

Gas Chromatographic Determination of the Composition of Expired Air and Atmospheric Air Mixtures

Jonathan Lam and Paul Cucchiara

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ABSTRACT

Gas chromatography is a widely used analytical chemistry method for determining the composition of vaporizable mixtures, such as to determine the concentration of carbon dioxide in the air to study climate change, or to carefully measure the composition of pharmaceutical drugs. The method was used to determine the molar fractions of four major gases— carbon dioxide, CO_2 (g), oxygen, O_2 (g), nitrogen, N_2 (g), and water vapor, H_2O (g)— in samples of laboratory air and exhaled (expired) breath. In general, the standard deviations of the calculated mole fractions for different gas components were low, but the accuracy of the lab compared to literature values was variable: the percent error for some components of the analysis of atmospheric air was as high as 4900% for one component, while the maximum percent error for the exhaled breath sample was 7.6%. However, there were large potential errors, such as poor injection technique, that may have been the reason for the poor accuracy. If the gross errors could be corrected, this method could be used to better understand the composition of ambient air and of the human respiratory system.

EXPERIMENTAL METHODS

A Gow-Mac 400 gas chromatograph was used to generate traces for three different gas mixtures: a standard gas mixture (with known molar concentrations) and two solutions with an unknown composition: laboratory air and exhaled breath (of Prof. Topper). Because the area under the peak in a trace is proportional to the number of moles of the gas corresponding to the peak, the traces were used to determine the number of moles, and subsequently the mole fractions, of the component gases of the two mixtures with unknown concentration. The nitrogen, oxygen, and carbon dioxide peaks of the traces were analyzed. The traces of the unknown mixture were used to generate calibration curves of area versus calculated number of moles. These calibration curves were used to determine the number of moles of the three gases. A separate calculation was performed to estimate the amount of water vapor present in the samples, because the gas chromatograph setup could not detect it, and was factored into the final mole fraction calculation. The procedure followed was based on the procedure stated in “Gas Chromatographic Determination Of The Composition of Vapor Mixtures,” from *The Official Cooper Union General Chemistry Laboratory Guide* (1). The discussion behind the methods used are explained in more detail in the discussion section.

Rather than drawing standard gas samples from a polyurethane bag, they were obtained by injecting a syringe into a specialized pressurized container that automatically filled the syringe. Before the injection of any sample, the syringe was purged by partially filling and emptying with the gas sample. After the syringes were purged, they were filled slightly past the injection volume and emptied to the correct volume just before injection, in order to prevent contamination. The injection volumes used for the standard sample calibration curves were 3mL, 3mL, 4mL, and 5mL, and the injection volumes for the other samples were 4mL. Four samples of the standard solution were tested for the calibration curve; five samples of exhaled breath were tested; and four samples (three ordinary, and one spiked with carbon dioxide) of laboratory air were tested. The “spiked” laboratory air was an exercise not included in the official laboratory guide; it involved taking a sample of laboratory air very close to a sublimating chunk of solid carbon dioxide, giving a trace similar to that of laboratory air with a prominent peak for carbon dioxide. This provides a qualitative retention time for carbon dioxide, because a carbon dioxide peak would be otherwise unnoticeable in a trace of laboratory air.

During injection, most often the syringe was not pressed down as firmly and quickly as would be optimal. Several issues arose from this: first, occasionally the pressure of the gas from inside the injection port pushed back on the syringe and caused it to slightly refill. The speed of the injection may not have been entirely consistent, which may have caused broadening of the peaks— this gives an explanation of why there was some overlap between the peak for the initial rush of air and the peak for carbon dioxide (see Figures 1, 5, and 6), which may lead to gross

error during calculations of the area under the carbon dioxide peak. This slow injection may also have contributed to the very large initial peak

The flow rate for the first sample was slightly lower than the laboratory guide-recommended rate of 60-65mL/min, so it was increased to fit in that range. All of the following samples used the increased flow rate. This should not have any effect on the area under the peaks, but it does change the retention time of the gas. The retention time for the gases in this mixture should be ignored when used to identify gases by their respective retention times (see Table 3).

There was some random fluctuation up to 0.1mV on the readings on the standard gas traces. The source of this fluctuation was not found, but the fluctuation was not observed in the samples of mixtures of unknown composition.

For the third sample of the exhaled breath, the experimenter injecting the volume was switched. The goal was to inject the gas more quickly than the other experimenter to avoid the broadening of the peaks. However, the carbon dioxide peak for this was fairly smaller than the other peaks for the exhaled breath samples (see Table 5 and Figure 6 for the CO₂ area for sample 3).

RESULTS

The molar composition of the standard gas was identified from the standard (reference) gas sample can. The molar compositions can be found in Table 1.

Table 1. Compositions of Standard Gas

	CO ₂	CO*	CH ₄ *	O ₂	N ₂
Mole Fraction (%)	15	7	4.5	4	69.5

The laboratory and gas chromatograph conditions during the injections of the standard gas are listed in Table 2. The pressure, temperature, and relative humidity were measured with a digital sensor. The detector current was displayed on the gas chromatograph (on a digital display). The flow rate of the gas was determined with a bubble meter (see A.1.5. for calculation of flow rate).

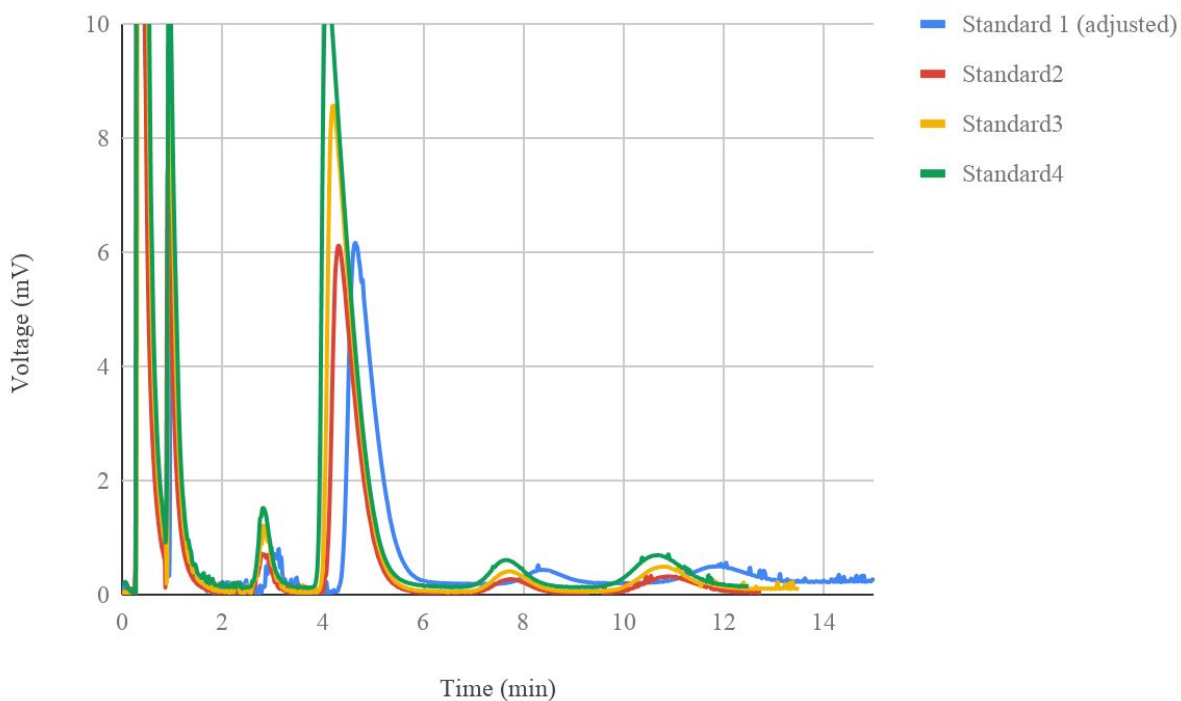
Table 2. Standard Gas Injection Conditions

	Ambient Pressure (in. Hg)	Ambient Temperature (K)	He Flow Rate (mL/min)	Detector Current (mA)	Relative Humidity (%)
Sample 1 (3mL)	29.88	297.65	57.6*	100.	29
Sample 2 (3mL)	29.88	297.65	60.6	100.	29
Sample 3 (4mL)	29.88	297.65	60.6	100.	29
Sample 4 (5mL)	29.88	298.15	60.6	100.	29

Traces of the standard samples can be found in Figure 1. Note that there are peaks for carbon monoxide and methane, but these are not analyzed in this experiment (this experiment focuses on carbon dioxide, oxygen, and nitrogen gases, as well as calculations for the amount of water vapor). The graph of the trace shows expected results, with consistent peaks throughout each sample: the first peak is the rush of air from the injection; the second peak is carbon dioxide; the third peak is oxygen; the fourth peak is nitrogen; and the last two peaks are carbon monoxide and methane. The peak of the 5mL injection is higher than the peaks for the 4mL injection, which are in turn higher than the peaks for the 3mL injections, as expected.

Except for sample 1, the peaks all roughly line up. (Sample 1 had a slightly slower flow rate; see experimental methods). However, because the identity of the gases is still apparent from the trace, its data is still used in the calibration curves.

Figure 1. Standard Gas Samples Trace



To generate a calibration curve, two pieces of data about each component gas were calculated: the area under the peak of the component gas, and the number of moles of the component gas. The area under the peaks of standard gas may be determined using the trapezoidal rule (calculation outlined in A.1.2). The moles of each component gas were calculated using the mole fraction of the gas in the standard gas mixture and the ideal gas law (see calculation in A.1.3).

The retention time of each component is calculated by subtracting the time of the start of the of the peak caused by the initial rush of air from the time of the start of the component's peak. See A.1.5. for the corresponding equation.

A summary of the areas, moles, and retention times of each peak for each standard gas sample are summarized in Table 3.

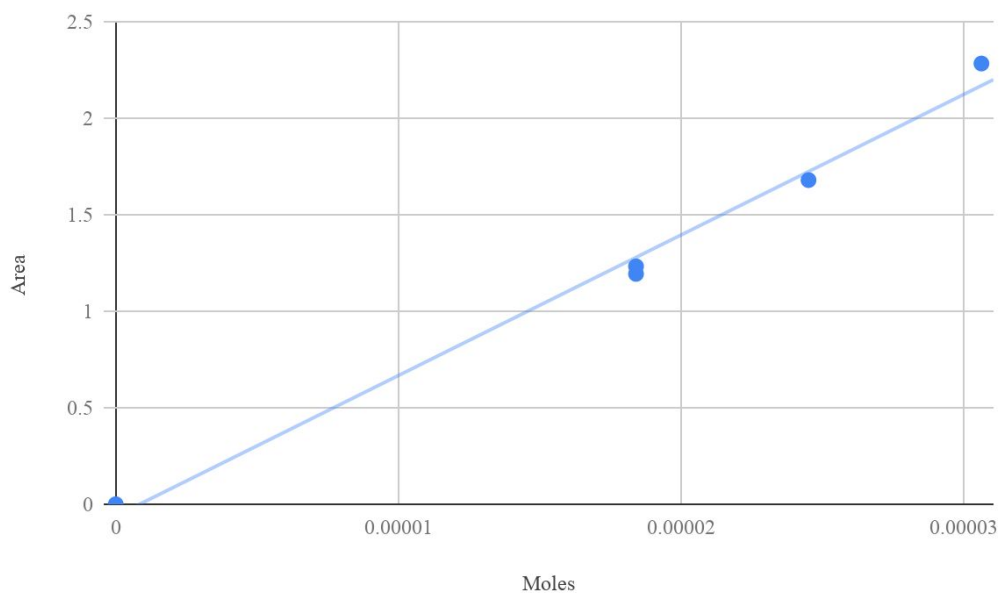
Table 3. Areas and Calculated Molar Compositions of Standard Gas Injection Samples

	CO ₂	O ₂	N ₂	Total
Sample 1 (3mL)				
Moles	0.0000184	0.00000491	0.0000853	0.000123
Volume (L)				0.0030
Retention Time (min)*	0.722	2.425	4.100	
Area of Peak	1.19	0.236	3.94	
Sample 2 (3mL)				
Moles	0.0000184	0.00000491	0.0000853	0.000123
Volume (L)				0.0030
Retention Time (min)	0.625	2.375	3.700	
Area of Peak	1.23	0.244	3.49	
Sample 3 (4mL)				
Moles	0.0000245	0.00000654	0.000114	0.000164
Volume (L)				0.0040
Retention Time (min)	0.625	2.375	3.650	
Area of Peak	1.68	0.367	5.16	
Sample 4 (5mL)				
Moles	0.0000306	0.00000816	0.000142	0.000204
Volume (L)				0.0050
Retention Time (min)	0.625	2.375	3.600	
Area of Peak	2.28	0.501	6.95	

* The retention time for this sample is not helpful because of the different flow rate for this sample— see the Experimental Methods for a brief discussion on this.

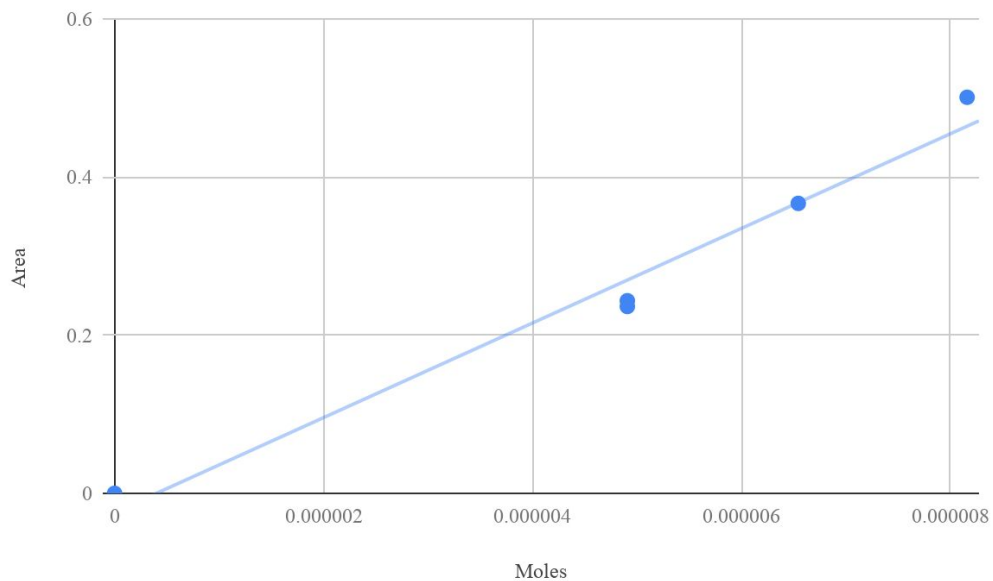
A plot of the area under the component peak as a function of number of moles of the component gives the calibration curve for a component. Figures 2, 3, and 4 show the calibration curves for CO₂, O₂, and N₂, respectively. The best fit lines and R² values are also shown. (Note that the point (0, 0) was included as a valid point on the curve; see the Discussion.)

Figure 2. Standard Gas CO₂ Calibration Curve



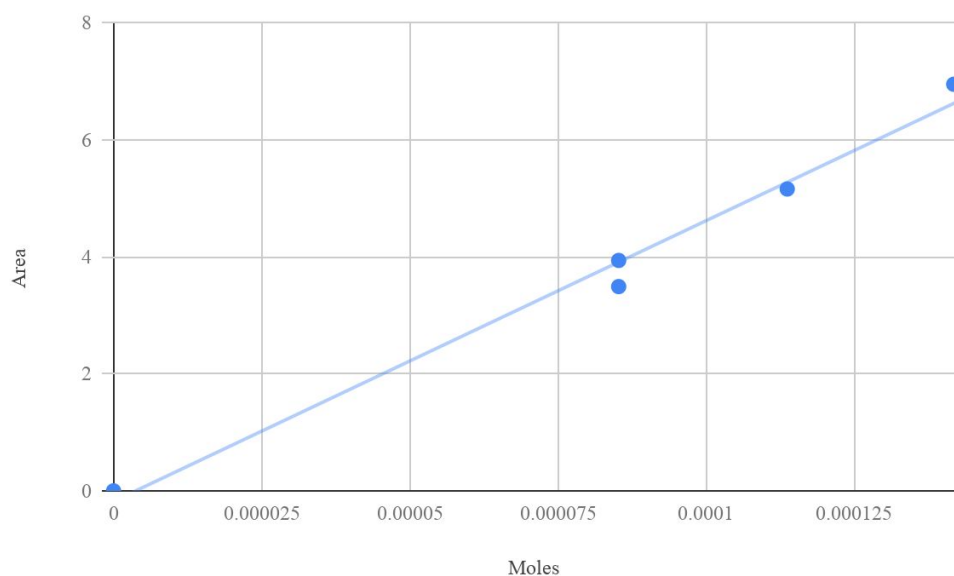
Best-Fit Line Equation: $A = 72876n - 0.0614$; $R^2: 0.99$

Figure 3. Standard Gas O₂ Calibration Curve



Best-Fit Line Equation: $A = 59861n - 0.0239$; $R^2: 0.973$

Figure 4. Standard Gas N₂ Calibration Curve



Best-Fit Line Equation: $A = 47939n - 0.178$; $R^2: 0.987$

Because the coefficient of determination of each calibration curve is fairly high (all are greater than 0.973), and the y-intercept of each graph is close to zero (y-intercepts lie between -0.178 and 0), the calibration curves are likely fairly reliable. The best-fit lines for these calibration curves will be inverted (see A.1.3) for use in calculating the number of moles of a component gas in a sample from the area under a peak on its trace.

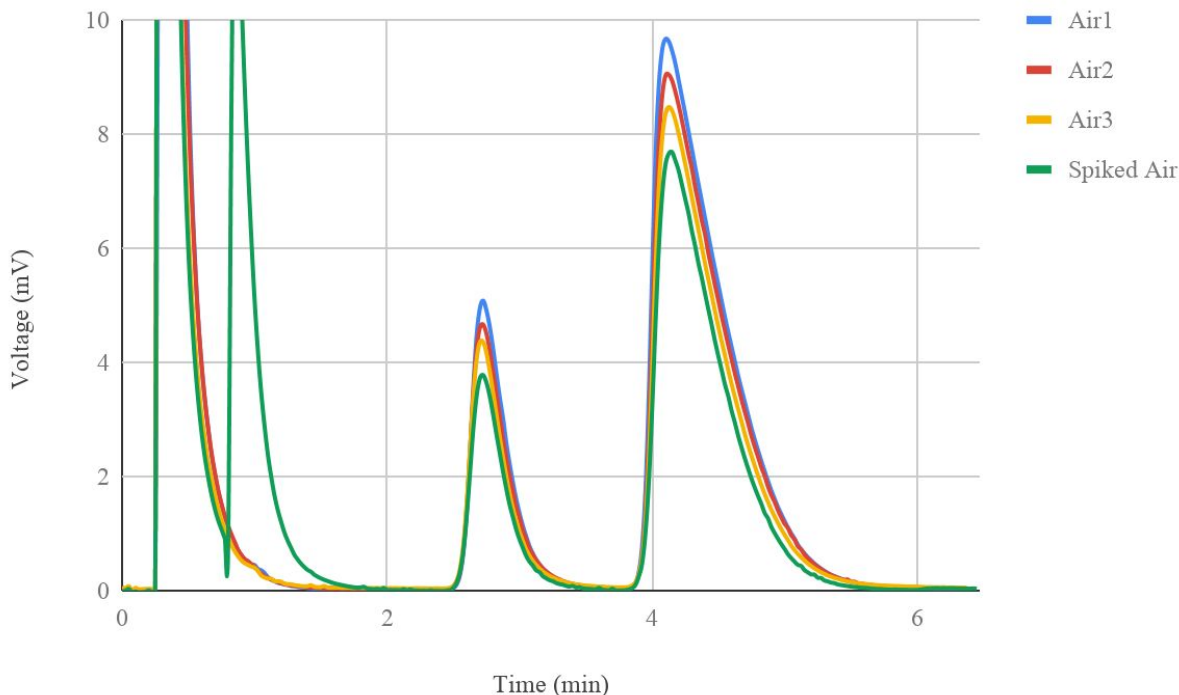
The laboratory conditions while taking the exhaled breath and laboratory air samples are displayed in Table 4. There was no change in any of the conditions between any of the sample trials, so they are consolidated into one table.

Table 4. Conditions for Exhaled Breath and Laboratory Air Samples

Injection Volume (L)	0.0040
He Flow Rate (mL/min)	60.6
Pressure (in. Hg)	29.88
Temperature (K)	298.15
Relative Humidity (%)	29

A trace of the laboratory air samples is shown in Figure 5.

Figure 5. Laboratory Air Samples Trace



The retention time of each component was calculated the same way as for the standard samples in Table 3. Peak area was found using the trapezoidal rule (see A.1.2.). The number of moles of gas were found using the inverse of the best-fit line of the corresponding component calibration curve (see A.1.3.). The mole fraction is calculated by dividing the number of moles by the total number of moles in the gas (see A.1.4.); note that this includes the calculated moles of water. The number of moles of water were calculated in A.1.1.

The sample with the spiked laboratory air was used to determine the retention time of CO_2 , but its data was not used to calculate the component mole fractions.

The average retention times, peak area, and moles for each component time are displayed in Table 5. Also displayed are the calculated mole fraction of each component for every sample. The mole fraction takes into account the number of moles of water.

Table 5. Retention Time and Composition of Laboratory Air

		CO ₂	O ₂	N ₂	H ₂ O
Mean retention time (min.)		0.600	2.250	3.625	
Mean peak area		0.187	1.48	5.54	
Mean moles		0.00000253	0.0000251	0.000119	0.00000148
Mole Fraction	Sample 1	0.00537	0.171	0.814	
	Sample 2	0.0228	0.168	0.800	
	Sample 3	0.0242	0.168	0.797	
Mean Mole Fraction		0.0171	0.169	0.804	0.00997

Figure 6 shows the traces of the exhaled breath samples, similar to Table 5. Table 6 shows the retention time and composition of exhaled breath, similar to Table 5.

Figure 6. Exhaled Breath Samples Trace

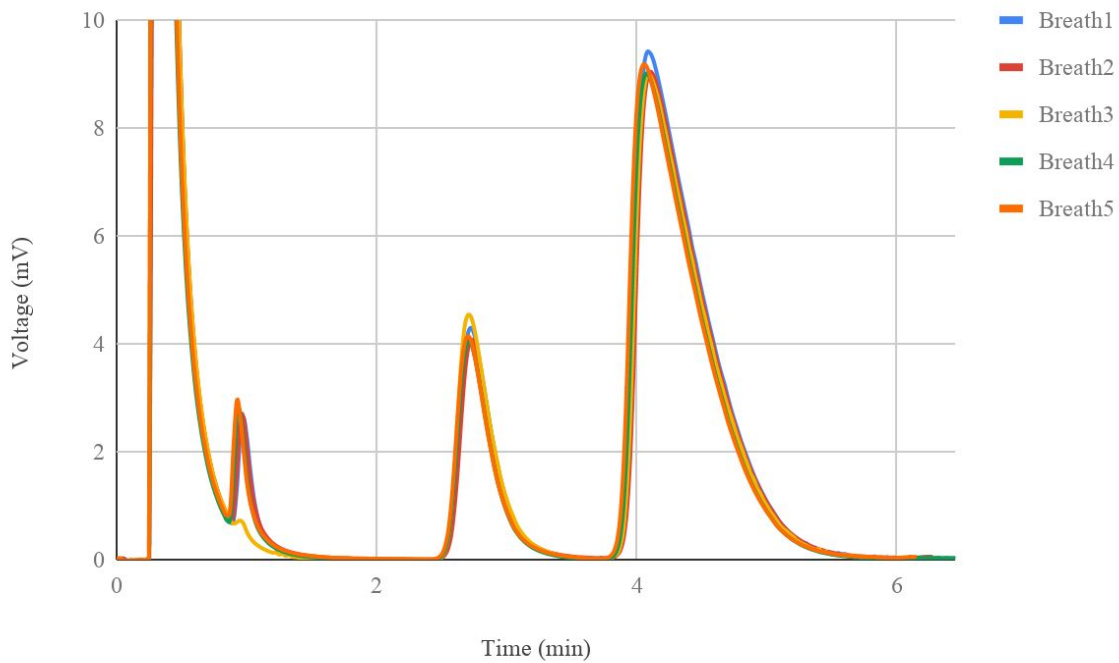


Table 6. Retention Time and Composition of Exhaled Breath

		CO ₂	O ₂	N ₂	H ₂ O
Mean retention time (min.)		0.600	2.250	3.550	
Mean peak area		0.370	1.304	5.524	
Mean moles		0.00000592	0.0000222	0.000119	0.0000101
Mole Fraction	Sample 1	0.0405	0.138	0.759	
	Sample 2	0.0432	0.138	0.755	
	Sample 3	0.0190	0.157	0.759	
	Sample 4	0.0423	0.137	0.755	
	Sample 5	0.0432	0.138	0.755	
Mean Mole Fraction		0.0377	0.141	0.757	0.0642

Table 7 compares the calculated mole fractions with literature mole fractions. The literature mole fractions and references are stated in Appendix 3.

Table 7. Comparison of Calculated Mole Fractions to Literature Values

	Laboratory Air		Exhaled Breath	
	Calculated Mole Fraction	Literature Mole Fraction	Calculated Mole Fraction	Literature Mole Fraction
CO ₂	0.0171	0.0003	0.0377	0.036
O ₂	0.169	0.2085	0.141	0.153
N ₂	0.804	0.7862	0.757	0.749
H ₂ O	0.00997	0.0005	0.064	0.062

DISCUSSION

Much of the insight on the discussion of the experimental method was based off of content from *Fundamentals of Analytical Chemistry* (2).

The studied gases were nitrogen gas, oxygen gas, carbon dioxide gas, and water vapor. While significant amounts of methane and carbon monoxide gas were present in the standard gas mixture, these are ignored in the calculations. Because these four gases comprise more than 99% of air (see Appendix 3), the mole fraction of each component gas in the entire sample of gas is estimated to be its mole fraction out of the total number of moles of these four gases.

The expectation is that the compositions of the two mixtures of unknown composition (exhaled breath and laboratory air) should have similar concentrations of nitrogen gas, because it is not involved in metabolic processes. However, since oxygen gas is used up in respiration, and because water vapor and carbon dioxide are formed, it is expected that the concentration of water vapor and carbon dioxide will be higher and that the concentration of oxygen will be lower in the exhaled breath sample than in the atmospheric air sample. (If the concentrations of other gases, such as methane or carbon monoxide, were studied, the result would be similar to nitrogen because they are also not involved in metabolic processes).

The specific gas chromatography column used does not detect water vapor. This will cause a systematic error to the results, because the mole fraction of water will be too low (if it is not detected, its mole fraction is 0%) and the mole fractions of the other gases will be too high (because the number of moles of water vapor are not considered in the total number of moles). In samples with a higher concentration of water vapor (i.e., the samples of exhaled breath), the error is more significant.

The correction is performed by determining the amount of water in the sample. The partial pressure of water can be found using the relative humidity of the sample (the relative humidity of the laboratory air was measured with a digital sensor; the relative humidity of the exhaled breath is estimated to be 100%) and the vapor pressure of water at the ambient temperature. The partial pressure of water vapor, volume of the injection, and ambient temperature can be used to calculate the number of moles of water vapor in the injection. These calculations are found in A.1.1. This amount of water can be added onto the calculated number of moles of the other gas components, and the relative humidity was calculated out of this corrected total number of moles of gas. This calculation is described in A.1.4.

In the calibration curves, an extra point at (0mol, 0mV) was added to increase the strength of the correlation. This point makes sense because an 0mol of gas should correspond with a 0mV reading.

In Tables 3, 5, and 6, the retention time of a component gas is calculated by subtracting the start of the component gas peak from the start of the initial gas peak. The start of the peak was chosen because that is when the component gas begins to reach the detector—the spread of the peak is dependent on how quickly the sample is injected.

In Tables 3, 5, and 6, the area under the peak is calculated using the trapezoidal rule (see A.1.2.). The units for the area are $mV \cdot min$. This unit is irrelevant because only the fact that the area is proportional to the number of moles of gas is important, and hence is not included in the tables.

As expressed in the experimental methods section, there was the potential for a large gross error due to poor injection technique, causing broad bands that had some overlap. The main overlap was between the peaks of the initial rush of air and the peak for the carbon dioxide gas. The calculated area of the CO₂ may therefore have contained some of the area of the peak of the initial air, making a systematically high value for the CO₂.

There were many assumptions made in the calculations. For example, the ideal gas law was used to calculate number of moles of gas from pressure, volume, and temperature measurements, but the behavior of real gases (especially H₂O) is not ideal. However, because of the small volumes, the correction factors using the Van der Waals equation are likely negligible compared to other errors caused by technique. This may cause some random error compared to the literature values.

Another assumption made was that laboratory air is very close to the literature atmospheric air, and that Prof. Topper's expired breath is close to the literature expired air compositions. It is unknown in which conditions the literature values were obtained, and it is unknown how the composition in the laboratory and Prof. Topper's breath compares to the average atmospheric and breath values. It is only known that Prof. Topper attempted to augment the CO₂ levels in his breath's sample by breathing in and out multiple times quickly before creating the sample. This may be the cause of some random error in contrast to the literature values.

Another potential error is that the calculated amount of water vapor in the air was calculating using the vapor pressure of water at body temperature (310.15K), but the sample had cooled down to the temperature of the surrounding air (298.15K) by the time the sample was used. Some of the water may have condensed, leaving in the gas mixture a lower amount of water vapor than calculated, causing a systematically high estimate.

A major error is that there was overlap between the first peak (of the initial air rush) and the second peak (for carbon dioxide). This means that the calculated area for carbon dioxide is systematically high, because some of that area is from gas in the first peak. While this was not a major problem in the standard gas and in the breath sample, where the concentrations of CO₂ are relatively large compared to the overlap from the first peak, there was a massive error in the calculated carbon dioxide mole fraction in the sample of atmospheric air. In this sample, the atmospheric air has very little carbon dioxide (0.03% as opposed to 15% in the standard sample), so a tiny overlap has a very large relative effect on the calculation of the carbon dioxide peak.

The errors, ranked from most to least significant, are the overlap between peaks, bad injection technique, assuming the ideal behavior of gases (i.e., using the ideal gas law), the calculation of water the fluctuation in the detector voltage output and other laboratory equipment precision errors.

Because there was a long sequence of dependent calculations, it is possible that there was a propagation of uncertainty from early calculations to the final calculated mole fraction. Any error in the calibration curve would have resulted in error in the calculation of the number of moles of a component gas, and therefore the overall mole fraction. An error in the calculation of the water pressure and number of moles of water would have affected the mole fraction. There is also the propagation of uncertainty from the small fluctuations inherent to the gas chromatograph (see Figure 1). However, there is nothing to suggest that any of these error calculations were systematic and would cause an overall systematic bias: the calibration curves had a high coefficient of determination and y-intercepts with small magnitudes, as expected, and there was no pattern in the fluctuations of the gas chromatograph reading. Because the general variance in the data were small (see Table 8), it is likely that any propagation of error was not very significant.

The calculated percentages of error (see Table 8) indicate that the data taken for the ambient air were much more accurate than those for the laboratory air. For the ambient air, all of the percent errors were under 10%. The laboratory had percent errors that far exceeded 10%, with a maximum of 4900% for carbon dioxide because of the overlap mentioned above. These large values are probably a result of the previously mentioned gross errors affecting these sample collections, creating significantly greater variances among the collected data.

CONCLUSIONS

The experimentally determined mole fractions for the mole fractions of gases had a distribution more similar to the literature values for the exhaled breath samples than for the laboratory air samples. The 90% confidence intervals for the exhaled breath was determined to be 0.037 ± 0.009 for carbon dioxide, 0.141 ± 0.008 for oxygen, 0.757 ± 0.002 for nitrogen, and 0.064 ± 0.001 for water. The confidence interval just barely miss (within ± 0.005) the literature values for all of the components, which are 0.036, 0.153, 0.749, and 0.062, respectively. The molar fraction values for the laboratory air was determined to be 0.02 ± 0.01 for carbon dioxide, 0.169 ± 0.003 for oxygen, 0.81 ± 0.01 for nitrogen, and 0.0097 ± 0.0005 for water. The carbon dioxide and water confidence intervals greatly fail to capture the literature values of 0.0003 and 0.0005, respectively, but the oxygen and nitrogen confidence intervals were close to (but still missed) the the literature values of 0.2085 and 0.7862, respectively. The wide range of accuracies indicates that this method is not entirely reliable; however, there were a number of potential gross errors which may have contributed to much of this error.

A potential improvement to the procedure is to encourage a slower flow rate, which will allow the gases more time to separate and reduce the overlap between different peaks. This may also counter some of the band broadening that may be caused when a sample is injected too slowly, such as that from some of the first samples in this report. The largest percent error for any mole fraction was for carbon dioxide in the air sample, where its peak was the smallest and the overlap from the initial air rush peak caused a massive error, so a method of reducing overlap would most likely have the greatest beneficial result on the accuracy of this method.

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Jonathan Lam was responsible for authoring the Experimental Methods, Results, Discussion, Acknowledgements, Appendix I, and Appendix II sections of this laboratory report, and served as editor for the sections written by Paul Cucchiara.

Paul Cucchiara was responsible for authoring the Abstract, Experimental Methods, Results, Conclusion, References, and Appendix III sections of this laboratory report, and served as editor for the sections written by Jonathan Lam.

Sun Hung Zhao was consulted for some of the considerations in the Discussion section.

The format of this laboratory report is based off of the “Laboratory Report” section of The Official Cooper Union General Chemistry Laboratory Guide, 19th edition, by Marcus Lay et al.

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- [2] Kimball, J. W. Kimball's Biology Pages. <http://www.biology-pages.info/> (accessed Dec 10, 2018).

APPENDIX I

CALCULATION METHODS AND REPRESENTATIVE CALCULATIONS

A.1.1. Correction for Water Vapor

The gas chromatograph column used is not able to detect water vapor. A correction is necessary to adjust the mole fractions of the gases to take into account the humidity of the sample.

Relative humidity is defined in Equation 1. In the sample of breath (at metabolic temperature), the relative humidity is roughly 100%. The relative humidity of the laboratory air was measured using a digital sensor.

Equation 1. $RH (\%) = \frac{P_{H_2O}}{P_{vap}} \times 100\%$

where P_{H_2O} is the partial pressure of water and P_{vap} is the vapor pressure of water at the sample's temperature.

The vapor pressure of water can be calculated from temperature. An estimate is given in Equation 2.

Equation 2. $P_{vap} = \exp(77.345 + 0.0057T - 7235T^{-1}) \div T^{8.2}$

Equation 1 can be rewritten in the following form to solve for the partial pressure of water if relative humidity and vapor pressure are known, which will be used to calculate the number of moles of water vapor in the air.

Equation 1a. $P_{H_2O} = \frac{RH}{100\%} \times P_{vap}$

The ideal gas law is used to estimate the number of moles of water in the sample using Equation 3.

Equation 3. (Ideal Gas Law) $n_{H_2O} = \frac{P_{H_2O}V}{RT}$

where V and T are the volume and temperature of the injection, respectively.

A representative calculation of number of moles of water is shown below, using Equations 1a, 2, and 3. The calculation is that of the number of moles of H₂O in the laboratory air. The laboratory temperature is 298.15K, the pressure is 101185Pa, and the (injection) volume is 4.0mL.

$$P_{vap} = \exp(77.345 + 0.0057(298.15K) - 7235(298.15K)^{-1}) \div (298.15K)^{8.2} = 3158Pa$$

$$P_{H_2O} = \frac{29\%}{100\%} \times 3158Pa = 916Pa$$

$$n_{H_2O} = \frac{(916Pa)(0.040L)}{(8314Pa \times L \times mol^{-1} \times K^{-1})(298.15K)} = 0.00000148mol$$

A.1.2. Calculation of Area Under Peak

The area under a peak on a chromatogram trace is proportional to the number of moles of the corresponding gas (the gas with the same retention time as the peak). Therefore, the number of moles may be calculated from the area if a known number of moles produces a known area. This calibration was carried out using the standard gas solution.

To calculate the area under a peak, an approximate upper and lower limit for the peak were chosen by observation of the graph to include as much of the peak without overlap with other peaks. (Overlap was only an issue with the CO₂ peak — see the Discussion.) Then, the trapezoidal rule (Equation 4) was performed to determine the area under one peak.

Equation 4 (Trapezoidal Rule).
$$A = \sum_{k=a}^{b-1} \left(\frac{1}{2}(V_k + V_{k+1})\Delta t \right)$$

where a is the lower limit for retention time, b is the upper limit for retention time, V_n is the detector voltage at the n retention time, and Δt is the interval between two consecutive detector readings.

A representative calculation for the CO₂ peak of the exhale sample 1 is illustrated below.

$$A = \sum_{k=36}^{54} \left(\frac{1}{2}(V_k + V_{k+1})(0.025min) \right) = 0.0178875 + 0.0261375 + \dots + 0.0024875 = 0.418025$$

A.1.3. Standard Gas Calibration Curve and Mole Calculation

The number of moles of each gas in the standard gas samples were estimated from the injection volumes of the samples using the ideal gas law (similar to equation 3) and the mole fractions of each gas.

Equation 5:
$$n_{gas} = \chi_{gas} \times \frac{PV}{RT}$$

where n_{gas} is the number of moles of one gas, χ_{gas} is the mole fraction of the gas, and P , V , and T are the pressure, (injection) volume, and temperature, respectively, of the sample.

The area under the peak of the each gas versus the number of moles of the corresponding gas (calculated from Equation 5) for each major gas (CO_2 , O_2 , and N_2) are plotted as a calibration curve. For each gas, a least-squares linear regression is performed (using the Google Sheets spreadsheet program) to produce a function mapping areas to moles.

The function is then inverted to create a function mapping moles to areas. This inverse function is used to calculate the number of moles of the corresponding gas in a sample given its area (see A.1.2.)

A representative calculation is shown below for the number of moles of carbon dioxide, using the inverse best-fit calibration curve for carbon dioxide.

Calibration curve: $A = 72876n - 0.0614$

Inverse calibration curve: $n_{\text{gas}} = \frac{A+0.0614}{72876}$

$$n_{\text{CO}_2} = \frac{0.418+0.0614}{72876} = 0.00000658 \text{ mol}$$

A.1.4. Mole Fraction Calculation with Correction

The mole fraction of the solution is the ratio of the number of moles of one gas to the total number of gases. The total number of moles includes the calculated number of moles of water vapor (see A.1.2.) using Equation 6.

Equation 6. $\chi_{\text{gas}} = \frac{n_{\text{gas}}}{n_{\text{total}}}$

A representative calculation is shown below for the mole fraction of carbon dioxide in the first breath sample.

$$\chi_{\text{gas}} = \frac{0.000162}{0.00000658} = 0.0405$$

A.1.5. Miscellaneous Calculations

The bubble meter was used to determine flow rate. The flow rate is the quotient of the volume of air by the amount of time it takes for a bubble to travel through that volume.

Equation 7. $FR = \frac{V}{t}$

The retention time of a component gas is the difference between the beginning of the component gas's peak and the beginning of the peak of the initial gas rush. An explanation of this reasoning is described in the Discussion.

Equation 8. $RT = (\text{time of start of component gas peak}) - (\text{time of start of initial gas peak})$

APPENDIX II COMPUTATION OF STATISTICAL MEASURES OF PRECISION

Statistical measures were calculated for each of the calculated mole fractions of a component gas for each of the two gas mixtures with unknown compositions. The calculations for the statistical measures for the CO₂ gas in exhaled air are shown below as a representative sample.

The calculations for the mean (\bar{x}), sample standard deviation (s), standard error (s_m), variance (s^2), and relative standard deviation of the calculated mole fraction values are shown below.

Because the data points are roughly spread uniformly over a small range and not heavily skewed, the mean will be used to represent the center and the standard deviation will be used to estimate the range (as opposed to the median and IQR). The data analyzed here is from Table 6.

$$n = 3$$

$$\bar{x} = \frac{1}{n} \sum_{k=1}^n (\text{calculated } \chi)_k = 0.0376$$

$$s = \sqrt{\frac{1}{n-1} \sum_{k=1}^n ((\text{calculated } \chi)_k - (\text{mean } \chi))^2} = 0.0105$$

$$s_m = \frac{s}{\sqrt{n}} = 0.00468$$

$$s^2 = 0.000110$$

$$\text{rel. std. dev. (ppt)} = 1000 \times \frac{s}{\bar{x}} = 278$$

The calculation of a 90% confidence interval of the mole fractions is shown below.

$$90\% \text{ confidence level } t_{n-1} = t_2 = 2.92$$

$$\text{uncertainty (u)} = t_{n-1} \times \frac{s}{\sqrt{n}} = 0.00999$$

$$90\% \text{ confidence interval} = 0.04 \pm 0.01$$

The percent error is calculated using the literature value from Table 8 (Appendix III).

$$\% \text{ Error} = \frac{|0.0376 - 0.036|}{0.036} \times 100\% = 4.5\%$$

The intermediate calculation results are displayed rounded, but all calculations are all only rounded at the end.

A summary of all of the statistical measures, for the mole fractions of the three gases, for the two different gas mixture samples, is displayed in Table 8.

Table 8. Summary of Statistical Values for Mole Fraction Calculations

	Atmospheric Air			
	CO ₂	O ₂	N ₂	H ₂ O
<i>n</i>	3	3	3	3
\bar{x}	0.0151	0.169	0.806	0.00974
<i>s</i>	0.00889	0.00180	0.00736	0.000290
<i>s_m</i>	0.00513	0.00104	0.00425	0.000167
<i>s</i> ²	0.0000791	0.00000322	0.0000541	0.0000000838
<i>rel. std. dev. (ppt)</i>	589	10.597	9.129	29.73
<i>90% confidence interval</i>	0.02 ± 0.01	0.169 ± 0.003	0.81 ± 0.01	0.0097 ± 0.0005
<i>% Error</i>	4900%	19%	2.5%	1800%
	Exhaled			
	CO ₂	O ₂	N ₂	H ₂ O
<i>n</i>	5	5	5	5
\bar{x}	0.0376	0.141	0.757	0.0643
<i>s</i>	0.0105	0.00850	0.00226	0.00137
<i>s_m</i>	0.00468	0.00380	0.00101	0.000611
<i>s</i> ²	0.000110	0.0000722	0.00000511	0.00000187
<i>rel. std. dev. (ppt)</i>	278	60.1	2.99	21.3
<i>90% confidence interval</i>	0.037 ± 0.009	0.141 ± 0.008	0.757 ± 0.002	0.064 ± 0.001
<i>% Error</i>	4.5%	7.6%	1.0%	3.7%

APPENDIX III
GAS MIXTURE LITERATURE VALUES

In Table 9, a list of literature values for the percentage by volume of CO₂, O₂, and NO₂ are shown. The values were obtained from [2].

Table 9. Gas Mixture Literature Values

	Mole Fraction	
	Atmospheric Air	Expired Air
CO ₂	0.0003	0.036
O ₂	0.2085	0.153
N ₂	0.7862	0.749
H ₂ O	0.0005	0.062