leaks of all sizes in product configurations having different initial headspace conditions and headspace volumes.**

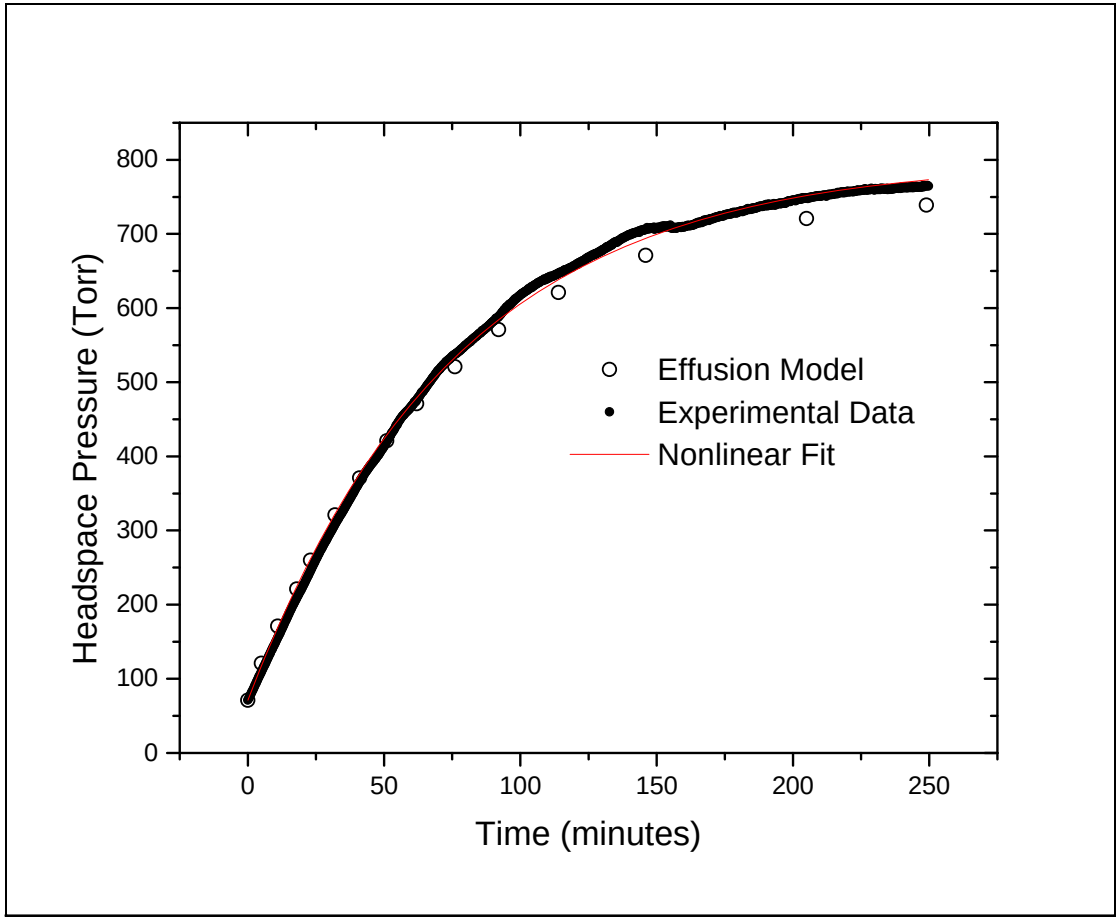
Book Chapter Reference:
"New Inspection Techniques For Aseptic Processing"
by James Veale

Practical Aseptic Processing, Vol 1
Edited by
Jack Lysfjord

Available at the PDA Bookstore

Effusive Flow: Vials under vacuum

$$\Delta P > 0 \qquad \Delta p > 0$$



$$\frac{\partial P}{\partial t} = \frac{kT}{V}\frac{\partial N}{\partial t}$$

$$\frac{\partial N}{\partial t} = ZA_0 = \frac{PA_0}{(2\pi mkT)^{\frac{1}{2}}}$$

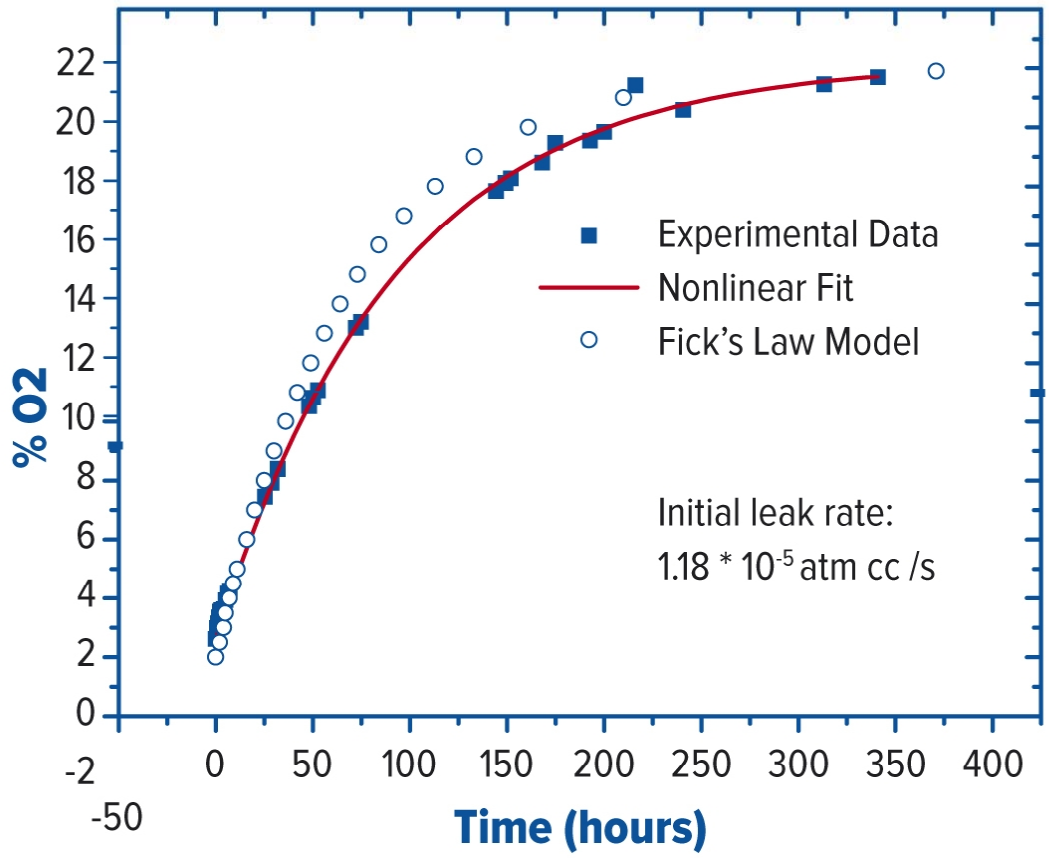
$$t = -\ln\left[\frac{P_0 - P_i}{P_0 - P_f}\right]\left(\frac{2\pi m}{kT}\right)^{\frac{1}{2}}\frac{V}{A_0}$$

5 micron diameter defect in a 10cc vial. Starting pressure: 71 torr (100mbar)

Leak times for various defect diameters: 10cc vial-Effusion Model

Pressure rise (torr)	Oxygen rise (% atm)	t(minutes)	t(hours)	t(hours)	t(days)
0	0.0	0	0	0	0
50	1.3	5	1	8	2
100	2.6	10	1	17	4
150	3.9	16	2	26	7
200	5.3	22	2	34	9
250	6.6	28	3	47	12
300	7.9	36	4	60	16
350	9.2	44	5	73	19
400	10.5	53	6	89	23
450	11.8	64	7	107	28
500	13.2	77	8	128	33
550	14.5	92	10	153	40
600	15.8	111	12	185	48
650	17.1	138	14	230	60
700	18.4	181	19	302	79
750	19.7	309	32	515	134
		5 micron hole	**2 micron hole**	**0.5 micron hole**	**0.2 micron hole**

Diffusive Flow: Vials not under vacuum



$$\Delta P = 0 \quad \Delta p > 0$$

$$J_z = -D\frac{\partial n}{\partial z}$$

$$\frac{\partial N}{\partial t} = J_z A_0 = -DA_0 \frac{\partial n}{\partial z}$$

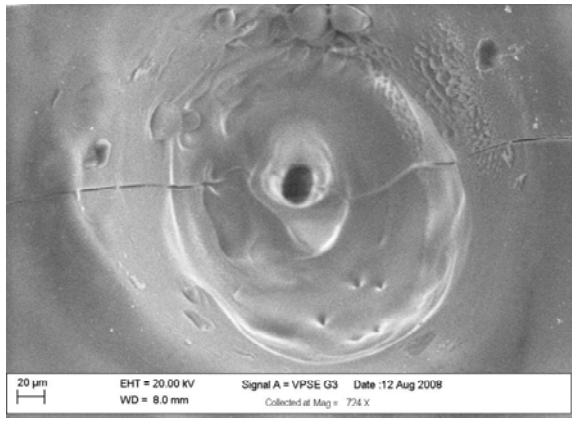
$$t(s) = -\ln\left[\frac{p_0 - p_i}{p_0 - p_f}\right]\frac{Vz}{DA_0}$$

6 micron defect in a 10cc vial. Starting O2% is 0%.

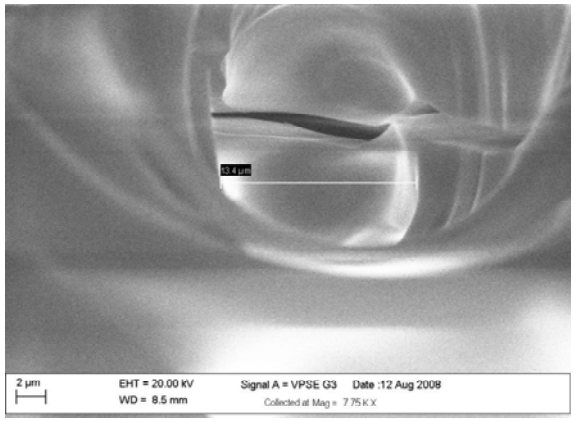
Leak times for various defect diameters: 10cc vial-Diffusion Model

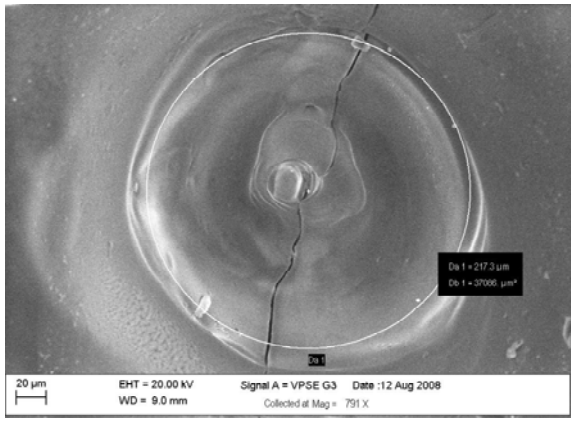
Partial Pressure Rise(atm)	Oxygen Concentration Rise (% atm)	t(days)	t(days)	t(weeks)	t(years)
0	0	0	0	0	0
0.005	0.5	<1	4	9	1
0.01	1	1	8	19	2
0.02	2	3	17	39	5
0.04	4	6	36	81	10
0.08	8	13	81	185	22
0.12	12	23	143	327	39
0.15	15	34	212	484	58
0.20	20	84	527	1204	145
		5 micron hole size	**2 micron hole size**	**0.5 micron hole size**	**0.2 micron hole size**

Micrograph of 5µm laser drilled holes

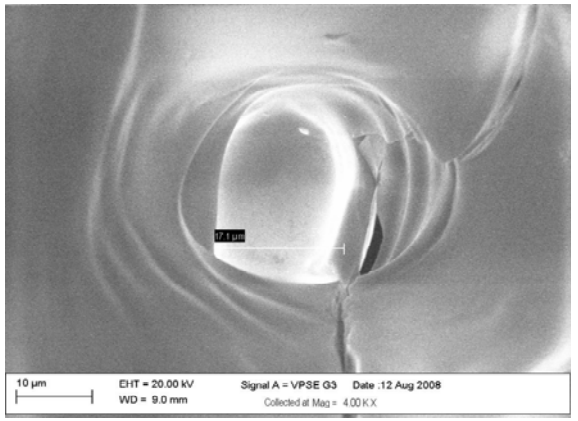


106

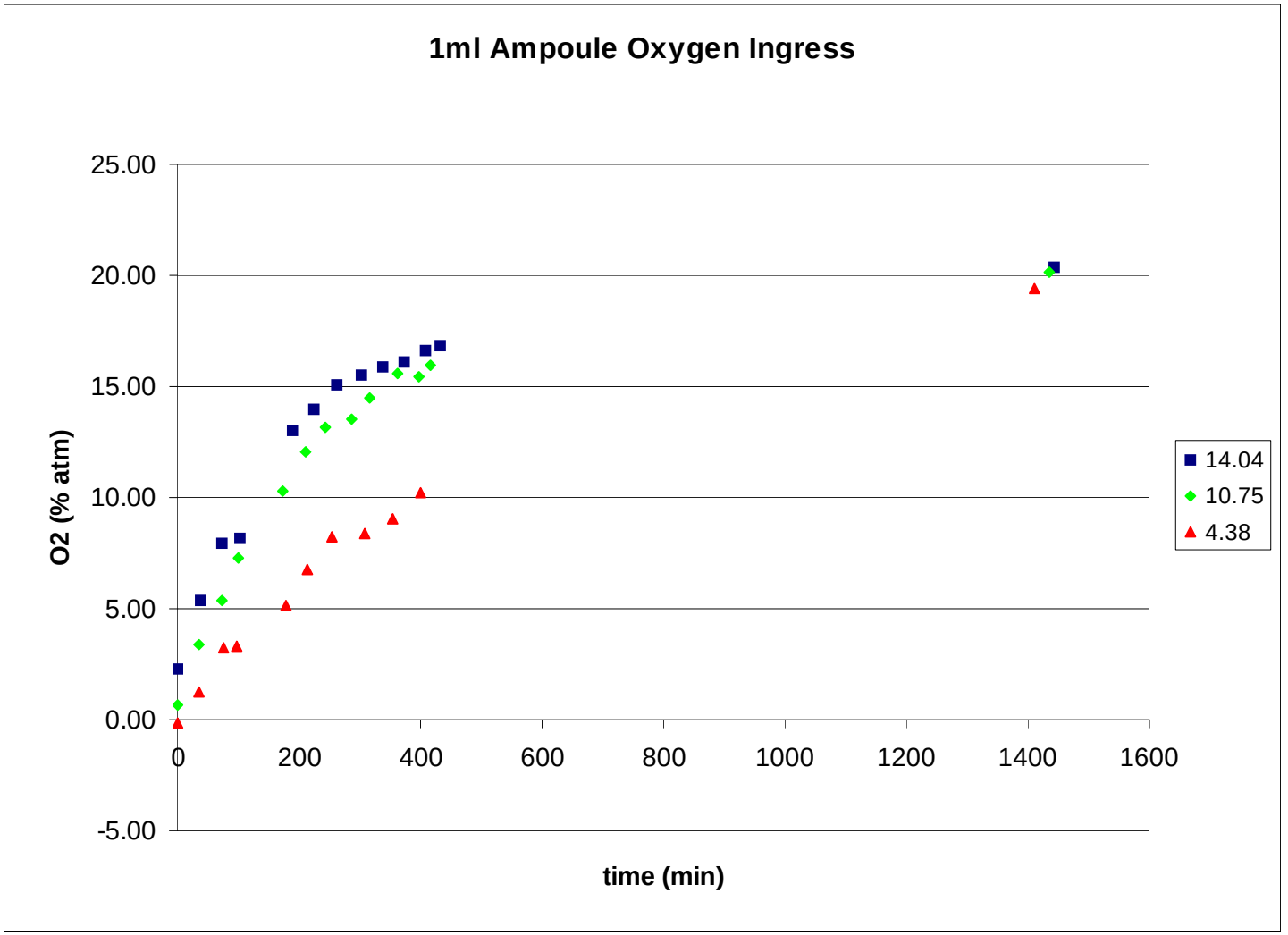




107

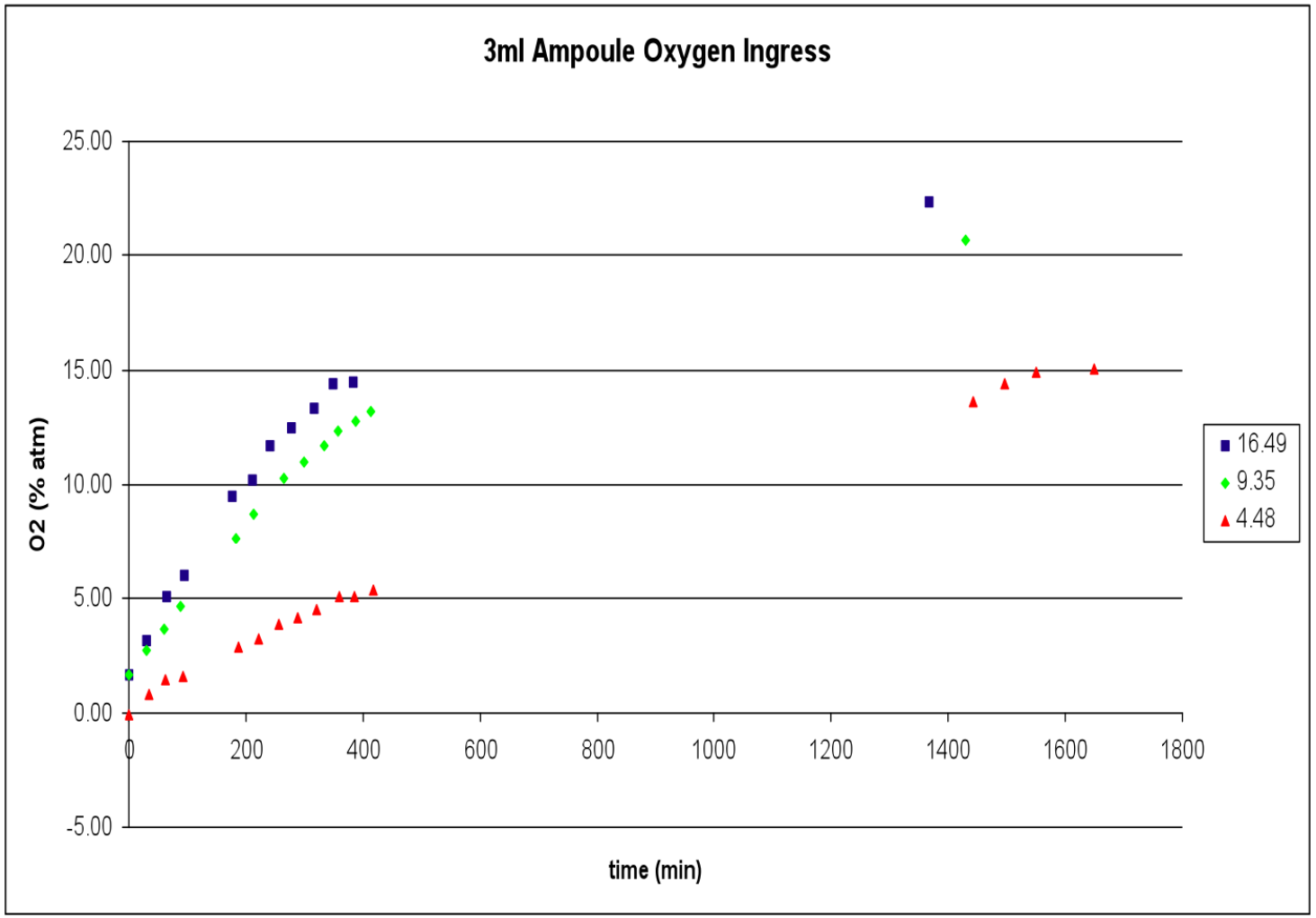


O_2 ingress by diffusion through laser drilled defects



Defect sizes (microns)

O_2 ingress by diffusion through laser drilled defects



Defect sizes (microns)

Experimental data compared to headspace model predictions

Volume (ml)	Hole Size (microns)	Measured vs Theoretical Oxygen Concentration					
		Initial (% atm)		8 hours (% atm)		24 hours (% atm)	
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
3	4.48	0	0	5.41	6.36	13.62	14.54
	9.35	1.71	1.71	13.17	11.72	20.63	19.23
	16.49	1.7	1.7	14.45	15.06	22.34	20.9

Comparing different container closure methods for detecting 5, 10, 15 micron leaks

Reference: Dana Guazzo, 'Nondestructive Container Closure Integrity Tests For Prefilled Syringes', PDA conference October, 2008

1. **Vacuum Decay Leak Detection**
2. **High Voltage Leak Detection**
3. **Dye Ingress**
4. **Microbial Ingress**

- **Methods 1 and 2 sensitive down to 5 microns, lower limit for method 2 not defined in this study.**
- **Method 3 reliable down to 10 um.**
- **Method 4 most sensitive but not as reliable as methods 1 and 2.**

Headspace Analysis

⇨ **Sensitive to all leak sizes with the appropriate waiting period.**

⇨ **Identifies permanent and temporary leaks.**

Container Closure Studies

Pressure rise in lyo vial due to injected air

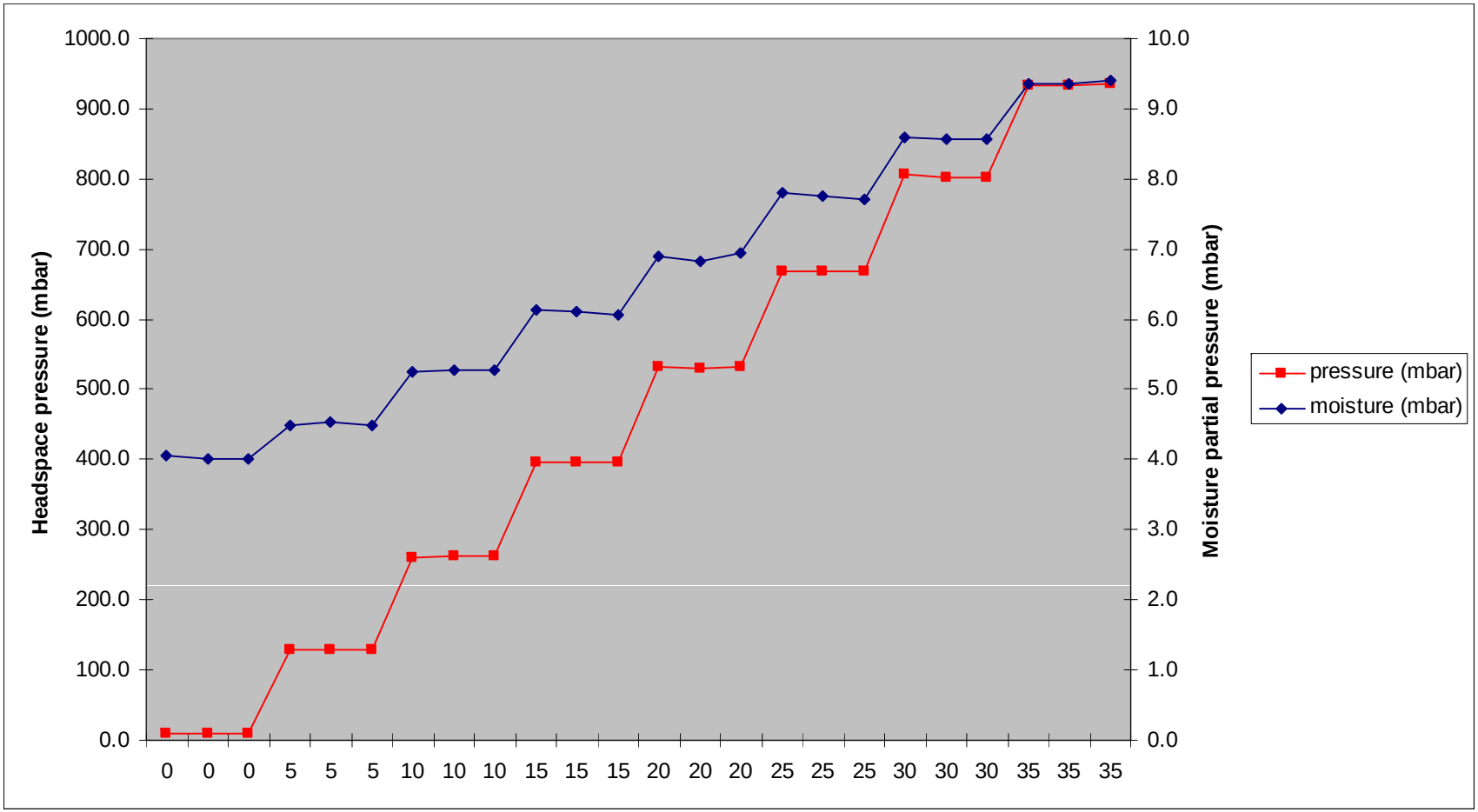
label	power	pressure (torr)	pressure (mbar)	moisture (torr)	moisture (mbar)
Sample 11	66.9	7.7	10.2	3.05	4.1
Sample 11	62.4	7.8	10.4	3.02	4.0
Sample 11	62	7.8	10.4	3.01	4.0
User comment:	5cc air injected				
Sample 11	64.2	97.3	129.4	3.38	4.5
Sample 11	63.7	97.4	129.5	3.41	4.5
Sample 11	62.9	97.3	129.4	3.37	4.5
User comment:	5cc air injected				
Sample 11	63	196.4	261.2	3.94	5.2
Sample 11	61.8	196.5	261.3	3.96	5.3
Sample 11	62.4	196.5	261.3	3.96	5.3
User comment:	5cc air injected				
Sample 11	65.9	298.2	396.6	4.61	6.1
Sample 11	61.8	298.7	397.3	4.59	6.1
Sample 11	63	298.1	396.5	4.55	6.1
User comment:	5cc air injected				
Sample 11	62.6	399.4	531.2	5.18	6.9
Sample 11	65.4	398.6	530.1	5.14	6.8
Sample 11	68.2	399.8	531.7	5.23	7.0
User comment:	5cc air injected				
Sample 11	65.7	502.1	667.8	5.87	7.8
Sample 11	62.3	501.6	667.1	5.83	7.8
Sample 11	62.1	502.1	667.8	5.8	7.7
User comment:	5cc air injected				
Sample 11	61.2	605.9	805.8	6.46	8.6
Sample 11	63.8	603.7	802.9	6.44	8.6
Sample 11	66.2	602.3	801.1	6.45	8.6
User comment:	5cc air injected				
Sample 11	64.2	701.7	933.3	7.04	9.4
Sample 11	66.9	700.8	932.1	7.03	9.3
Sample 11	64.4	702.8	934.7	7.07	9.4

20cc empty evacuated vials
Incremental air injection

Sample 11	Mean Pressure (mbar)	SD Pressure (mbar)
Intact	10.3	0.06
Plus 5cc air	129.5	0.06
Plus 10cc air	261.3	0.06
Plus 15cc air	396.8	0.32
Plus 20cc air	531.0	0.61
Plus 25cc air	667.6	0.29
Plus 30cc air	803.3	1.81
Plus 35cc air	933.3	1.00

Incremental pressure & moisture change

Sample 11 (incremental 5cc air injection)

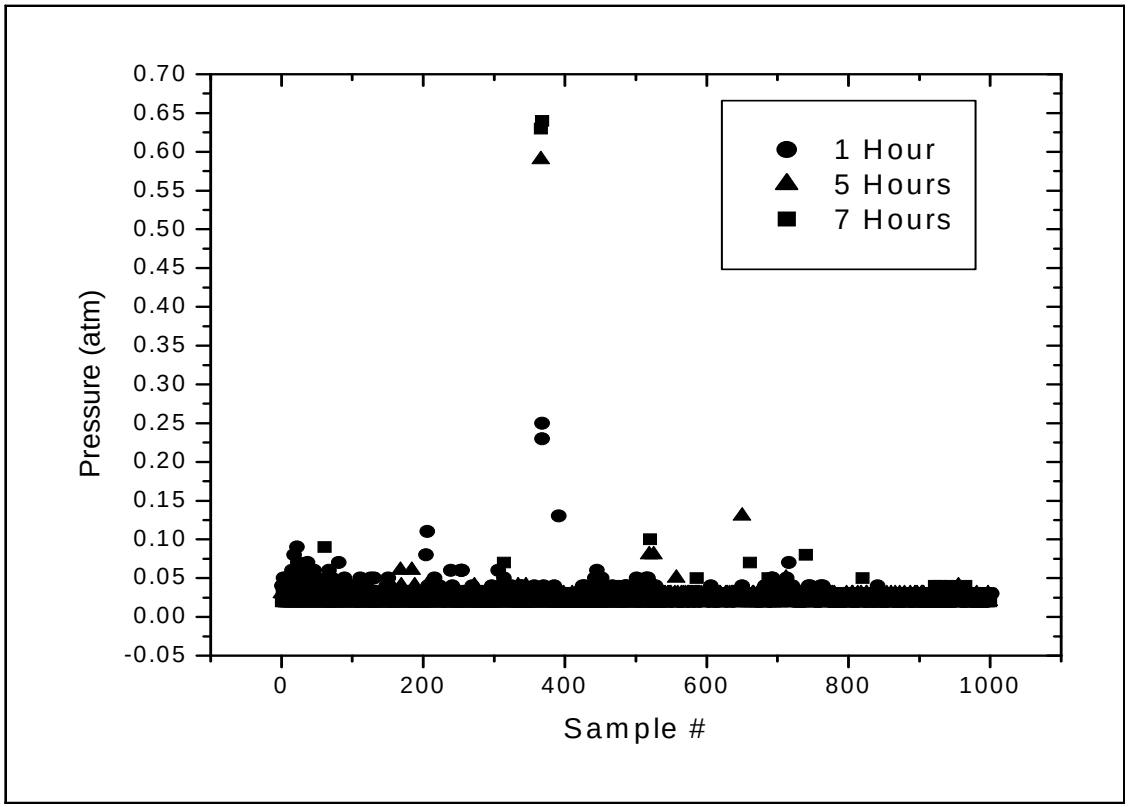


Container Closure Study:
Vacuum Retention Butyl Rubber Stoppers

- **The Problem:**
  - Gain insight into failure rate of packaging components used for lyophilized products

- **The Experiment:**
  - Evacuated 1,000 15cc vials to 0.5 torr
  - Stoppered and removed from chamber
  - Measured pressure at 1, 5 and 7 hour intervals

Vacuum Retention Results



The Results:

One vial found to be leaking (0.10%)

"Stopper Pop-Up" Study in Uncapped Vials Using Barrier-coated Stoppers

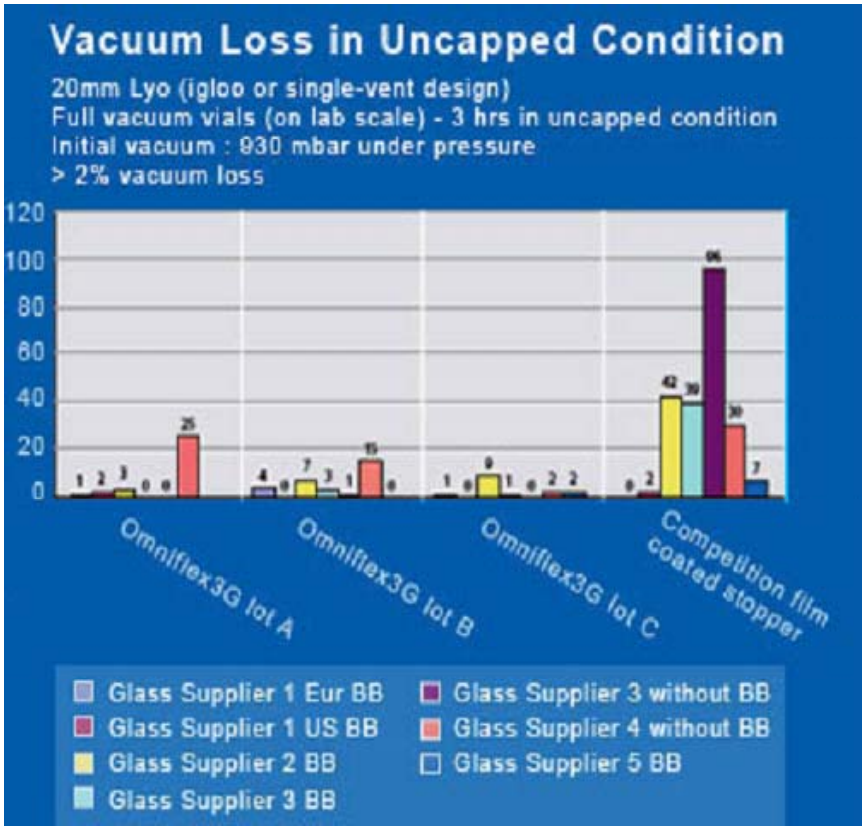
Graph shows percentage of vials suffering from vacuum loss after 3 hrs in the uncapped condition.

- **Container closure studies crucial for indentifying appropriate vial/stopper combination**

Why does vacuum loss happen?

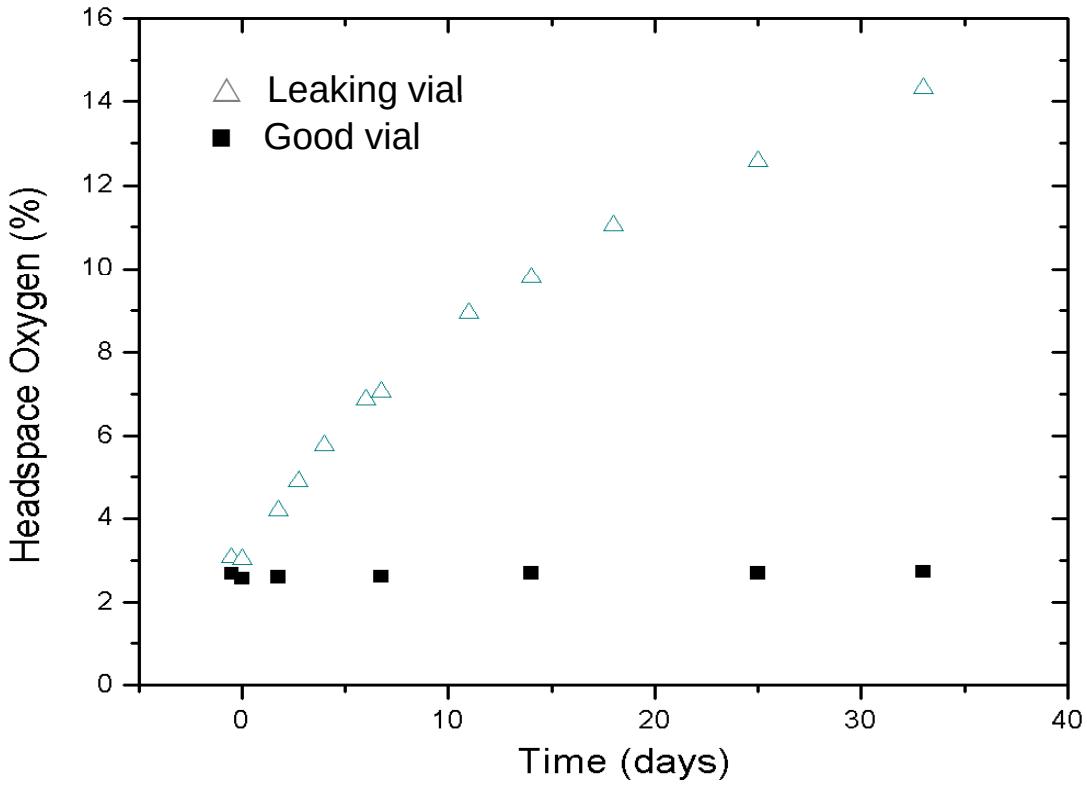
Hypothesis:

In the uncapped situation there can be a slight force upwards exerted on the stopper. This causes the stopper to "pop up" resulting in loss of closure and therefore loss of vacuum.



Graph courtesy of Helvoet Pharma Omniflex3G website

Correlating Leak Rate To Microbial Ingress Probability



Results:

Leak rate of $3x10^{-6}$ sccs; correlates to hole size < 0.2 microns

Correlating Leak Rate To Microbial Ingress Probability

An empirically determined microbial ingress probability function:

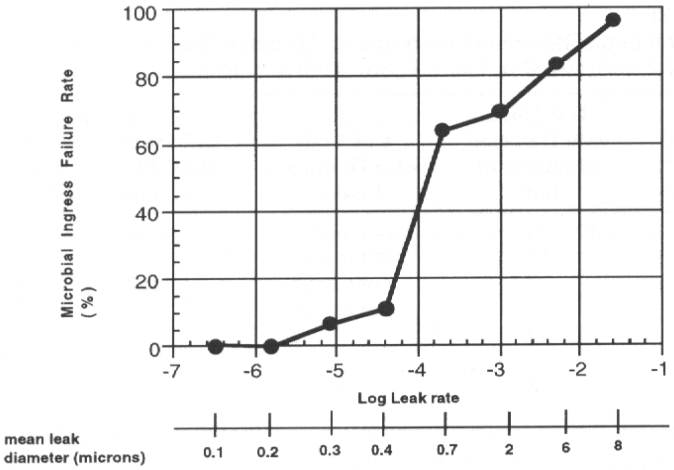


Figure 2—The correlation of microbial failure rate (%) and the mean logarithm of the absolute leak rate and nominal leak diameter for modified SVPs. The absolute leak rate (standard cubic centimeters per second) was determined by mass spectrometry-based helium leak rate detection. Microbial failure was measured by microbial ingress after 24 hour immersion in a bath (37°C) containing 10^8 to 10^{10} *P. diminuta* and *E. coli* organisms/mL and a 13 day, 35°C incubation.

Kirsch, et al, *PDA J Pharm Sci & Technol* 51, 5, 1997 p. 200

Conclusions: Container Closure and Microbial Testing

- **Potential for streamlining microbial testing using headspace container closure measurements - see FDA guidance "Container and Closure System Integrity Testing in Lieu of Sterility Testing as a Component of the Stability Protocol for Sterile Products"**

- **Validation experiments need to be done correlating headspace container closure measurements to microbial ingress.**

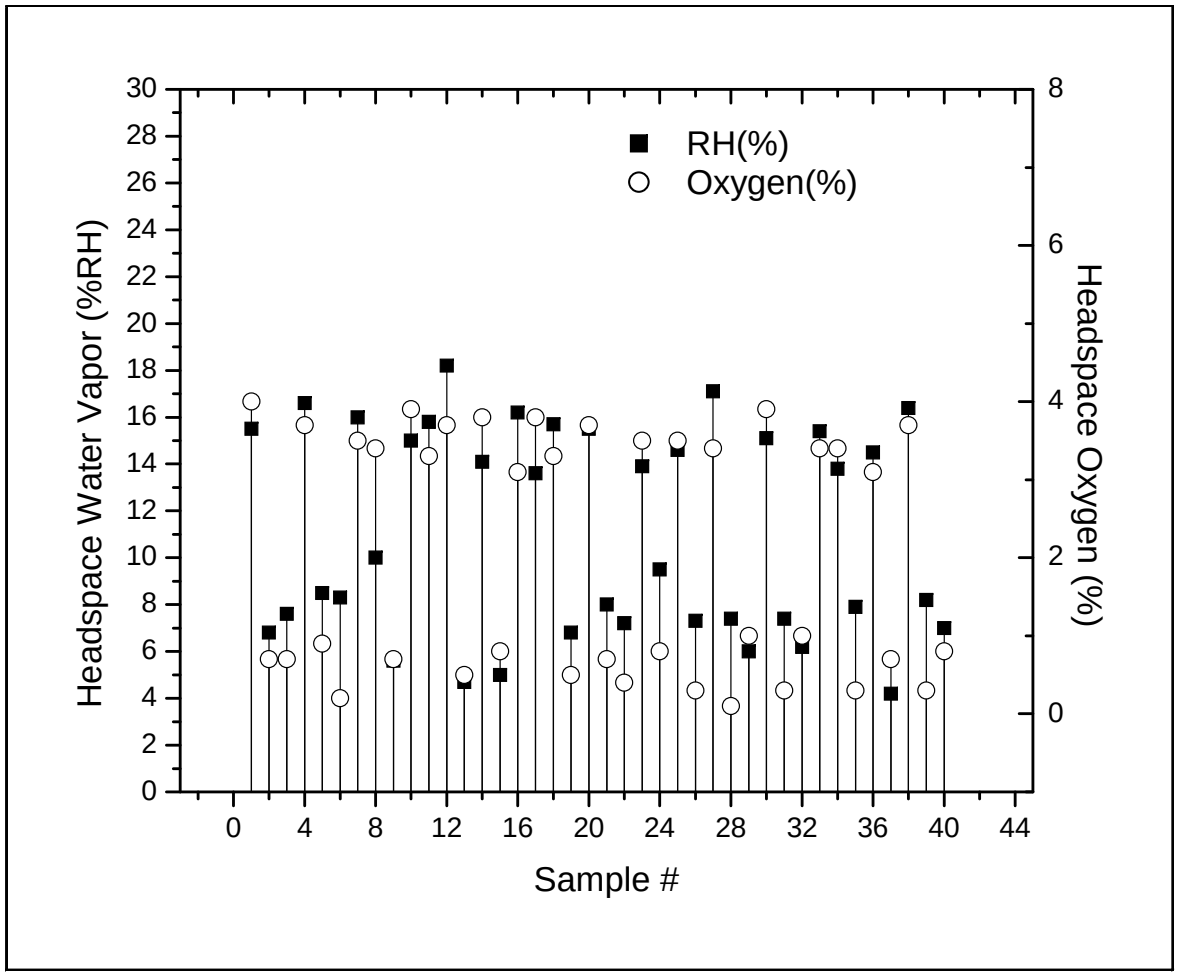
Case Study: End of Shelf Life Stability Study

- **The Objective:**
  - Assess headspace moisture & oxygen levels in lyo formulation samples for end of shelf life stability study application.

✓ **The Experiment:**

- Two blind sets of lyophilized product (recently manufactured and past shelf life) delivered for analysis.
- Measure moisture and oxygen in headspace.

End of Shelf Life Results



Conclusions: End of Shelf Life Stability Study

- **Conclusions:**
  - Old & new lyo product easily distinguishable with headspace measurement.
  - 4x increase of oxygen: permeation through stopper
  - 2x increase of moisture: permeation & desorption of stopper
  - Knowledge of headspace dynamics contributes to better assessment of shelf life

Benefits of Rapid Non-Destructive Headspace Method for Container Closure Studies

It is a quantitative physical test for container closure integrity calibrated with traceable standards

- **Ability for multiple measurements on same container.**
  - Trends over time, under different storage conditions.
  - Reduction in sample preparation time & material.
  - Increased accuracy: no sample-to-sample variability.
- **Ability to rapidly perform 100% inspection.**
  - Gives science-based insight into process and component variability, enabling efficient optimisation and validation.
  - Not only identifies sterility risk by identifying leaking containers but also identifies product stability risk in cases of oxygen/moisture sensitivity

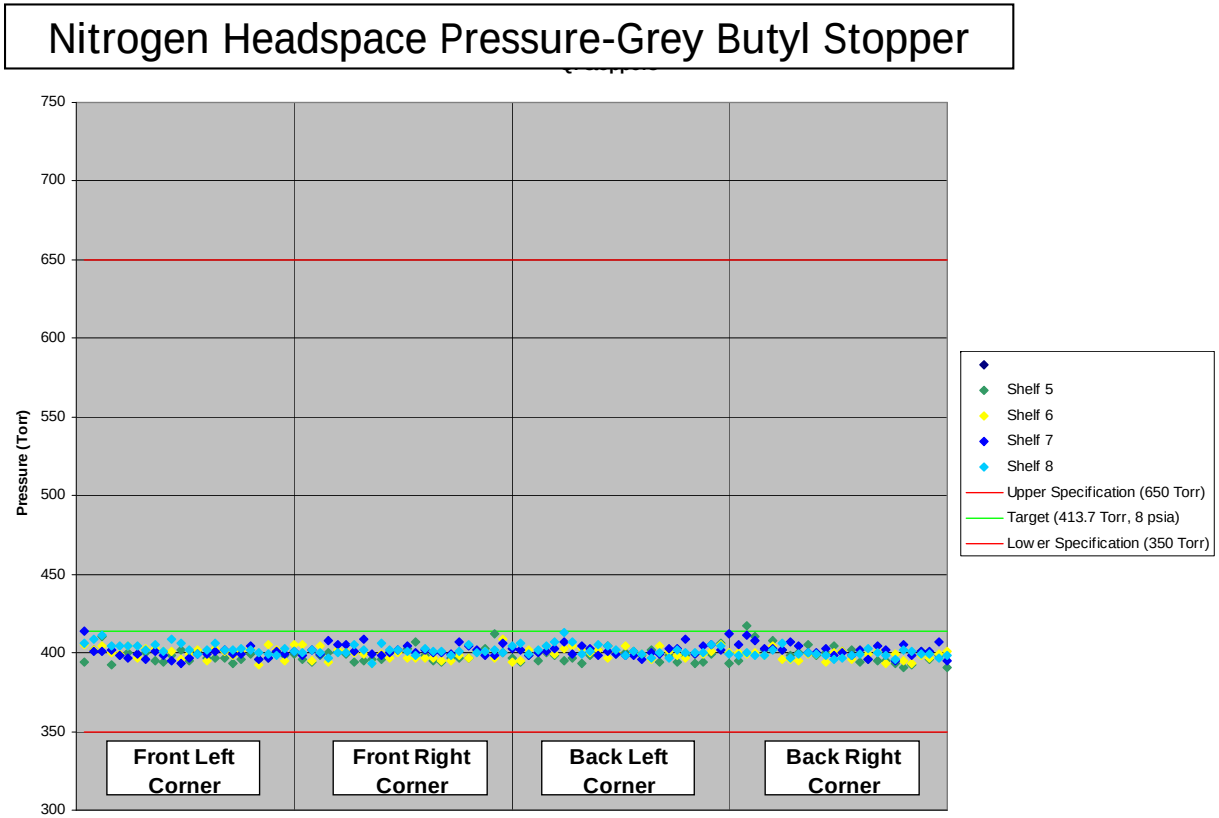
PDA
Parenteral Drug Association

Laser-based
Headspace Inspection

Scale Up of Headspace Analysis for Automated 100% Container Closure Inspection in Manufacturing

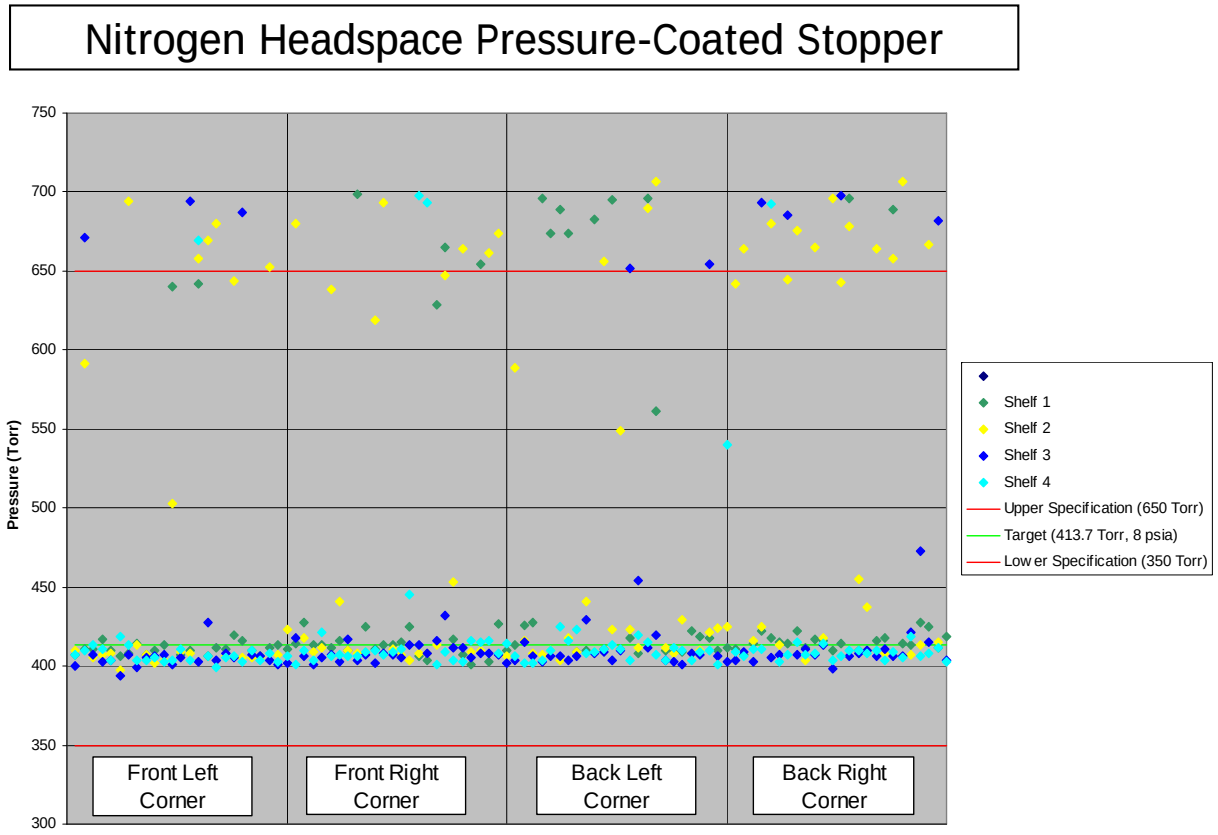
Manufacturing Inspection Case Study: Raised stopper issue in lyo validation batch

- Blue dye test performed in container closure validation studies.
- However, closure integrity issue identified in validation batches of a lyophilized product packaged at 550 mbar of N2.
- Test stopper configurations (Butyl and coated) for ability to hold vacuum.



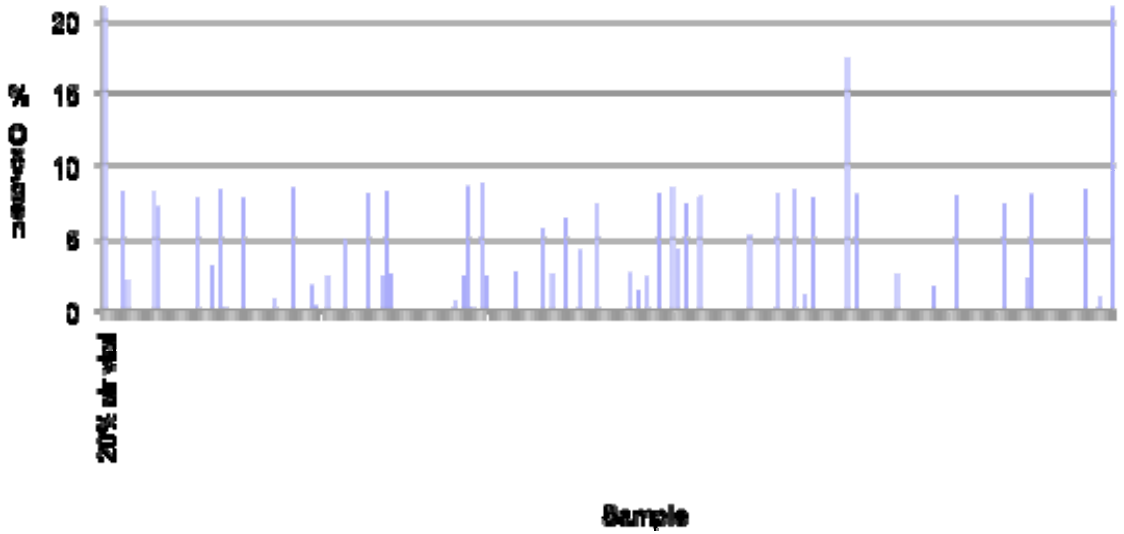
Manufacturing Inspection Case Study: Raised stopper issue in lyo validation batch

- Headspace inspection quickly characterized closure failure issue in 20% of vials
- Further studies demonstrated a process change could reduce closure failure to 1%.
- 100% Headspace Inspection Machine validated for final product inspection.



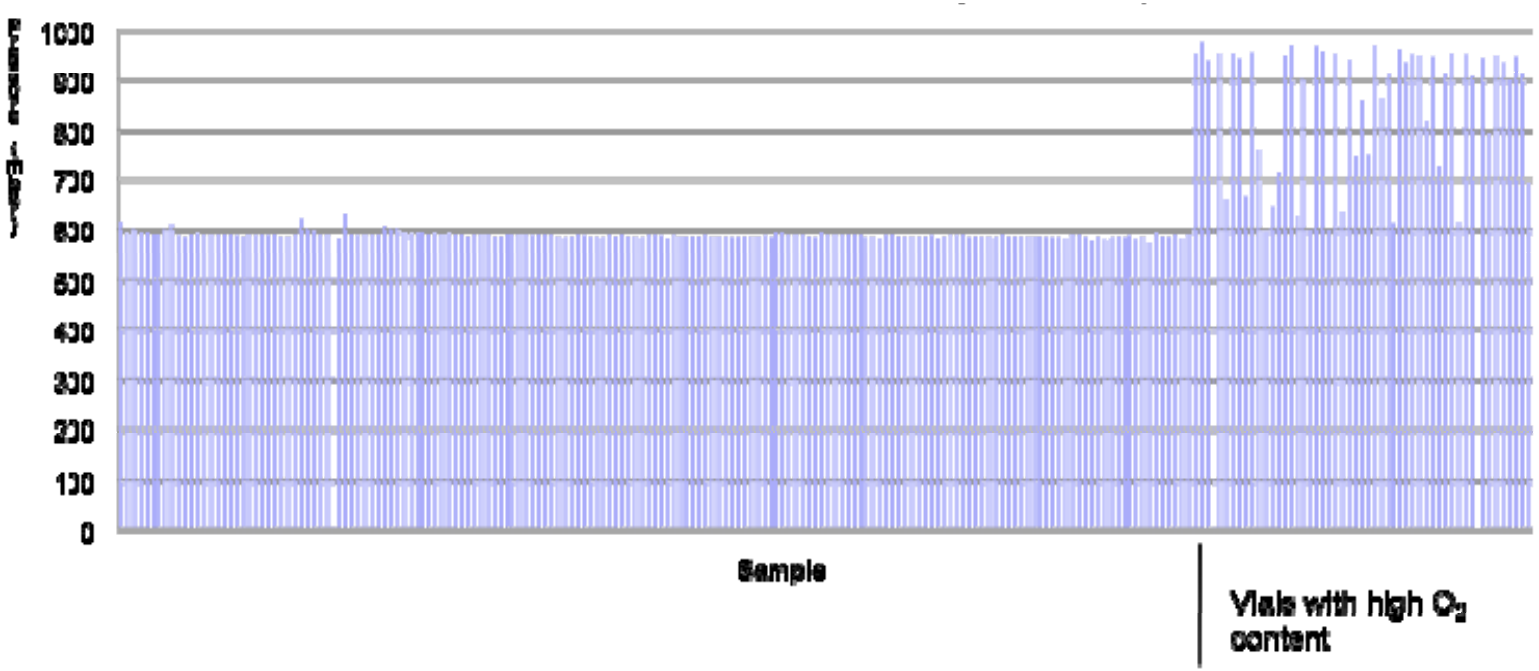
Manufacturing Inspection Case study: Raised stopper issue commercial batch of cytotoxic lyo

- Troubleshoot a lyophilized product batch of 11,000 vials packaged at 600mbar
- A suspected raised stopper issue motivated 100% inspection
- Total Headspace Characterization™ was performed.



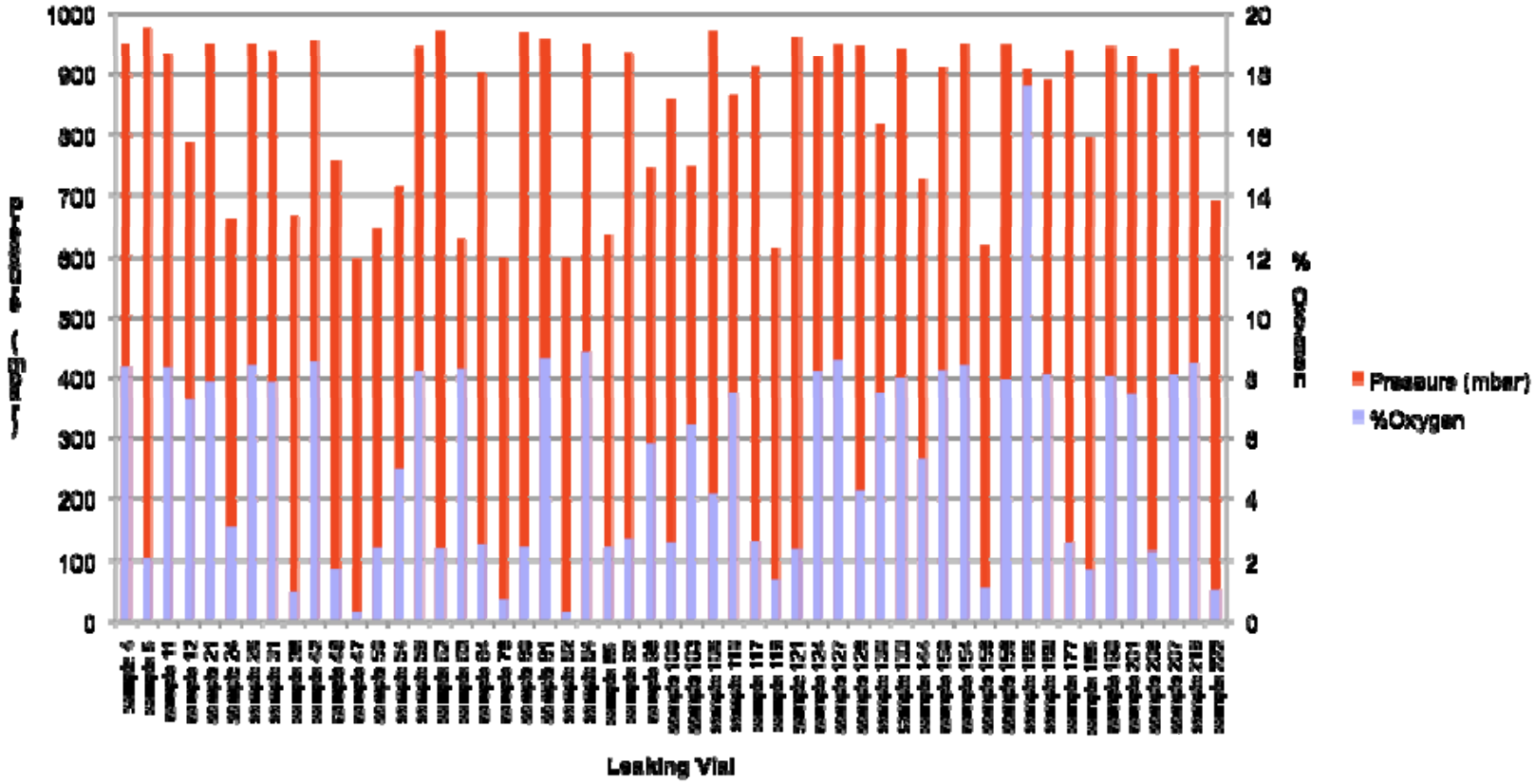
Headspace oxygen analysis showed ~25% of the vials had raised Oxygen levels due to a leak occurring in air

Manufacturing Inspection Case study:
Raised stopper issue commercial batch of cytotoxic lyo



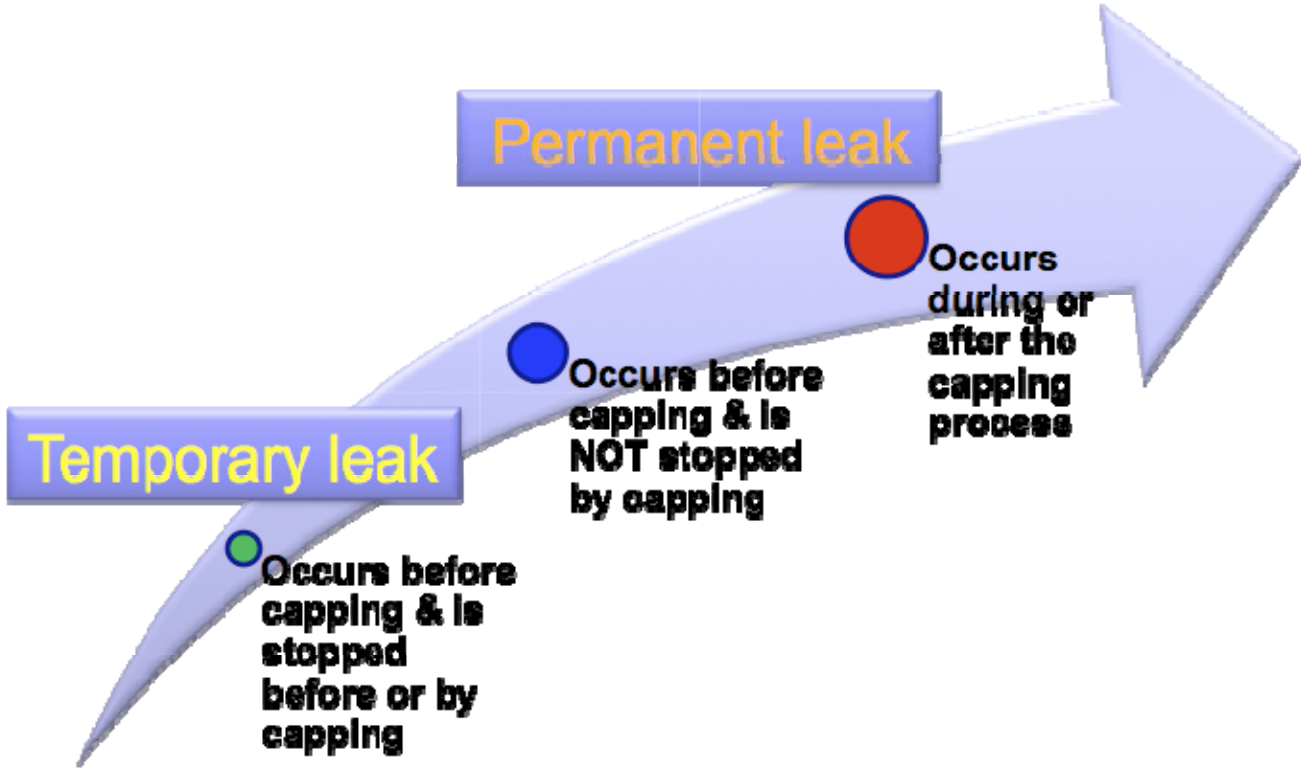
Vials with raised O_2 levels showed partial or full vacuum loss

Manufacturing Inspection Case study: Raised stopper issue commercial batch of cytotoxic lyo



Correlating headspace oxygen and pressure measurements identifies the process issue (raised stopper coming out of the freeze dryer) and the type of leak (temporary or permanent)

Container leak scenarios



Headspace inspection at a later stage (after capping) will identify all containers which have lost of integrity

Case Study Conclusions

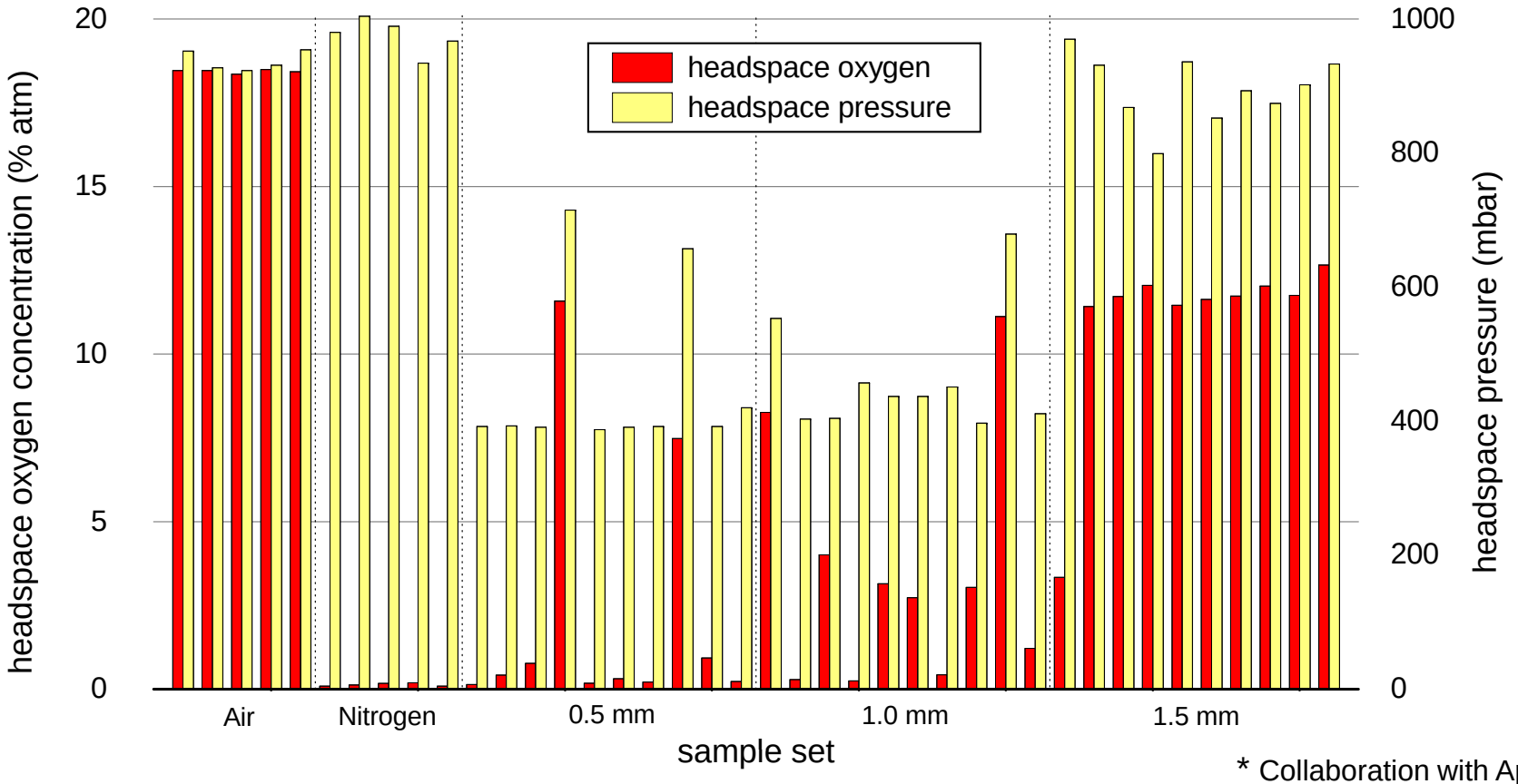
- 100% laser-based headspace inspection after capping identified all vials suffering from container closure issues due to raised stoppers in the capping area.
- In contrast to visual methods, headspace inspection directly measures loss of closure.
- Such an inspection process robustly accomplishes the objectives of the Revised Annex 1 with respect to ensuring good container closure and therefore maintenance of sterility.

Raised Stopper Study: Correlating stopper height to loss of closure

- Samples prepared in a lyo chamber with headspace conditions of 420 mbar of nitrogen.
- Stopper heights set at (0.5, 1.0, and 1.5 mm) using plastic spacers
- Samples exposed to air for one hour before the spacers were removed and the vials were capped
- Two control vial sets contained air and nitrogen respectively
- Headspace oxygen and pressure was measured in the control and the experimental vials

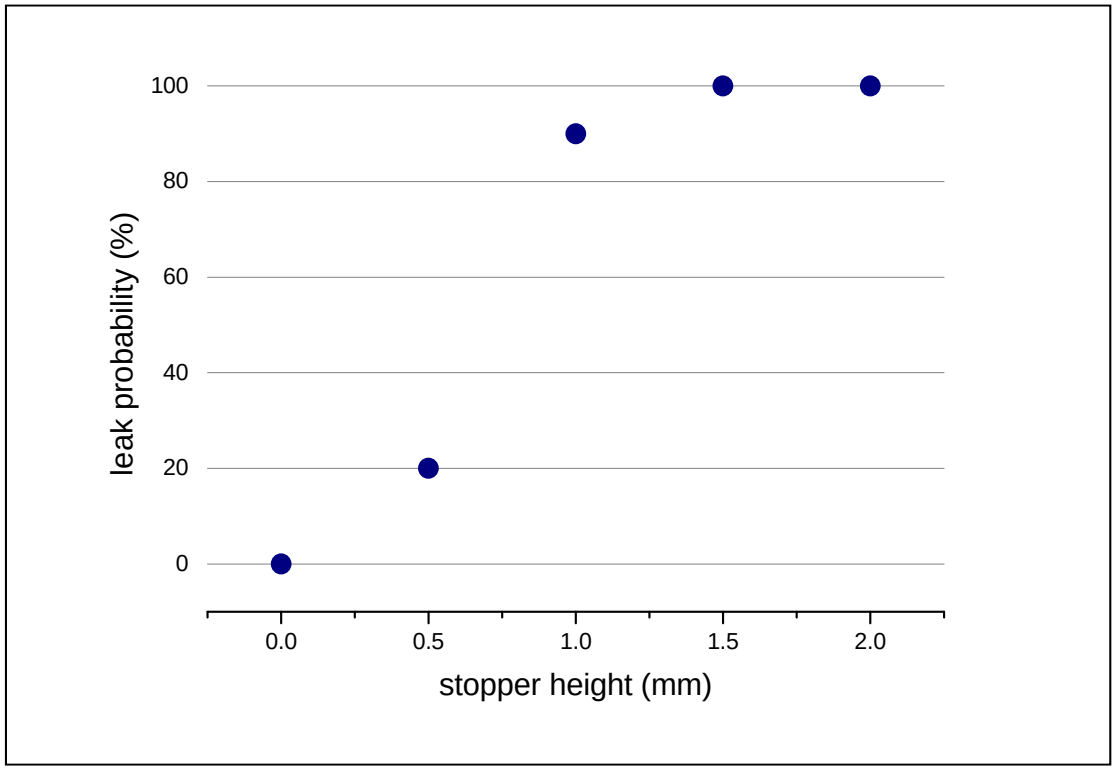
* Collaboration with Aptuit

- Initial headspace conditions 420 mbar of nitrogen in 5 ml lyo vial
- Headspace oxygen and pressure analysed for indications of leaks



* Collaboration with Aptuit

Probability of gas ingress as a function of raised stopper height



Collaboration with Aptuit

- Even slightly raised stoppers (0.5 mm) have some probability of leaking
- Headspace inspection identifies leaks at all raised stopper heights.

Stopper Pop-up Study

Samples/Materials/Equipment:

- **10cc clear tubing vials/grey siliconized stoppers/Lyostar I/benchtop FMS**
- **plastic shims (0.5, 1.0, 1.5, 2.0mm)**

Parameters:

- **N=30 vials*40 conditions=1200 measurements**
- **Five stopper heights (0-2mm)**
- **Four vial headspace pressures (0-570 torr)**
- **Two closure processes:**
  - Back fill with N2, **Raise, Release** and Vent with air
  - Back fill with N2, **Raise, Hold** and Vent with air

Analysis:

- **Leak probability (did a vial leak?) Measurable rise in pressure or oxygen**
- **Leak rates (how much did a vial leak?) Equivalent defect diameter**

Experimental Equipment

Lyo Star I Freeze Dryer

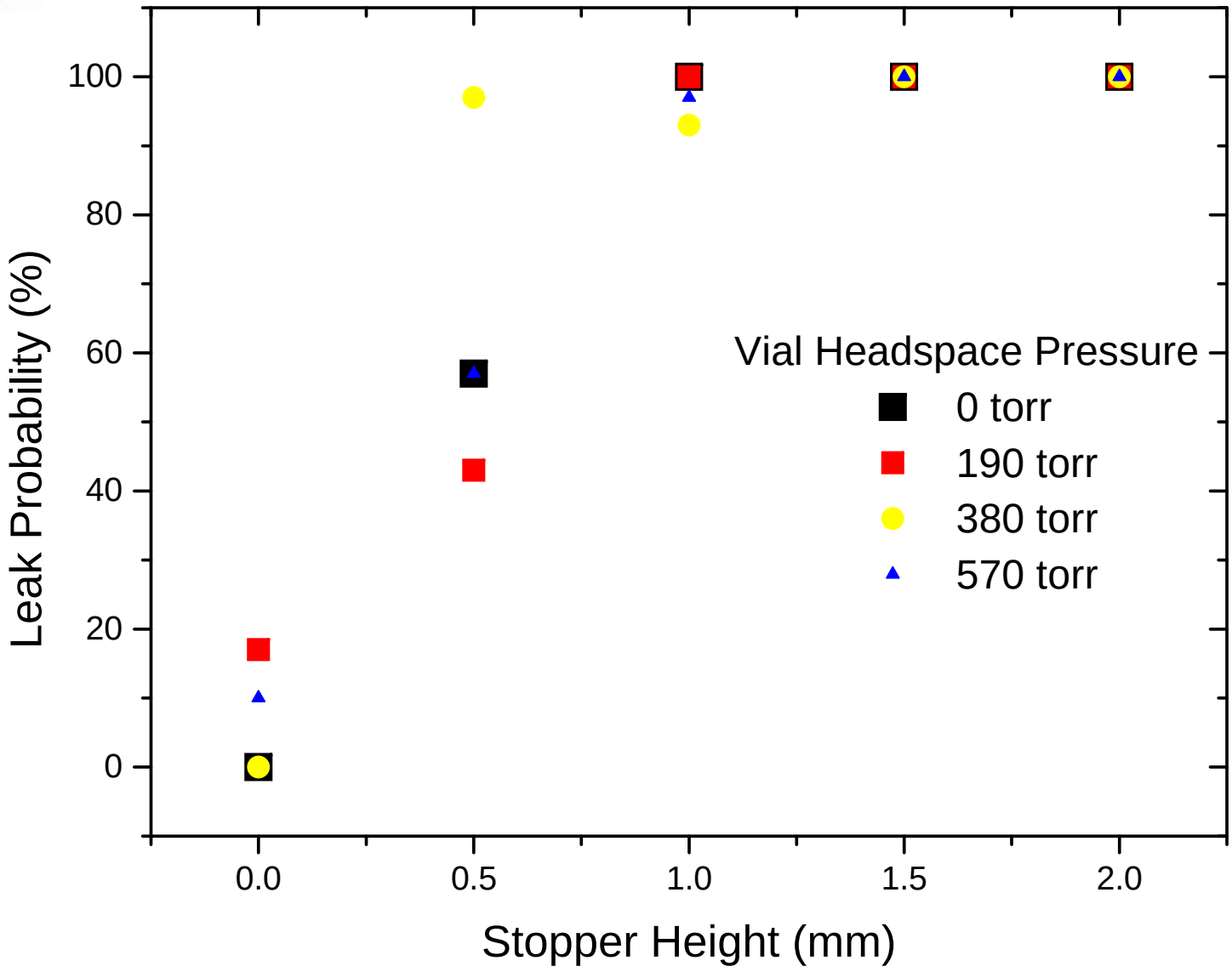
FMS-760 Headspace Oxygen Analyzer

FMS-1400 Headspace Pressure Analyzer

Data Table-Close and release

	Close + Release				
			P=P0	P0<P<Atm	P=Atm
P=0	Shim	Avg O2	No Leak	Effusive Leak	Total Leak
	0.0	0.1	100%	0%	0%
	0.5	10.2	43%	7%	50%
	1.0	18.8	0%	3%	97%
	1.5	18.2	0%	0%	100%
	2.0	18.6	0%	0%	100%
P=190	Shim	Avg O2	No Leak	Effusive Leak	Total Leak
	0.0	0.8	83%	13%	3%
	0.5	6.0	57%	3%	40%
	1.0	19.4	0%	0%	100%
	1.5	19.1	0%	0%	100%
	2.0	19.4	0%	0%	100%
P=380	Shim	Avg O2	No Leak	Effusive Leak	Total Leak
	0.0	0.2	100%	0%	0%
	0.5	17.8	3%	0%	97%
	1.0	16.7	7%	0%	93%
	1.5	19.2	0%	0%	100%
	2.0	19.2	0%	0%	100%
P=570	Shim	Avg O2	No Leak	Effusive Leak	Total Leak
	0.0	0.5	90%	n/a	10%
	0.5	11.2	43%	n/a	57%
	1.0	18.7	3%	n/a	97%
	1.5	19.4	0%	n/a	100%
	2.0	19.5	0%	n/a	100%

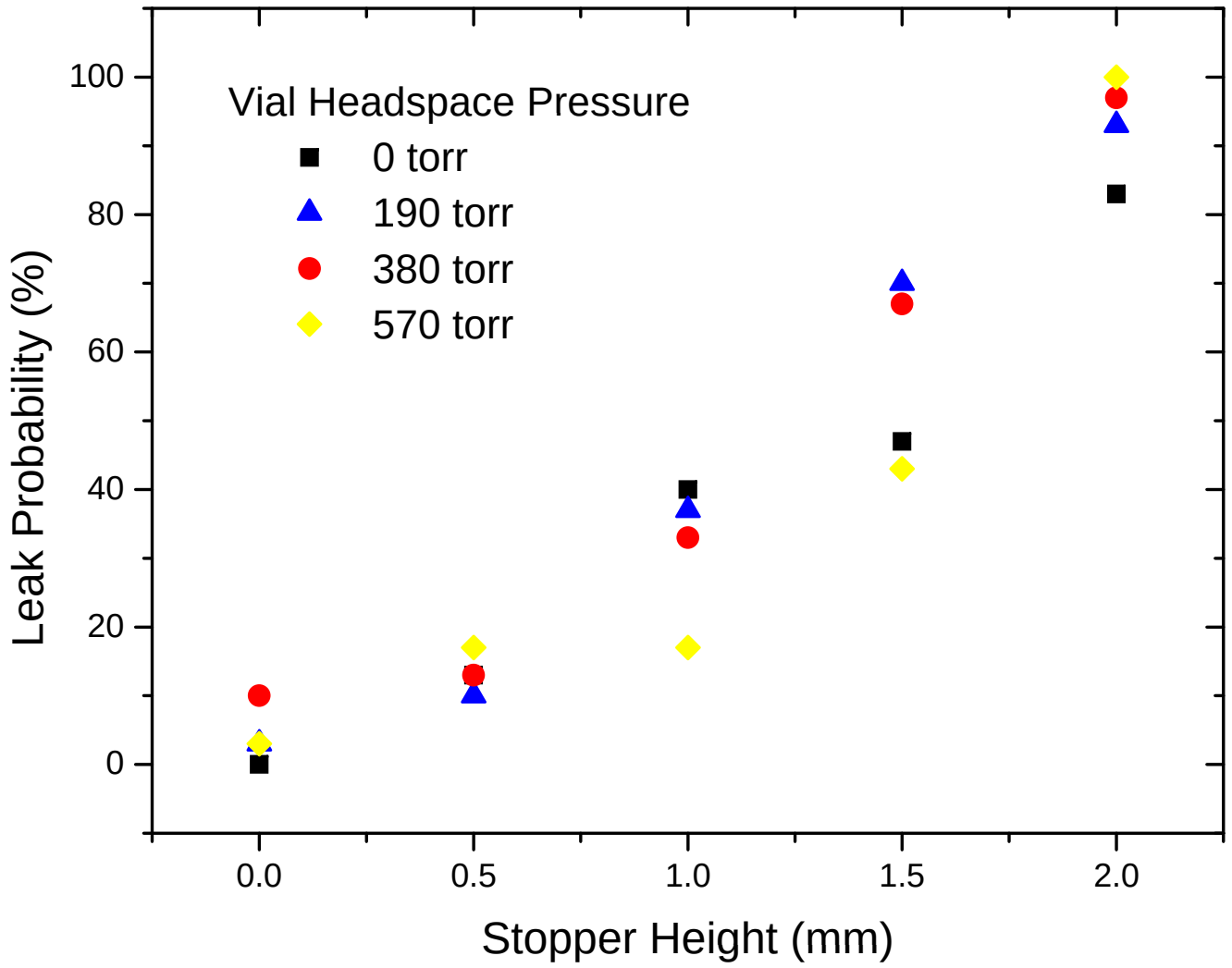
Data Graph-Close and release



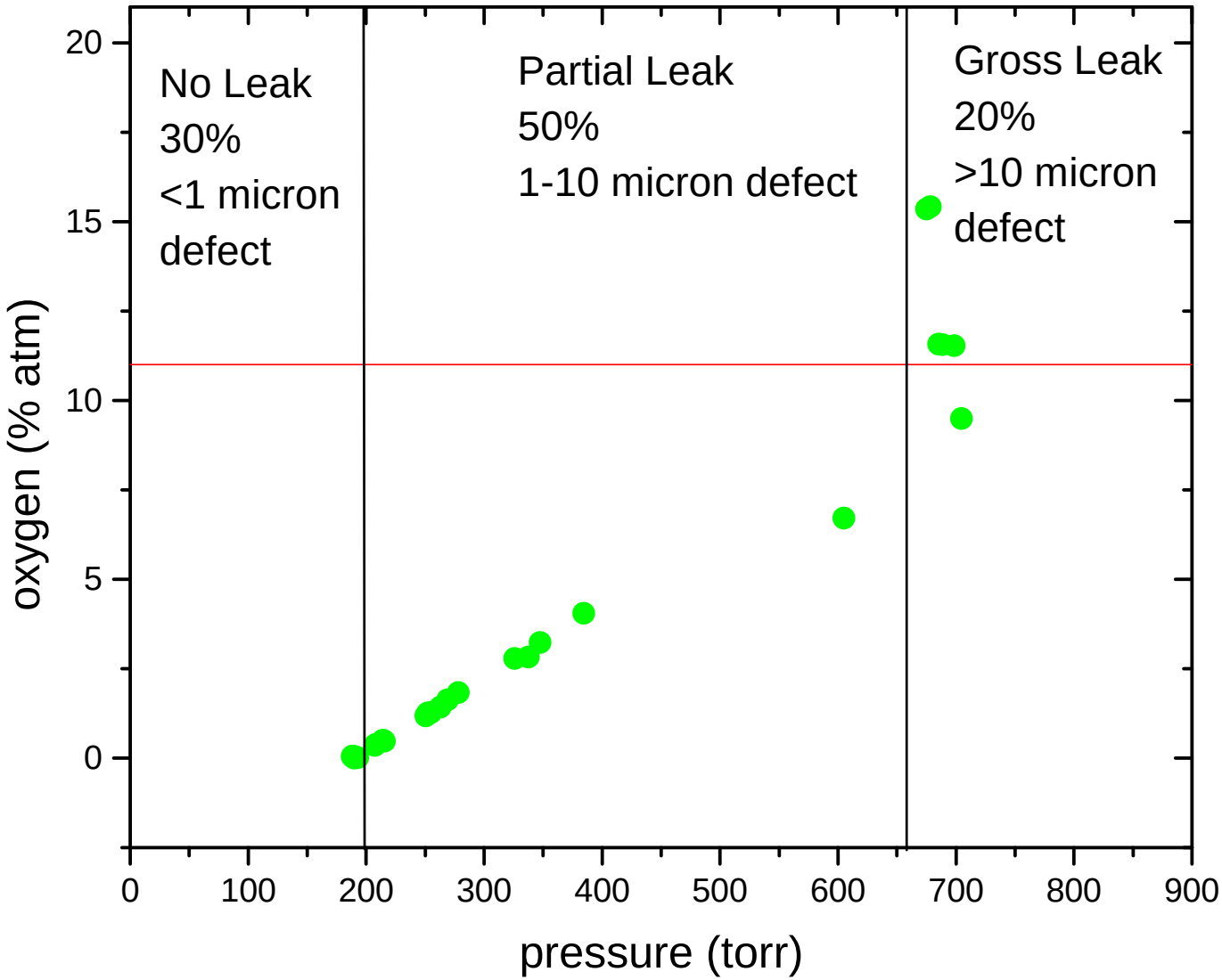
Data Table-Close and hold

	Hold Closed				
			P=P0	P0<P<Atm	P=Atm
P=0	Shim	Avg O2	No Leak	Effusive Leak	Total Leak
	0.0	0.1	100%	0%	0%
	0.5	0.8	87%	13%	0%
	1.0	1.9	60%	37%	3%
	1.5	6.7	53%	17%	30%
	2.0	14.8	17%	7%	77%
P=190	Shim	Avg O2	No Leak	Effusive Leak	Total Leak
	0.0	0.2	97%	0%	3%
	0.5	1.0	90%	7%	3%
	1.0	1.9	63%	23%	13%
	1.5	6.7	30%	50%	20%
	2.0	14.7	7%	17%	77%
P=380	Shim	Avg O2	No Leak	Effusive Leak	Total Leak
	0.0	0.4	90%	3%	7%
	0.5	0.6	87%	0%	13%
	1.0	0.7	67%	23%	10%
	1.5	3.7	33%	20%	47%
	2.0	17.4	3%	3%	93%
P=570	Shim	Avg O2	No Leak	Effusive Leak	Total Leak
	0.0	0.3	97%	n/a	3%
	0.5	0.5	83%	n/a	17%
	1.0	0.6	83%	n/a	17%
	1.5	2.2	57%	n/a	43%
	2.0	19.4	0%	n/a	100%

Data Graph-Close and hold



Characterizing the leak: Close and hold, P=190 torr, 1.5mm shim sample set



Designing a Robust Container Closure Inspection Process

- **Where in the process does / should headspace inspection occur?**
  - At capping: immediate raised stopper detection
  - In packaging: final quality inspection (detect raised stopper issues, only later in the process; also detects closure issues arising during or after capping)
- **Process / product parameters: line speed, container size, (holding) time before inspection**
- **Inputting the process / product parameters into the leak rate model allows one to conduct an inspection process feasibility**

Example: Inspection Process Feasibility

Example Process

Vent chamber to 700 mbar nitrogen, lower shelves, vent chamber to atmosphere with NITROGEN, hold vials for 30 minutes, remove and cap. First vial arrives at capper and headspace inspection system in 40 minutes and last vial (assuming batch size of 120,000 and line speed of 425 vpm) in 4.7 hours. Oxygen detection or pressure rise could be used to detect leaking vials. Choice depends on required leak rate sensitivity and the performance of the headspace system for this particular product configuration.

Defect Diameter (micron)	%O2 1st vial	Pressure Rise (mbar)	%O2 last vial	Pressure Rise (mbar)
5	**0.6**	**260**	**5.5**	**305**
10	**0.7**	**305**	**13**	**305**
50	**13**	**305**	**20**	**305**
100	**20**	**305**	**20**	**305**

Example: Inspection Process Feasibility

Modified Process to accelerate oxygen ingress

Vent chamber to 700 mbar nitrogen, lower shelves, vent chamber to atmosphere with AIR, hold vials for 30 minutes, remove and cap. First vial arrives at capper and headspace inspection system in 40 minutes and last vial (assuming batch size of 120,000 and line speed of 425 vpm) in 4.7 hours. In this process, the detection of oxygen in a leaking vial to identify a leaker is greatly improved compared to the initial process.

Defect Diameter (micron)	%O2 1st vial	Pressure Rise (mbar)	%O2 last vial	Pressure Rise (mbar)
5	**5.4**	**260**	**8.3**	**305**
10	**6.8**	**305**	**15**	**305**
20	**11**	**305**	**20**	**305**
50	**20**	**305**	**20**	**305**
100	**20**	**305**	**20**	**305**

Headspace analysis…

- **…can be a powerful analytical tool for investigating container closure integrity.**
- **…physically characterises the headspace gases which identifies not only closure failures but also stability risks to the formulation as well as giving insight into the process.**
- **…scales for automated 100% container closure inspection in manufacturing guaranteeing closure quality of finished product.**

Thank you!