

This article describes the studies which were performed to verify the calibration of a H₂O₂ Vapor Monitor for the measurement of VPHP and water vapor concentrations.

Calibration of a Near-Infrared (NIR) H₂O₂ Vapor Monitor

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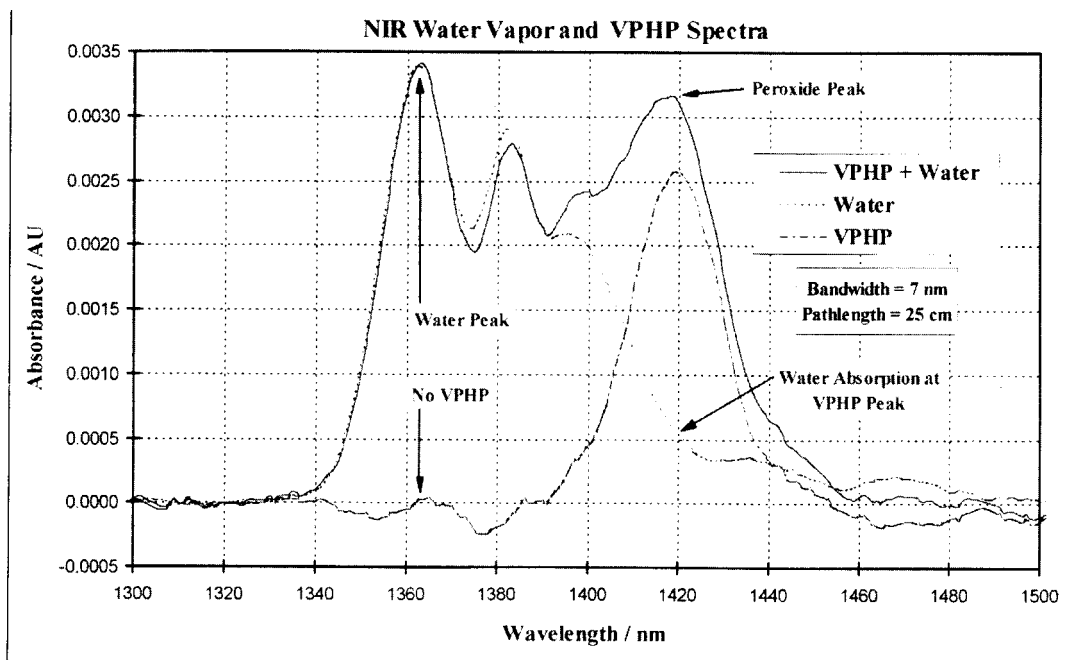
The H₂O₂ Vapor Monitor was designed using absorption parameters established by a combination of theoretical modeling and laboratory analysis with several different instruments. The following paragraphs describe a calibration procedure which was conducted with the H₂O₂ Vapor Monitor to confirm and/or improve these parameters. A statistical analysis showed that the Vapor Phase Hydrogen Peroxide (VPHP) and water vapor absorption coefficients already in use by the H₂O₂ Vapor Monitor were accurate, but that a change in another important calibration parameter (the water vapor peak ratio) was warranted.

Abstract

The H₂O₂ Vapor Monitor consists of a Near-Infrared spectrometer coupled by fiber optic cables to a 25 cm (10 in) pathlength stainless steel gas probe. The probe is placed inside an isolator to detect both VPHP and water vapor. For the VPHP calibration study, the gas probe was positioned on a test stand in the center of

a 50 W (1.5 m³) stainless-steel/glass isolator. During VPHP measurements by the H₂O₂ Vapor Monitor, samples of the vapor inside the isolator were extracted by pumping a known amount through a pair of impingers containing a given volume of water. The VPHP concentration was then determined by chemical analysis. A statistical analysis showed that the VPHP absorption coefficient already in use by the H₂O₂ Vapor Monitor was accurate. The water vapor calibration was performed by placing the gas probe inside a 6.25 W (0.18 m³) plexiglas chamber. Relative humidity was measured using a sling psychrometer. Water vapor concentration was determined by converting the relative humidity to absolute humidity using water vapor pressure/temperature tables and applying the ideal gas law. A statistical analysis showed that the water vapor absorption coefficient already in use by the H₂O₂ Vapor Monitor was accurate, but that a change in another important calibration parameter (the water vapor peak ratio) was warranted.

Figure 1. NIR water vapor and VPHP spectra.



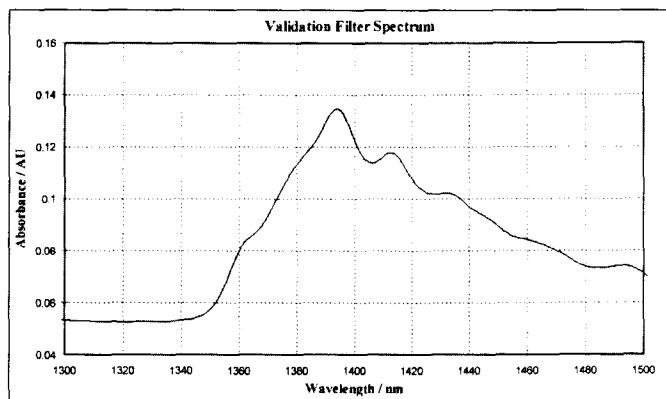


Figure 2. NIR validation filter spectrum.

Background

The use of VPHP sterilization is becoming more widespread in pharmaceutical manufacturing processes. VPHP sterilization is most efficient just below the point of saturation, which occurs at a maximum injection rate for the isolator's temperature. Generating the maximum VPHP concentration without condensation produces the most rapid and repeatable microbial kill. Isolator applications which use VPHP sterilization historically have been characterized by monitoring the liquid hydrogen peroxide injection rate. Theoretical VPHP concentration is based upon the measured injection rate and air flow. Due to adsorption, absorption and decomposition of the VPHP onto the isolator and its internal components (load), this theoretical value does not reflect the actual concentration inside the isolator. By evaluating surface area and type of load, an estimation of the half-life of VPHP can be determined. This data, along with the theoretical concentration, can be used to calculate a D-value for *Bacillus stearothermophilus*, one of the more VPHP resistant spores. The D-value, which is the time required to inactivate 90% of a microbial population, is essential for process validation. Thus, a method of quantitatively measuring isolator VPHP concentration could result in more efficient sterilization cycles as well as more accurate D-values.

The Steris VHP/1000 Decontamination System operates in a closed-loop fashion in which the VPHP is introduced into the enclosure through an inlet and then returns to the VPHP Generator where it is catalytically decomposed into water and oxygen. The water is then absorbed by a desiccant. The VPHP Generator generates a programmed amount of VPHP every minute at a designated air flow. The sterilization cycle includes four phases: dehumidification, conditioning, sterilization and aeration. The dehumidification phase removes water vapor from the isolator and increases the isolator surface temperature. As a result, higher VPHP concentrations may be achieved. During the conditioning phase, VPHP is introduced at a high injection rate into the isolator for a short period of time. This conditioning phase helps reduce the time to reach the maximum concentration. As the cycle proceeds into the sterilization phase, a lower VPHP injection rate is used to produce a steady-state concentration without condensation. Lastly, the aeration phase removes the VPHP after the programmed sterilization time has expired.

NIR spectroscopy has been shown to be an effective method of directly measuring VPHP and water vapor concentrations.²⁴ A device has been produced (UOP Guided Wave H₂O₂ Vapor Monitor), using the NIR technique, specifically for the measurement of VPHP and water vapor inside an enclosure.

This article describes the studies which were performed to verify the calibration of that device for the measurement of VPHP and water vapor concentrations. The VPHP calibration was performed at Baxter Healthcare, Round Lake, Illinois; while the water vapor calibration took place at UOP Guided Wave, El Dorado Hills, California.

The H₂O₂ Vapor Monitor Measurement Technique

The H₂O₂ Vapor Monitor provides a direct in-situ measurement of VPHP and water vapor concentrations. The monitor operates on the principle of absorption of near-infrared radiation by molecular vibration-rotation bands. Figure 1 shows the NIR spectra of VPHP and water vapor as measured by a scanning spectrometer using a 25 cm (10 in) gas probe. The 7 nm spectral bandwidth of the spectrometer resolves three water vapor peaks and one VPHP absorption peak.

The H₂O₂ Vapor Monitor is a dual-beam, post dispersed, fixed wavelength fiber array spectrometer. Fiberoptic cables fixed in the light dispersion plane inside the instrument determine the measurement wavelengths and bandwidth. These wavelengths are 1320 nm, 1362 nm, 1422 nm and 1460 nm. The 1320 nm and 1460 nm measurements are used as baseline correction points. The 1362 nm measurement coincides with the NIR water vapor peak, while the VPHP absorbance peak is centered at 1419 nm. The H₂O₂ Vapor Monitor operates at a 6 nm bandwidth.

Since the water vapor spectrum is quite broad and has significant absorbance at 1419 nm, it interferes with the measurement of VPHP - Figure 1. To reduce the amount of interference, the analytical measurement of hydrogen peroxide vapor is made at 1422 nm. Nevertheless, it is still necessary to make a water vapor correction at 1422 nm to obtain an accurate measurement of VPHP concentration. This correction can be made by measuring the absorbance at 1362 nm (comprised solely of water vapor), dividing that absorbance by the ratio, *WaterRat*, of the water vapor absorbance coefficients at 1362 nm and 1422 nm, and subtracting this estimated water vapor absorbance at 1422 nm from the measured absorbance at 1422 nm (comprised of both water vapor and VPHP). Then the VPHP concentration can be computed accurately using its absorption coefficient at 1422 nm.

Quantification of VPHP concentration is determined by applying Beer's Law. Beer's Law states that concentration is proportional to the negative logarithm of the transmittance. The intensity of the light transmitted through the sample, *S*, is

$$S(\lambda) = S_0(\lambda) \cdot e^{-\alpha(\lambda)\rho\ell} \quad (1)$$

where *S*₀ is the light intensity incident on the sample, λ is the wavelength, $\alpha(\lambda)$ is the wavelength-dependent absorption coefficient, ρ is the concentration and ℓ is the pathlength. Solving for concentration yields

$$\rho = \frac{-\log[S(\lambda)/S_0(\lambda)]}{\alpha(\lambda)\ell \log(e)} \quad (2)$$

It is customary to include the $\log(e)$ term in the absorption coefficient, $\alpha(\lambda)$, and to define $S(\lambda)/S_0(\lambda)$ as the transmittance, *T*. Absorbance is then defined as $A = -\log(T)$, resulting in the more familiar form of Beer's Law:

$$\rho = A(\lambda) / \alpha(\lambda)\ell \quad (3)$$

Impingers in Series (Carryover) Test VPHP Results					
Sample Name	Avg [VPHP] (mg/L)	Std Dev (mg/L)	Theoretical [VPHP] at STP (mg/L)	% of Theoretical	% Diff between Concurrent Samples
NONMIX-A	1.545	0.030	1.686	91.6	—
MIX-A	1.554	0.058	1.686	92.2	0.6
NONMIX-B	1.579	0.026	1.686	93.6	—
MIX-B	1.586	0.096	1.686	94.0	0.4
NONMIX-C	1.603	0.029	1.686	95.0	—
MIX-C	1.735	0.214	1.686	102.9	7.6

Table A.

If we define the term $Water_{Ratio}$ to be the ratio of the water vapor absorbance at the water vapor peak (1362 nm) to the water vapor absorbance at the VPHP peak (1422 nm), then

$$Water_{Ratio} = \alpha_{water}(\lambda_{1362}) / \alpha_{water}(\lambda_{1422}) \quad (4)$$

The VPHP concentration is then given by:

$$\rho_{VPHP} = [A_{Measured\ 1422} - A_{Measured\ 1362} / Water_{Ratio}] / \alpha_{VPHP}(\lambda_{1422}) \ell \quad (5)$$

where $A_{Measured\ 1422}$ is the total measured absorbance at the VPHP peak (1422 nm), and $A_{Measured\ 1362}$ is the total measured absorbance at the water vapor peak (1362 nm) minus the respective total absorbances measured when the instrument was referenced.

The H_2O_2 Vapor Monitor measures water vapor concentration relative to the water vapor concentration that existed at the time the instrument was referenced. If the instrument is referenced in dry air or nitrogen, then it will report absolute water vapor concentrations. If referenced under wet conditions, the instrument will report relative water vapor concentrations. These 'relative' water vapor concentrations are given by:

$$\rho_{Water} = A_{Measured\ 1362} / \alpha_{Water}(\lambda_{1362}) \ell \quad (6)$$

As can be seen from equations (5) and (6), to determine the VPHP and water vapor concentrations, accurate values for the water vapor ratio, $Water_{Ratio}$, and absorption coefficients, α_{VPHP} and α_{Water} must be known. The precise values for the absorption

(or extinction) coefficients and water vapor ratio are wavelength and bandwidth dependent. For this reason, they must be determined for a particular instrument design. Once the wavelength and instrument bandwidth are determined, the accuracy of any given production instrument can be demonstrated by showing similarity to the photometric properties of the device on which the water vapor ratio and absorption coefficients were determined.

The determination of preliminary absorption coefficients for the H_2O_2 Vapor Monitor was done by a combination of theoretical modeling and laboratory analysis." The preliminary VPHP coefficient was developed by a vapor phase analysis using the scanning spectrometer and correlation of the data with the Xylenol Orange Photometric Assay method.' The water vapor absorption coefficient and ratio, $Water_{Ratio}$, were developed by a vapor phase analysis using the scanning spectrometer and correlation of the data with psychrometric humidity measurements. This article describes the verification and development of final values for these parameters.

To ensure the measurement accuracy of the H_2O_2 Vapor Monitor, an automatic validation procedure has been built into each instrument. The validation procedure involves the measurement of a polymeric filter with absorbance characteristics shown in Figure 2. The polymeric material was chosen for its thermal stability and non-hygroscopic property. Prior to VPHP concentration measurements by the H_2O_2 Vapor Monitor, the instrument measures the absorbance of this filter at each of its fixed wavelengths. These measured absorbance values are compared to factory-set values stored in the instrument's non-volatile memory at the time of manufacture. The factory-set values are established by careful alignment of

Impinger Sets in Parallel (Repeatability) Test VPHP Results					
Sample Name	Avg [VPHP] (mg/L)	Std Dev (mg/L)	Theoretical [VPHP] at STP (mg/L)	% of Theoretical	% Diff in Concurrent Samples
A1	1.327	0.016	1.686	78.7	—
A2	1.386	0.012	1.686	82.2	4.3
B1	1.320	0.017	1.686	78.3	—
B2	1.449	0.022	1.686	85.9	8.9
C1	1.377	0.013	1.686	81.7	—
C2	1.478	0.002	1.686	87.7	6.8

Table B.

each wavelength, followed by precise absorbance measurements of the validation filter at each wavelength. In this way, any change in the wavelength characteristics of the H₂O₂ Vapor Monitor can be detected. Because the validation filter peak absorbance is located mid-way between the peak absorbances of water vapor at 1362 nm and VPHP at 1422 nm, this feature increases the sensitivity of the validation measurement since a wavelength shift in either direction will cause the measured absorbance values to change in opposing directions.

VPHP Calibration

Vapor Sample Extraction Method Validation

The vapor sampling method validation, as well as the VPHP calibration work, was carried out in a 50 ft³ (1.5 m³) rigid-walled, sterility test isolator. The isolator was constructed of 316L stainless steel with glass windows and two opposing glove sets.

Impingers were passivated with concentrated nitric acid. All other laboratory glassware and Teflon sample bottles were 10% HNO₃ acid-washed. The impinger pairs were manually assembled with different lengths and diameters of Tygon tubing. A Tygon tube was used to connect the impinger pairs to the Primary Flow Calibrator for pump pre- and post-calibration. Two identical sampling tubes (Pharmed tubing) were assembled to connect (via a lattice fitting) the impingers inside the isolator to the pumps, which were located outside the isolator. Two sample pumps were used in every run; thus providing concurrent sampling. By placing the impingers inside the isolator (as opposed to drawing the sample from the isolator through impingers located external to the isolator), sample degradation was avoided by not allowing the vapor sample to contact any plastic tubing and possible adverse absorption to occur. Figure 3 shows the Impinger/Pump calibration configuration and the Impinger/Pump sampling configuration. Isolator pressure was controlled (at essentially ambient) by supplying a 1.5 in (3.8 cm) diameter flexible hose from the isolator to a fume hood. The isolator set-up also is depicted in Figure 3.

The single lot of 31% Liquid H₂O₂ used throughout the testing was assayed in triplicate via the Xylenol Orange (XO) Photometric Assay method.¹ The average H₂O₂ volumetric concentration was 31.84% (102.7% of nominal) with a 0.679% standard deviation. The Assay QC result was 99.5% of a prepared standard solution.

This liquid assay value was used in Equation 7 to calculate the theoretical VPHP concentrations generated by the bio-decontamination unit:

$$[\text{VPHP}]_{\text{theor}} = \left(\frac{\text{IR}}{\text{Q}} \right) \times \left(\frac{1000 \text{ mg}}{\text{g}} \right) \times \left(\frac{\text{cf}}{28.32 \text{ L}} \right) \times \left(\frac{\text{A}\%}{100} \right) \quad (7)$$

where, $[\text{VPHP}]_{\text{theor}}$ = Theoretical VPHP Concentration, mg/L
 IR = VPHP Generator Injection Rate, g/min
 Q = VPHP Generator Air Flow Rate, cfm
 A% = 31.84% = Assayed Liquid H₂O₂ Average Concentration, vol%

Impingers in Series (Carryover) Test

A carryover test was performed to ensure negligible vapor carry-over from the first impinger to the second serially located impinger. Twenty (20) mL (0.7 oz) de-ionized (DI) water was pipetted into 12 impingers (6 pairs). A water control sample was analyzed in triplicate with the Xylenol Orange

Photometric Assay. Negligible quantities of liquid H₂O₂ were found in this water control sample, and all subsequent water control samples. The Impinger/Pump combinations were pre-calibrated external to the isolator by connecting each impinger pair's outlet to the sample line and then the sample line to one of the sample pumps. The impinger pair's inlet was then connected to a Primary Flow Calibrator - Figure 3. Both sample pumps were set to a nominal 1.0 L/min flow rate. Following a one minute pump warm-up period, three flow rate measurements were made with the Primary Flow Calibrator. The Impinger/Pump combinations also were post-calibrated in this same manner.

The impinger pairs were then stoppered and placed into the center of the isolator. The two sample tubes were connected to the isolator via double-barbed lattice fittings. The VPHP Generator was programmed with the following parameters:

de-humidification:	20 cfm (34 cmh), 10 min, 2.3 mg/L
conditioning:	None
sterilization:	20 cfm (34 cmh), 2 hrs, 3.0 g/min
aeration:	20 cfm (34 cmh), 5 min
pressure control:	None (isolator vented to fume hood).

VPHP injection parameters and measurements were logged to paper tape by the VPHP Generator. The sterilization phase of the cycle consisted of the following:

30 minute equilibration period
 10 minute impinger sampling period A
 10 minute delay
 10 minute impinger sampling period B
 10 minute delay
 10 minute impinger sampling period C

For each 10 minute sampling period (periods A, B, and C), three samples were taken simultaneously. The contents from both impingers used with Pump 1 were combined and analyzed together. The contents from individual impingers used with Pump 2 were analyzed separately. Each quantitative liquid H₂O₂ analysis was performed in triplicate.

The analysis results showed negligible carryover (<1%) of vapor from one impinger to the next; hence, the 2-impinger method was adequate.

VPHP Concentrations based upon the Impingers in Series (Carryover) Test Results

The results from the impingers which were analyzed separately (non-mixed) were now combined to calculate a VPHP concentration. The pump sample volumes were calculated by averaging the pre- and post-calibration flow rate measurements (in L/min) and multiplying these values by 10 minutes per sampling period. The pump sample volumes were then corrected for isolator temperature using Equation 8:

$$V_{\text{corr}} = Q_{\text{pump}} \times 10\text{min} \times \left(\frac{298 \text{ K}}{\text{T}} \right) \quad (8)$$

where, V_{corr} = Adjusted Sample Volume, L
 Q_{pump} = Average of the Pre- and Post-Calibration Sample Pump Flow Rates, L/min
 T = Average Isolator Temperature during Sample Period, K

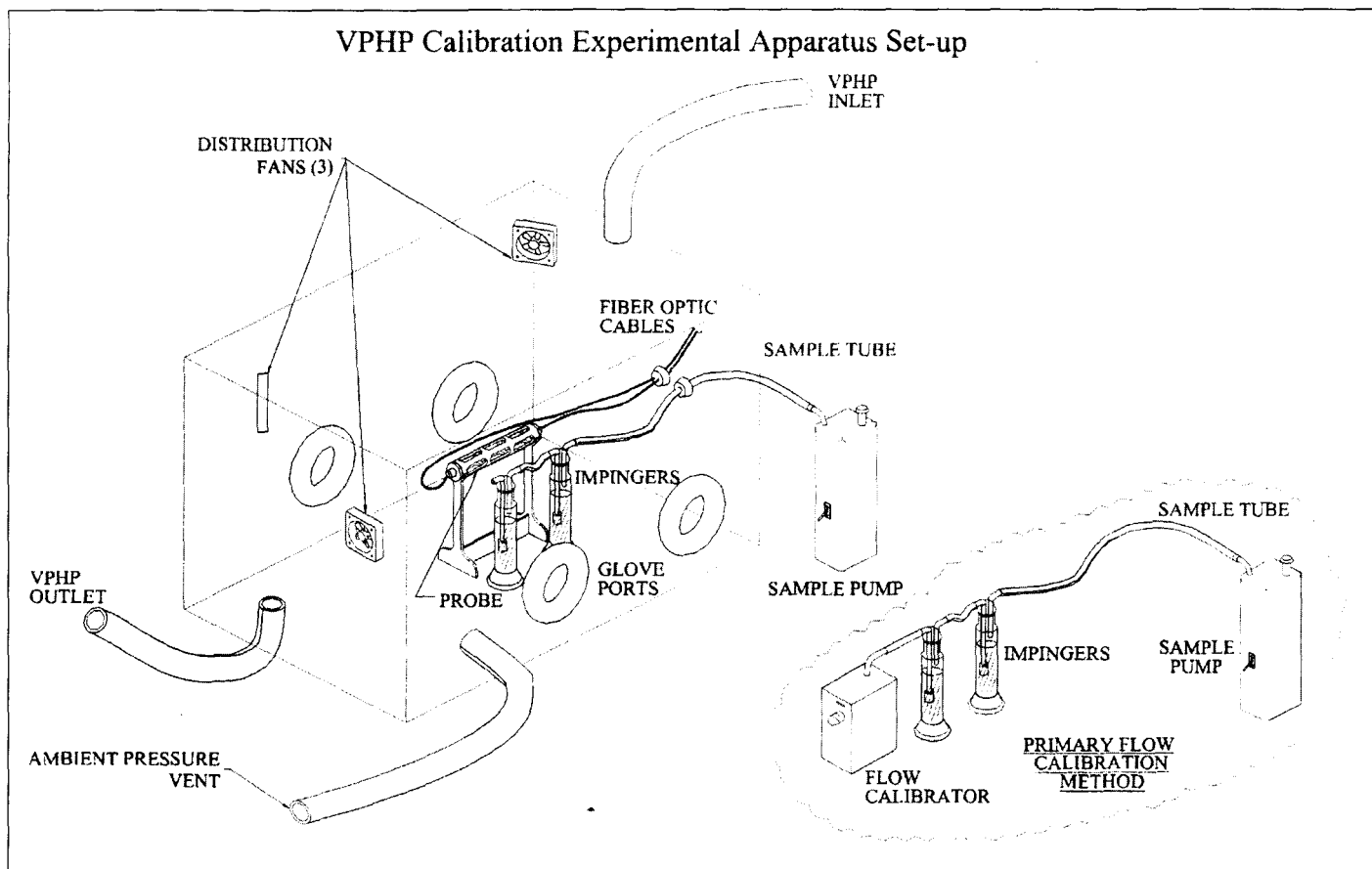


Figure 3. VPHP calibration experimental apparatus set-up.

Since the isolator gauge pressure increased less than 0.1% over ambient (408 in wc, 101325 Pa) and only during glove movement, it was not necessary to correct the pump sample volumes for pressure. The isolator temperature for each 10 minute sampling interval was determined from an average of 10 RTD measurements taken in the Isolator VPHP exit line.

Isolator VPHP concentration for each sampling interval was determined by using Equation 9:

$$[\text{VPHP}]_{\text{isol}} = \frac{[\text{H}_2\text{O}_2]_{\text{Liq Extract}} \times V_{\text{Liq Extract}} \times \left(\frac{L}{1000 \text{ mL}}\right)}{V_{\text{corr}}} \quad (9)$$

where, $[\text{VPHP}]_{\text{isol}}$ = Isolator VPHP Concentration, mg/L

$[\text{H}_2\text{O}_2]_{\text{Liq Extract}}$ = H_2O_2 Concentration in Liquid Extract, mg/L

$V_{\text{Liq Extract}}$ = Liquid Extract Sample Volume, mL

V_{corr} = Adjusted Sample Volume from Equation (8), L

The VPHP concentration results, based upon the 'Impingers in Series (Carryover) Test' results, are displayed in Table A.

Impinger Sets in Parallel (Repeatability) Test

Due to the different operators, tubing lengths and diameters, and impinger port sizes used throughout the calibration procedure, this test was performed to demonstrate repeatability among concurrent samples. The same procedure described above for the 'Impingers in Series (Carryover) Test' was repeated, except:

For each 10 minute sampling period (A, B, and C), two

samples were taken simultaneously. The contents of both impingers used with Pump 1 and the contents of both impingers used with Pump 2 were each combined and analyzed for liquid H_2O_2 in triplicate. The isolator VPHP concentration for each sampling interval was determined by applying Equation 9. Results are displayed in Table B.

When the results from the 'Impingers in Series' and 'Impinger Sets in Parallel' Tests were combined, an average 4.8% Difference between Concurrent Samples was determined. Since the VPHP Concentrations agreed to within 5%, the results were deemed reproducible and were not dependent on the operator, tubing length or diameter or impinger port diameter.

VPHP Calibration Data Collection

Experimental Set-Up

The 25 cm (10 in) gas probe was fastened to a test stand in a horizontal position so that the centerline of the probe was at the same height as the inlet ports on the impingers - Figure 3. The stand was placed in the center of the isolator floor. Two 200 μm diameter fiber optic cables were connected from the gas probe to the H_2O_2 Vapor Monitor. The fiber optic cables were sealed into the isolator with a bored-out rubber stopper and RTV. The H_2O_2 Vapor Monitor was set for a 20 second integration time with data logged to a computer.

Three thermocouples were affixed to the probe so that one junction was located on each side of the probe and one junction on the end of the gas probe. Another thermocouple measured ambient temperature outside the isolator. The thermocouples were connected to a datalogger. A computer was used to log

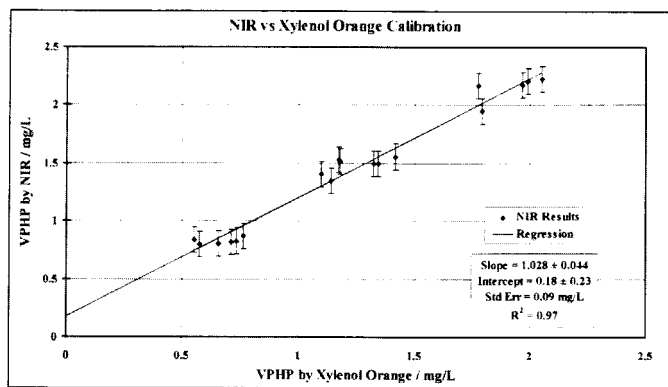


Figure 4. NIR vs Xylenol Orange calibration.

temperature data at a rate of 1 sample per minute per thermocouple. The isolator set-up is depicted in Figure 3.

Data Capture

The same procedure described previously for the 'Impingers in Series (Carryover) Test' was repeated, except:

The VPHP Generator was programmed with a 200 min, 1.5 g/min injection rate for the sterilization phase. The sterilization phase of the cycle consisted of the following:

- 30 minute equilibration period
- 10 minute impinger sampling and H₂O₂ Vapor Monitor testing period A
- 10 minute delay
- 10 minute impinger sampling and H₂O₂ Vapor Monitor testing period B
- VPHP Generator Injection Rate at 3.0 g/min
- 30 minute equilibration period
- 10 minute impinger sampling and H₂O₂ Vapor Monitor testing period C
- 10 minute delay
- 10 minute impinger sampling
- H₂O₂ Vapor Monitor testing period D
- VPHP Generator Injection Rate at 4.5 g/min
- 30 minute equilibration period
- 10 minute impinger sampling and H₂O₂ Vapor Monitor testing period E
- 10 minute delay
- 10 minute impinger sampling and H₂O₂ Vapor Monitor

These parameters produced a 0.6 mg/L to 2.3 mg/L VPHP concentration range and a 31°C to 36°C temperature profile. The H₂O₂ Vapor Monitor was referenced two minutes prior to sterilization start. At 20 minutes into the sterilization phase, the isolator gloves were entered and movement was initiated to cause several +/-0.6 in we (150 Pa) pressure swings. No unusual fluctuations were observed in the H₂O₂ Vapor Monitor data. It was concluded that glove movement did not affect the H₂O₂ Vapor Monitor results.

For each 10 min sampling period, one sample was taken. The contents of both impingers of each pair were combined and quantitatively analyzed for liquid H₂O₂ concentration in triplicate. The isolator temperature for each 10 minute sampling interval (for pump flow corrections) was determined from an average of the 30 thermocouple values.

The Isolator VPHP concentration for each sampling interval (as reported by the wet chemistry method) was determined

by applying equation 9. The Isolator VPHP concentration for each sampling period (as reported by the H₂O₂ Vapor Monitor) was determined by averaging the 30 VPHP concentration readings collected by the H₂O₂ Vapor Monitor during each individual sampling interval.

The preceding 'Data Capture' procedure was repeated three times to yield a total of four calibration data sets (designated Run 1, Run 2, Run 3, and Run 4). The results are summarized in Table C.

Stopper Control Study

A stopper control study was conducted to determine if the stoppers were effective in sealing the impinger inlets and outlets against adverse VPHP penetration. Three (3) stopper control impinger pairs (6 impingers total) were subjected to the same sterilization cycle used for the H₂O₂ Vapor Monitor data capture runs. The combined contents of each impinger pair were analyzed for liquid H₂O₂ concentration in triplicate. Since the VPHP allowed to pass through the stoppers (as measured by the liquid H₂O₂ concentration) did not exceed 0.15% of the lowest liquid H₂O₂ assay value from any of the previous tests, the stoppers were deemed effective in preventing H₂O₂ vapor penetration into the impingers.

System Control Study

A system control study was conducted to determine if the thermocouples or the H₂O₂ Vapor Monitor gas probe and fibers affected the observed VPHP concentration. The same procedure described previously in the 'Data Capture' section was repeated, except:

The H₂O₂ Vapor Monitor and thermocouples were not used. The sterilization phase of the cycle consisted of the following:

- 30 minute equilibration period
- 10 minute impinger sampling period A
- VPHP Generator Injection Rate at 3.0 g/min
- 30 minute equilibration period
- 10 minute impinger sampling period B
- VPHP Generator Injection Rate at 4.5 g/min
- 30 minute equilibration period
- 10 minute impinger sampling period C

For each 10 minute sampling period, two samples were taken simultaneously. The contents of both impingers used with Pump 1 and the contents of both impingers used with Pump 2 were each combined and analyzed in triplicate for H₂O₂ liquid concentration.

The isolator temperature for each 10 minute sampling interval (for pump flow corrections) was determined from an average of 10 RTD measurements taken in the isolator VPHP exit line. The Isolator VPHP concentration for each sampling interval was determined by applying equation (9). Results are displayed in Table D.

These 'System Control' results were slightly higher than 'Data Capture' Run 1 and Run 2 results, roughly the same as Run 3 results, and slightly lower than Run 4 results. Since the results were mixed, it was concluded that the presence of the H₂O₂ Vapor Monitor equipment in the isolator during testing (fiberoptic cables, thermocouples, gas probe, racks and tape) did not adversely affect the observed VPHP concentrations.

VPHP Concentration Data Analysis

A comparison plot of the VPHP concentrations as measured by the H₂O₂ Vapor Monitor and the Xylenol Orange Photometric Assay method is shown in Figure 4. The data is contained in

VPHP Calibration Data Collection Results

			WET CHEMISTRY RESULTS			UOP H ₂ O ₂ MONITOR RESULTS		
Run	Sample Name	Theoretical [VPHP] at STP (mg/L)	Avg [VPHP] (mg/L)	Std Dev (mg/L)	% of Theoretical	Avg [VPHP] (mg/L)	Std Dev (mg/L)	% of Theoretical
1	A	0.843	— ¹	— ¹	— ¹	0.61	0.05	72.4
	B	0.843	— ¹	— ¹	— ¹	0.61	0.05	72.4
	C	1.686	1.097	0.029	65.0	1.41	0.05	83.6
	D	1.686	1.138	0.018	67.5	1.35	0.09	80.1
	E	2.530	1.776	0.087	70.2	2.17	0.05	85.8
	F	2.530	1.793	0.089	70.9	1.95	0.04	77.1
2	A	0.843	0.552	0.083	65.4	0.84	0.04	99.6
	B	0.843	0.573	0.038	67.9	0.80	0.03	94.9
	C	1.686	1.178	0.039	69.9	1.52	0.05	90.2
	D	1.686	1.170	0.037	69.4	1.53	0.05	90.7
	E	2.530	1.691	0.070	66.8	1.57 ²	0.09	62.1
	F	2.530	1.730	0.059	68.4	1.10 ²	0.04	43.5
3	A	0.843	0.655	0.095	77.7	0.81	0.05	96.1
	B	0.843	0.709	0.040	84.0	0.82	0.04	97.3
	C	1.686	1.322	0.048	78.4	1.50	0.05	89.0
	D	1.686	1.344	0.042	79.7	1.50	0.05	89.0
	E	2.530	1.990	0.077	78.7	2.21	0.05	87.4
	F	2.530	1.967	0.096	77.8	2.18	0.04	86.2
4	A	0.843	0.761	0.055	90.2	0.87	0.03	103.2
	B	0.843	0.733	0.032	87.0	0.83	0.05	98.5
	C	1.686	1.418	0.037	84.1	1.56	0.04	92.5
	D	1.686	— ³	— ³	— ³	1.58	0.04	93.7
	E	2.530	2.053	0.100	81.1	2.23	0.05	88.1
	F	2.530	— ⁴	— ⁴	— ⁴	2.29	0.05	90.5

¹ Results not valid. Measurements were very close to the low end of the xylenol orange calibration curve (1 out of 3 replicates was outside the calibration range)

Severe baseline drift was experienced during this sampling period. Results were not used for further analysis.

³ Impinger set accidentally used twice for sampling.

⁴ Impinger set never used for sampling.

Table C.

Table C. Each point in the Xylenol Orange data set represents the VPHP concentration determined by continuous impinger sampling over each 10 minute data collection period. Each point in the H₂O₂ Vapor Monitor data set represents the average of 30 readings collected over the 10 minute data collection period. The error bars depict intervals of one standard deviation for the 30 H₂O₂ Vapor Monitor readings col-

lected during each 10 minute data collection period. The straight line represents a least-squares linear regression of the data. The regression correlation coefficient (R²) indicates the degree of agreement between the two VPHP concentration measurement methods. The regression results yield a slope of 1.028 with a ± 0.044 uncertainty (standard error of the slope), an intercept of 0.18 with an uncertainty of ± 0.23 (standard

System Control Study VPHP Results

Sample Name	Avg [VPHP] (mg/L)	Std Dev (mg/L)	Theoretical [VPHP] at STP (mg/L)	% of Theoretical
A1	0.653	0.086	0.843	77.5
A2	0.758	0.059	0.843	89.9
B1	1.349	0.004	1.686	80.0
B2	1.332	0.004	1.686	79.0
C1	1.989	0.101	2.530	78.6
C2	1.987	0.069	2.530	78.5

Table D.

error of the intercept), and an R^2 (correlation coefficient) of 0.97.⁶ It is clear from the uncertainties that the slope and intercept are not significantly different from 1 and 0 respectively, and that 97% of the uncertainty in the data is explained by the regression. Therefore, the VPHP absorption coefficient currently in use by the H₂O₂ Vapor Monitor [$7.00 \times 10^{-5} \pm 3.1 \times 10^{-6}$ AU/cm/(mg/L) at 1422 nm wavelength and 6 nm bandwidth] was found to be accurate and no change was warranted. This coefficient is valid over the range of temperatures (31-36°C) and concentrations (0.6-2.3 mg/L) encountered in this experiment. The estimated standard error of regression (or accuracy of the instrument) over this range of values was 0.09 mg/L. This calculated accuracy was limited by the actual accuracy of the impinger sampling and Xylenol Orange Photometric Assay methods, and not by the spectroscopy.

Water Vapor Calibration

As can be seen from equation (6), to determine the water vapor concentration, an accurate value for the water vapor absorption coefficient, $\alpha_{\text{Water}}^{1362}$, must be known. And, to determine the VPHP concentration, an accurate value for the water vapor ratio, $\text{Water}_{\text{Ratio}}$, must be known for equation (5). From prior experiments using a scanning near-infrared spectrometer and from the characterization of early prototype H₂O₂ Vapor Monitors, fairly accurate values for these two water vapor parameters were already known. The objective of this experiment was to confirm or improve those values for the production H₂O₂ Vapor Monitors. The procedure entailed generating water vapor in an enclosure, independently measuring the water vapor concentration via a sling psychrometer, and correlating these independent measurements to the output of three H₂O₂ Vapor Monitors.

Experimental Set-Up

The calibration method described below is simple and direct, using readily available equipment. For these measurements, a plexiglas aquarium, a mechanical-type room humidifier, a sling psychrometer, 3 fans, a beaker of DI water, an air recirculating blower, a container of desiccant, three H₂O₂ Vapor Monitors (two of which were previously unavailable for the VPHP calibration), and a digital timer were used. With this equipment, a range of humidities from 5 mg/L to 25 mg/L was generated, covering a range of temperatures from 22°C to 34°C.

The experimental apparatus is shown in Figure 5. A 6.25 ft³ (0.18 m³) commercial aquarium tank was fitted with a plexiglas cover, a blower, a desiccant canister containing silica gel and associated valves. Located in upper diagonally opposite cor-

ners were two 2.5" (6.4 cm) square tube-axial fans to provide continuous and uniform vapor distribution in the enclosure. The fans were aimed at the diagonally opposite lower corners of the enclosure. A sling psychrometer (wet bulb/dry bulb hygrometer) was mounted in one corner of the enclosure. This hygrometer consisted of two mercury thermometers (0.5°C resolution each) mounted 1/2" (1.3, cm) apart. The lower end of the cloth sleeve on the wet bulb thermometer was dipped and secured into a beaker of DI water. A constant flow of air over the hygrometer was maintained by placing a 2.5" (6.4 cm) square tube-axial distribution fan 4" (10 cm) from the thermometer bulbs. Water vapor was generated with a spinning-disk room humidifier. A remote switch permitted the humidifier to be turned on and off as required to generate the small amount of water vapor necessary for this size of enclosure. Three H₂O₂ Vapor Monitor gas probes were bundled together and located in the center bottom of the enclosure. Digital data from the H₂O₂ Vapor Monitors were collected every 30 seconds and loaded to a computer.

Experimental Procedure

The H₂O₂ Vapor Monitors were referenced at ambient conditions with the blower off and all three fans operating. The thermometer temperatures were periodically recorded along with the corresponding times. Approximately 15 minutes into the experiment, all clock (the digital timer and the three internal H₂O₂ Vapor Monitor clocks) times were recorded to provide an initial time synchronization. The blower was then turned on with the valves in the desiccant by-pass mode.

Since the blower added heat to the system, the temperature inside the enclosure increased as a result of its operation. The blower remained operating for the remainder of the experiment. At 44 minutes into the run (13:14 on Figure 6), the desiccant by-pass valve was closed and the desiccant inlet valve was opened. The humidity decreased, as a result of the desiccant, to approximately 5 mg/L. After the system stabilized, the valves were returned to the desiccant by-pass mode and the humidity began to rise again as a result of the suction on the desiccant canister (there was no outlet valve on the desiccant canister). At 13:40, the humidifier was turned on for 60 s. The humidity spiked up, then decreased to a steady-state value. This procedure was followed by another 60 second pulse and then a 90 second pulse of humidity. The final addition of humidity was for 6 min and 20 seconds starting at 14:59 and resulted in the formation of condensation on the walls of the enclosure. At 15:26, the blower and valves were placed in drying mode, then returned to desiccant by-pass

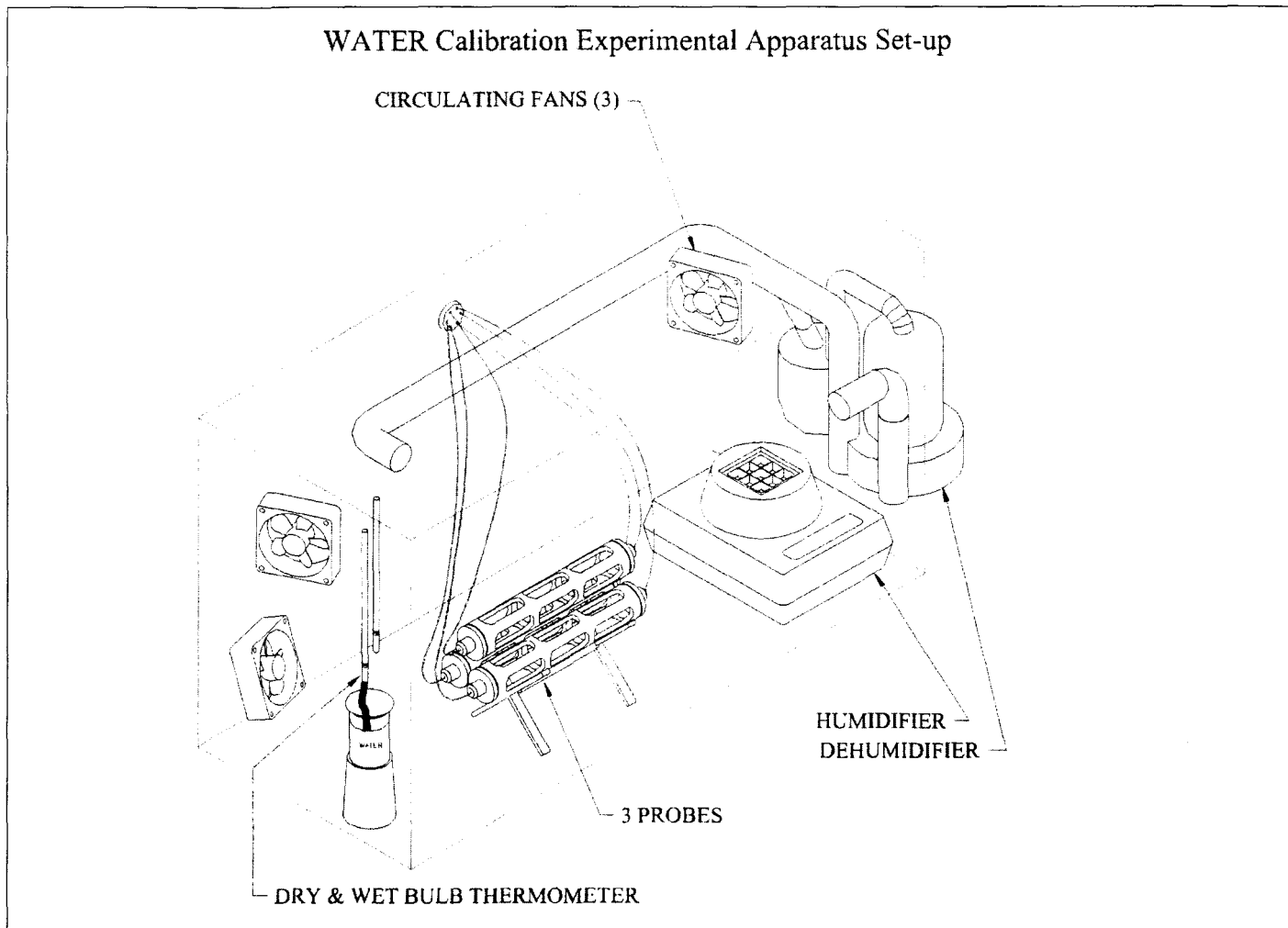


Figure 5. Water vapor calibration experimental apparatus set-up.

mode at 15:45. Finally, at 15:54, the intake piping to the blower was removed, permitting ambient room air to enter the enclosure. All data collection was stopped at the same time so that the last data point in each file would serve as a final time marker.

Data Analysis

The wet bulb/dry bulb temperature measurements were converted to relative humidity using RH charts from the CRC Handbook of Chemistry and Physics. Absolute humidity was computed using the saturated partial pressure of water tables and the ideal gas law. The data were corrected for the dry bulb temperature, but were not corrected for atmospheric pressure (standard pressure, 14.7 psi, 101325 Pa was used). To compare the hygrometry results with the H₂O₂ Vapor Monitor results, it was necessary to produce a common time axis. Using the initial time synchronization and the last datum received, it was possible to re-scale the H₂O₂ Vapor Monitor times to match the hygrometry times. This time synchronization had a ± 20 second uncertainty and combined to give a ± 20 second uncertainty.

Absorption Coefficient at 1362 nm

Since the optical path within the gas probe contained atmospheric water vapor at the time the H₂O₂ Vapor Monitors were referenced, the water vapor concentrations reported directly

by the H₂O₂ Vapor Monitors were relative to the absolute water vapor concentration which existed inside the enclosure at the time of reference. Therefore, the initial absolute water vapor concentration, as measured by hygrometry, was added to all of the H₂O₂ Vapor Monitor reported values. This offset did not affect the accuracy of the calibration since the water vapor

absorption coefficient is determined by the slope of the absorbance-concentration curve. Figure 6 compares these corrected H₂O₂ Vapor Monitor results with the hygrometry results.

To generate a parity plot comparing the H₂O₂

Vapor Monitor results with the hygrometry results, it was necessary to pair the individual hygrometry measurements with the closest (time-wise) H₂O₂ Vapor Monitor results. Figure 7 shows a least-squares fit in which the intercept was constrained to zero. The intercept was constrained to zero because of the adjustment made to the H₂O₂ Vapor Monitor reported values as described in the previous paragraph. The slope of this parity line was 1.002 with a ± 0.003 uncertainty (standard error of the slope). From the 0.003 uncertainty, it is clear that the slope is not significantly different from unity. Therefore, the water vapor absorption coefficient currently in use by the H₂O₂ Vapor Monitors [$3.61 \times 10^{-5} \pm 1.1 \times 10^{-7}$ AU/cm/(mg/L) at 1362 nm wavelength and 6 nm bandwidth] was found to be accurate and no change was warranted. This coefficient is valid over the range of temperatures (22-34°C) and concentrations (5-25 mg/

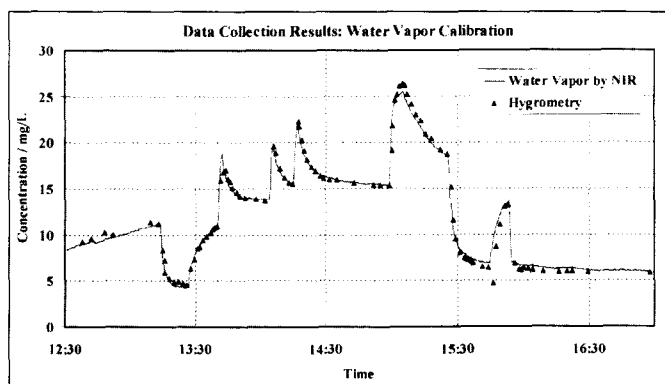


Figure 6. Water vapor calibration.

L) encountered in this experiment. The standard error of regression (or accuracy of the instrument) over this range of values was 0.83 mg/L. This calculated accuracy was limited by the actual accuracy of the hygrometry measurement, and not by the spectroscopy.

Ratio of the Absorption Coefficients at 1362 and 1422 nm

Water vapor has significant absorption at 1422 nm, where VPHP concentration is measured - Figure 1. Since the shape of the water vapor spectrum is relatively constant and VPHP does not absorb at 1362 nm, the absorbance at 1362 nm can be used to estimate the absorbance due to water vapor at 1422 nm. To determine the water vapor contribution at 1422 nm when VPHP is present, the absorbance at 1362 nm is divided by the ratio of the water vapor absorption coefficients at these two wavelengths. This ratio is the slope of the line relating absorbance at 1422 nm to absorbance at 1362 nm.

Figure 8 shows this relationship for H₂O₂ Vapor Monitor serial number 1003 using the data collected during the water vapor calibration. Note that the absorbance ratio is linear over the measured range. For clarity, only one H₂O₂ Vapor Monitor data set is shown. The linear relationships for the other two H₂O₂ Vapor Monitors were similar. For the three instruments employed (1002, 1003, and 1006), the reciprocal slopes, as determined by least-squares regression, are respectively 7.84, 8.02, and 7.16 with a mean of 7.67 and an estimated standard deviation (uncertainty) of 10.45. This result was significantly different than the 8.39 which was determined during earlier experiments and was currently in use by the H₂O₂ Vapor Monitor test units.

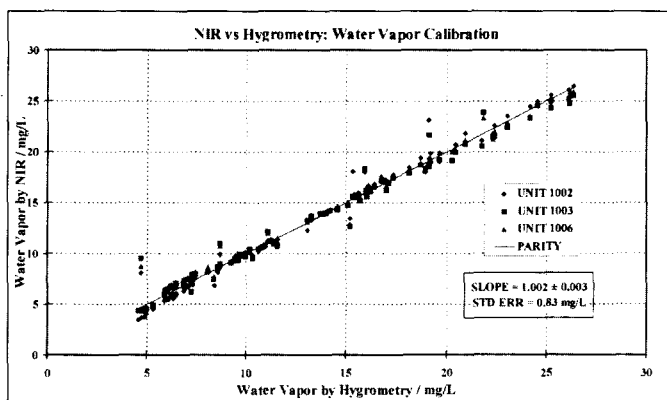


Figure 7. NIR vs. Hygrometry: Water vapor calibration.

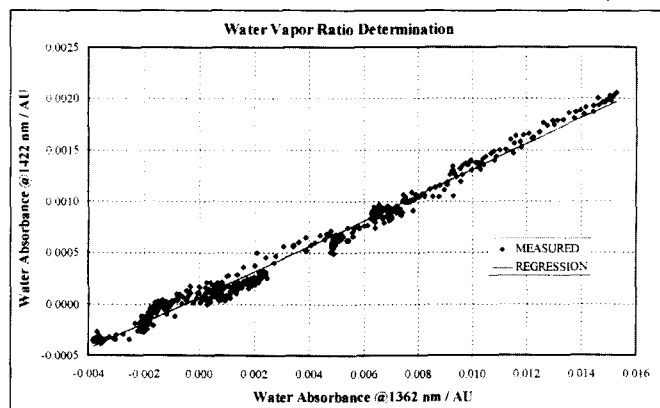


Figure 8. Water vapor ratio determination.

The uncertainty in this measurement was primarily due to the low water vapor absorbance at 1422 nm. The maximum observed absorbance was 0.0025 AU; which represented a transmission of 99.42%, or only a 0.58% drop in the amount of light transmitted through the 25 cm gas probe. Uncertainty in the water vapor ratio represents a second order error in the VPHP measurement. An uncertainty (one standard deviation) of ± 0.45 in the ratio represents less than a ± 0.02 mg/L error in the calculated VPHP concentration.

Conclusions

The foregoing paragraphs described a method to calibrate a NIR spectrometer for VPHP and water vapor concentrations. It is a simple and direct method which shows linearity and reproducibility. Due to the physics of spectroscopy and the instrument's design, direct calibration needs only to be performed once. A built-in validation method will shutdown the instrument if spectral tolerances, similar to those achieved by the three test units used in these calibration studies, are not met.

The calibration method employed here demonstrated that the VPHP and water vapor absorption (extinction) coefficients already in use by the H₂O₂ Vapor Monitors are valid. However, a change in another important calibration parameter (the water vapor peak ratio) was warranted. Although a minimal error in the reported VPHP concentrations would have resulted by not using this new value during the VPHP calibration studies, the statistical validity of the VPHP absorption coefficient determined earlier was not affected. This new water vapor peak ratio has been incorporated into all H₂O₂ Vapor Monitors.

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