Design and Implementation of a Laser-Based Ammonia Breath Sensor for Medical Applications

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ABSTRACT

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Laser-based sensors can be used as non-invasive monitoring tools to measure parts per billion (ppb) levels of trace gases. Ammonia sensors are useful for applications in environmental pollutant monitoring, atmospheric and combustion kinetic studies, and medical diagnostics. This sensor was specifically designed to measure ammonia in exhaled breath to be used as a medical diagnostic and monitoring tool, however, it can also be extended for use in other applications. Although ammonia is a naturally occurring species in exhaled breath, abnormally elevated levels can be an indication of adverse medical conditions. Laser-based breath diagnostics have many benefits since they are cost effective, non-invasive, painless, real time monitors. They have the potential to improve the quality of medical care by replacing currently used blood tests and providing immediate feedback to physicians.

This sensor utilizes a Quantum Cascade Laser and Wavelength Modulation Spectroscopy with second harmonic normalized by first harmonic detection in a 76 m multi-pass absorption cell to measure ppb levels of ammonia with improved sensitivity over previous sensors. Initial measurements to determine the ammonia absorption
This is the first experimental study of the ammonia absorption line transitions near 1103.46 cm$^{-1}$ with absorption spectroscopy. The linestrengths were measured with uncertainties less than 10%. The collisional broadening coefficients for each of the ammonia lines with nitrogen, oxygen, water vapor, and carbon dioxide were also measured, many of which had uncertainties less than 5%. The sensor was characterized to show a detectability limit of 10 ppb with an uncertainty of less than 5% at typical breath ammonia levels. Initial breath test results showed that some of the patients with chronic kidney disease had elevated ammonia levels while others had ammonia levels in the same range as expected for healthy patients. For all of the patients the breath ammonia level decreased during dialysis but the percent decrease varied considerably for each patient.

The sensor has demonstrated improved sensitivity and has been applied to measure ppb levels of ammonia in exhaled breath. Further tests have been designed to improve the sensor and continue to investigate the medical applications.
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Chapter 1

Introduction

1.1 Motivation

Ammonia is a naturally occurring species in exhaled breath; however, recent studies have shown that abnormally elevated levels of ammonia in exhaled breath can indicate adverse medical conditions [1 2 3]. The development of an accurate sensor for qualitative analysis of ammonia in exhaled breath would, therefore, be a valuable tool for diagnosing medical conditions as well as monitoring the progress and effectiveness of treatments [4]. Laser diagnostics enable scientists to study gases non-invasively while giving real-time results. New developments in quantum cascade lasers (QCL) enable laser-based sensors to be small and portable. Laser-based sensors have a variety of applications, one of which is the detection of parts per billion (ppb) levels of trace gases. The goal of this thesis is the design of a novel ammonia breath sensor based on laser diagnostics. The sensor can then be implemented to test potential medical applications by comparing ammonia levels in the exhaled breath of healthy patients and patients with clinically diagnosed medical conditions. The sensor will be employed to study the relationship between ammonia levels in exhaled breath to currently used blood tests in order to determine if this sensor would be a viable
alternative to the blood tests, offering greater ease of use and faster responses.

1.2 Organization of the Thesis

The purpose of this thesis is to develop a laser based sensor to measure ppb levels of ammonia in exhaled breath and implement it to study the relationship between breath ammonia levels and blood indicators currently used to diagnose and monitor chronic kidney disease (CKD) with potential applications for other medical conditions.

Chapter 2 describes the theory of direct absorption spectroscopy (DAS) as well as wavelength modulation spectroscopy (WMS). DAS will be used to study the selected ammonia absorption lines while WMS will be used in the final sensor design. This theory lays the foundation for the experimental work and describes parameters that must be known in order to implement sensors based on laser spectroscopy.

Chapter 3 describes the process to determine the characteristics of the sensor. Previous research aimed at designing similar laser-based ammonia breath sensors is first discussed. The rest of the chapter explains the simulations that were used to choose the ammonia line transitions to measure, the pressure the measurements would be taken at, the required path length to achieve the required sensitivity, and the strategy to be employed to improve the accuracy of the sensor.

The preliminary measurements used to determine the necessary ammonia line parameters for the sensor are described in detail in Chapter 4. The practical implementation of direct absorption spectroscopy to determine line parameters is explained. The unique method required to measure the selected lines, due to the adsorption of ammonia, is described in detail. The results for the values and uncertainties of the required line parameters are given as well.

The developed laser-based ammonia breath sensor is described in Chapter 5. A detailed explanation of the practical implementation of WMS is given which includes
the experimental determination of the laser parameters and the calculation of the mole fraction from the measured WMS signal. Sensitivity analysis is performed to determine the affect of uncertainties in the input parameters on the output measured value. The sensor is also characterized to determine the sensitivity and detectability limits.

Chapter 7 describes the medical applications of an ammonia breath sensor. Previous research on the medical significance of ammonia in breath is summarized. The methods for investigating the correlation between ammonia in breath and blood indicators used to diagnose and monitor CKD are also described. The initial results and analysis of these tests are then discussed. Chapter 8 summarizes this research and describes future work.
Chapter 2

Theory of Laser Spectroscopy

2.1 Theory of Direct Absorption Spectroscopy

Molecules absorb energy in discrete amounts corresponding to their possible energy levels. When light interacts with a medium the photons can either be reflected, absorbed, or transmitted. When a photon of light at a particular frequency, \( \nu \), with energy corresponding to the difference in two energy levels interacts with a molecule, the molecule absorbs this photon and is excited to a higher energy state as seen in Figure 2.1.

![Figure 2.1: Molecule undergoing energy transition by absorbing a quanta of light.](image)

A laser at a wavelength corresponding to this frequency/energy when passing through this gas will, therefore, have lower transmitted intensity than it would if the
gas were not present, as seen in Figure 2.2. This phenomenon is described by Beer’s Law,

$$\tau_\nu = \frac{I}{I_o} = \exp\left(-k_\nu L\right)$$

(2.1)

where the transmissivity, \( \tau_\nu \), is the fraction of laser intensity that is transmitted through the medium when the spectral absorption coefficient of the gas is \( k_\nu \) (cm\(^{-1}\)) and the path length of the laser through the absorbing gas is \( L \) (cm).

![Image of Figure 2.2: The transmitted intensity of the laser decreases at the wavelength where the molecule absorbs.](image)

The term in the exponential is also called the spectral absorbance, \( \alpha_\nu \), which is the fraction of incident light that is absorbed.

$$\alpha_\nu = k_\nu L = -\ln\left(\frac{I}{I_o}\right) = PX_iLS\phi_\nu$$

(2.2)

\( P \) (atm) is the pressure, \( X_i \) is the mole fraction of species \( i \), \( L \) (cm) is the path length, \( S \) (cm\(^{-2}/\)atm) is the linestrength, and \( \phi_\nu \) (cm) is the normalized lineshape.
function at frequency $\nu$. This is an important equation because, since $I$ and $I_0$ are measured experimentally, one of the terms on the right hand side of the equation can be determined if the others are known.

The linestrength is a fundamental parameter of a specific energy transition. It represents the strength of this transition based on the number of molecules in the lower energy state at a given temperature and the probability that the molecules will undergo the transition to the higher energy state. This parameter is typically determined experimentally. The lineshape function is included in the spectral absorbance equation because the energy transition does not occur at a finite frequency. Due to the Heisenberg Uncertainty Principle there is uncertainty in the energy levels between which the molecule is transitioning. This leads to the absorption being distributed over a range of frequencies. The lineshape function describes this distribution in relative terms since it is normalized to sum to one over all frequencies.

$$\phi \equiv \frac{k_\nu}{\int k_\nu d\nu}$$

$$\int \phi_\nu d\nu = 1$$

This broadening of the absorption line described by the lineshape function comes from three sources. Natural broadening based solely on the Heisenberg Uncertainty Principal is generally negligible when compared to the other two. Another source of broadening is called collisional or pressure broadening. As the pressure increases the increased number of collisions between molecules leads to an increase in the uncertainty of the energy states, which in turn leads to increased broadening. In this case
the lineshape function follows a Lorentzian shape.

\[ \phi_L(\nu) = \phi_L(\nu_o) \frac{1}{1 + 4 \left( \frac{\nu - \nu_o}{\Delta \nu_c} \right)^2} \]  

(2.4)

Where the lineshape at linecenter is

\[ \phi_L(\nu_o) = \frac{2}{\pi \Delta \nu_c} \]  

(2.5)

and the collisional linewidth, which is the full width at half maximum (FWHM) is

\[ \Delta \nu_c = P \sum_A X_A 2\gamma_{B-A} \]  

(2.6)

where \(2\gamma_{B-A}\) is the collisional broadening coefficient and is generally determined experimentally.

Another source of broadening, called Doppler broadening, comes from the random motion of the molecules. When a molecule has a velocity component in the same direction that the laser is propagating, the frequency of the absorption shifts. Doppler broadening results from many molecules with a velocity distribution, so the lineshape function follows a Gaussian shape.

\[ \phi_D(\nu) = \phi_D(\nu_o) \exp \left[ -4 \ln 2 \left( \frac{\nu - \nu_o}{\Delta \nu_D} \right)^2 \right] \]  

(2.7)

Where the lineshape at linecenter is

\[ \phi_D(\nu_o) = \frac{2}{\Delta \nu_D} \left( \frac{\ln 2}{\pi} \right)^{1/2} \]  

(2.8)
and the doppler linewidth (FWHM), is

\[ \Delta \nu_D = \nu_o (7.1623 \times 10^{-7}) \left( \frac{T}{M} \right)^{1/2} \]  

(2.9)

where \( T \) is the temperature and \( M \) is the molecular weight.

Collisional broadening increases with pressure while Doppler broadening increases with temperature, so in the low pressure, high temperature limit there is only Doppler broadening, while in the high pressure low temperature limit there is only collisional broadening. In most cases, neither is negligible so they must both be accounted for. One common method for combining the lineshapes is with the Voigt profile which is a convolution of the two.

\[ \phi_V(\nu) = \int_{-\infty}^{\infty} \phi_D(u)\phi_C(\nu - u)\,du \]  

(2.10)

Solutions are listed in tables and described by numerical approximations.

In this research, a laser-based sensor was developed to measure the mole fraction of one species in a mixture. As mentioned above, in order to determine the mole fraction the other parameters must also be known. These parameters can be determined with the same experiments given known mole fractions. The quantities of interest, as described above, are the linestrengths and the collisional broadening coefficients. These can each be determined independently by scanning the laser over an entire absorption lineshape. The linestrength can be determined by integrating to find the area under the curve, due to the definition of the lineshape function.

\[ \int \alpha_\nu d\nu = \int PX_iLS\phi_\nu d\nu = PX_iLS \int \phi_\nu d\nu = PX_iLS = Area \]  

(2.11)

The collisional broadening coefficient can be calculated from the collisional line width (FWHM) which is determined from the measured line shape using Equation 2.6.
2.2 Theory of Wavelength Modulation Spectroscopy

An improvement in the signal-to-noise ratio can be obtained through the use of wavelength modulation spectroscopy (WMS). In order to develop a calibration-free sensor, wavelength modulation spectroscopy with second harmonic normalized by first harmonic detection (WMS-2f/1f) will be used as described by Rieker [5].

The laser frequency is modulated with angular frequency $\omega_m$, by modulating the input current to the QCL, described by

$$\nu(t) = \nu + a \cos(\omega_m t)$$  \hspace{1cm} (2.12)

where $\nu$ (cm$^{-1}$) is the laser-center frequency and $a$ is the modulation depth. The intensity of the laser is then also modulated according to

$$I_o(t) = I_o [1 + i_o \cos(\omega_m t + \psi_1) + i_2 \cos(2\omega_m t + \psi_2)]$$  \hspace{1cm} (2.13)

where $I_o$ is the average laser intensity, $i_o$ is the normalized linear intensity modulation amplitude, $\psi_1$ is the linear phase shift, $i_2$ is the normalized non-linear intensity modulation amplitude, and $\psi_2$ is the non-linear phase shift.

By definition of the transmissivity, $\tau(\nu)$, the detected intensity is given by

$$I = \tau(\nu(t)) \cdot I_o$$  \hspace{1cm} (2.14)

Since the transmissivity is a function of the laser frequency and the laser frequency is being modulated sinusoidally, the transmissivity can be expanded in a fourier cosine series

$$\tau(\nu + a \cos(\omega_m t)) = \sum_{n=0}^{\infty} H_n(\nu, a) \cos(n\omega_m t)$$  \hspace{1cm} (2.15)
where
\[ H_0 = \frac{1}{2\pi} \int_{-\pi}^{\pi} \tau(\nu + a \cos \theta) d\theta \] (2.16)
\[ H_n = \frac{1}{\pi} \int_{-\pi}^{\pi} \tau(\nu + a \cos \theta) \cos(n\theta) d\theta \] (2.17)

The second harmonic signal is found by multiplying the detected signal by \( \cos(2\omega_m t) \) and \( \sin(2\omega_m t) \) to get the so called \( X \) and \( Y \) components, respectively, then calculating the magnitude.

\[ I_{2f} = \sqrt{X_{2f}^2 + Y_{2f}^2} = \sqrt{(\tau(\nu)I_o \cos(2\omega_m t))^2 + (\tau(\nu)I_o \sin(2\omega_m t))^2} \] (2.18)

The first harmonic signal is similarly found by multiplying by \( \cos(\omega_m t) \) and \( \sin(\omega_m t) \) instead.

Since the resulting signal will be filtered, only the non time varying terms are kept in the derivation of the magnitudes of the first and second harmonic signals. Useful trigonometric identities for the derivation include:

\[ \cos(\alpha) \cos(\beta) = \frac{\cos(\alpha - \beta) + \cos(\alpha + \beta)}{2} \] (2.19a)
\[ \cos(\alpha) \sin(\beta) = \frac{\sin(\alpha + \beta) - \sin(\alpha - \beta)}{2} \] (2.19b)
\[ \cos(-\alpha) = \cos(\alpha) \] (2.19c)
\[ \sin(-\alpha) = -\sin(\alpha) \] (2.19d)

The magnitude of the \( WMS 2f \) signal is

\[ R_{2f}(\tau) = \frac{G I_o}{2} \left\{ \left[ H_2 + \frac{i_o}{2} (H_1 + H_3) \cos \psi_1 + i_2 \left( H_0 + \frac{H_4}{2} \right) \cos \psi_2 \right]^2 \ight. \\
+ \left. \left[ \frac{i_o}{2} (H_1 - H_3) \sin \psi_1 + i_2 \left( H_0 - \frac{H_4}{2} \right) \sin \psi_2 \right]^2 \right\}^{1/2} \] (2.20)
where $G$ accounts for the opto-electrical gain of the system and losses due to scattering, beam steering, and window fouling. When there is no absorption, $H_o = 1$ and $H_n = 0$ so the background second harmonic signal, from the non-linear intensity modulation, is

$$R_{2f}^o(\nu) = \frac{GT_o i_2}{2}$$ (2.21)

The magnitude of the background subtracted second harmonic signal is then

$$S_{2f}(\nu) = \frac{GT_o}{2} \left\{ \left[ H_2 + i_o \left( H_1 + H_3 \right) \cos \psi_1 + i_2 \left( H_0 - 1 + \frac{H_4}{2} \right) \cos \psi_2 \right]^2 + \left[ i_o \left( H_1 - H_3 \right) \sin \psi_1 + i_2 \left( H_0 - 1 - \frac{H_4}{2} \right) \sin \psi_2 \right]^2 \right\}^{1/2}$$ (2.22)

The magnitude of the WMS $1f$ signal is

$$R_{1f}(\nu) = \frac{GT_o}{2} \left\{ \left[ H_1 + i_o \left( H_0 + \frac{H_2}{2} \right) \cos \psi_1 + i_2 \left( H_1 + H_3 \right) \cos \psi_2 \right]^2 + \left[ i_o \left( H_0 - \frac{H_2}{2} \right) \sin \psi_1 + i_2 \left( H_1 - H_3 \right) \sin \psi_2 \right]^2 \right\}^{1/2}$$ (2.23)

When there is no absorption, the background first harmonic signal, from the linear intensity modulation, is

$$R_{1f}^o(\nu) = \frac{GT_o i_o}{2}$$ (2.24)

The magnitude of the background subtracted first harmonic signal is then

$$S_{1f}(\nu) = \frac{GT_o}{2} \left\{ \left[ H_1 + i_o \left( H_0 - 1 + \frac{H_2}{2} \right) \cos \psi_1 + i_2 \left( H_1 + H_3 \right) \cos \psi_2 \right]^2 + \left[ i_o \left( H_0 - 1 - \frac{H_2}{2} \right) \sin \psi_1 + i_2 \left( H_1 + H_3 \right) \sin \psi_2 \right]^2 \right\}^{1/2}$$ (2.25)

The need for calibration to find $G$ is eliminated by normalizing the second har-
monic signal by the first harmonic signal giving

\[ C = \frac{S_{2f}}{S_{1f}} \]  

(2.26)

where \( C \) is a function of both laser parameters \((a, i_o, i_2, \psi_1, \) and \( \psi_2 \)) and gas parameters \((P, T, L, S, \phi, \) and \( X_i \)). The laser parameters can be determined before the measurements and therefore the sensor can be used to measure one of the gas parameters if the others are known. This strategy is called ”calibration free” because it allows for the measurement of concentration without the need to calibrate the signal to a known mixture as is necessary with traditional WMS [5].
Chapter 3

Ammonia Sensor Design

Laser based ammonia gas sensors have useful applications in many fields including combustion, atmospheric, and medical diagnostics. A review of the literature in the field shows that laser based ammonia sensors have been developed for a variety of applications using a variety of strategies [1, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17].

The sensor in this research has been designed with the medical application in mind to be used to study ammonia concentrations in exhaled breath. However, it can be extended for use in other applications. The application choice affects the design because it dictates the range of concentrations the sensor is attempting to measure as well as the other species present that may add interference. Ammonia concentrations in exhaled breath are on the order of hundreds of ppb in healthy patients to a few ppm in patients with related medical conditions. The main interfering species in exhaled breath include water vapor and carbon dioxide which each make up about 6%. These same species will be interfering species in both atmospheric and combustion studies as well, but at different relative concentrations. In order to apply this sensor for use in these other applications it would be necessary to study those mixtures and possibly adjust the measurement strategy.
3.1 Previous Research

The motivation for developing an ammonia sensor for medical diagnostics is that the concentration of ammonia in a person’s exhaled breath has been correlated to a number of adverse medical conditions, one such medical condition is chronic kidney disease (CKD). For healthy individuals, ammonia is present in exhaled breath at typical levels of a few hundred ppb. Research has linked CKD to elevated levels, greater than one ppm, of ammonia in breath [1]. Chapter 7 will give more details about the medical motivation and previous medical research. After this link between ammonia concentration in breath and medical conditions was established, further research has been done to develop improved sensors. Table 3.1 summarizes recent developments in laser based ammonia breath sensors. The above mentioned medical research was performed with an ammonia sensor based on a CO$_2$ laser operating at discrete wavelengths using an optoacoustic cell. An ammonia sensor was designed using a QCL with quartz-enhanced photoacoustic spectroscopy (QEPAS) at the ammonia absorption near 1046.4 cm$^{-1}$ which demonstrated improved sensitivity for ammonia measurements in the ppb range typical for breath [6]. Another ammonia sensor to study breath was designed with a QCL at 967.35 cm$^{-1}$ using pulsed cavity ring-down spectroscopy (CRDS) [4]. Mention was made of increased sensitivity at 1103.46 cm$^{-1}$ but at that time QCLs in that region were not readily available. They then designed another sensor at the same wavelength using intra and inter pulse techniques with a long path length herriot cell [7] to improve the sensitivity. Another ammonia breath sensor, utilizing the same ammonia absorption peak, was recently developed based on QEPAS and using a second harmonic wavelength modulation technique [8].
### Table 3.1: Summary of recent laser-based ammonia breath sensors

<table>
<thead>
<tr>
<th>Wavelength</th>
<th>Laser Method</th>
<th>Reported Sensitivity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discrete lines</td>
<td>CO₂ PAS</td>
<td>±10% for breath measurements as low as 100 ppb</td>
<td>[1]</td>
</tr>
<tr>
<td>9 - 10 µm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1046.4 cm⁻¹ QCL</td>
<td>QEPAS</td>
<td>20 ppb (1σ) detection limit</td>
<td>[6]</td>
</tr>
<tr>
<td>967.35 cm⁻¹ QCL</td>
<td>Pulsed CRDS</td>
<td>50 ppb detection limit</td>
<td>[4]</td>
</tr>
<tr>
<td>967.35 cm⁻¹ QCL</td>
<td>Inter and Intra Pulse</td>
<td>3 ppb detection limit   ±5% for breath measurements as low as 140 ppb</td>
<td>[7]</td>
</tr>
<tr>
<td>967.35 cm⁻¹ QCL</td>
<td>QEPAS with WMS 2f</td>
<td>6 ppb (1σ) detection limit for NH₃ in N₂ from 160 ppb to 5 ppm</td>
<td>[8]</td>
</tr>
</tbody>
</table>

### 3.2 HITRAN Simulations

Many factors go into designing a sensor, including the wavelength of the absorption line, optical cell path length, pressure of the gas sample, and spectroscopic strategy to be employed. The factors are chosen to maximize the absorbance of the target species and minimize the absorbance of the potentially interfering species. Before verifying with experiments, many of these factors can be chosen based on simulations of absorption lines with data provided in the HITRAN database [18]. The HITRAN database contains parameters specific to each energy transition for many molecules; some of the parameters include the line center frequency, the linestrength, and the air and self broadening coefficients. Based on these data the absorbance of a molecule over a range of frequencies can be simulated for a given temperature, path length,
pressure, and mole fraction. Simulations on theoretical breath mixtures of 73% N\textsubscript{2}, 15% O\textsubscript{2}, 6% H\textsubscript{2}O, and 6% CO\textsubscript{2} were used to design this sensor, while varying the frequency, mole fraction of NH\textsubscript{3}, pressure, and optical path length.

### 3.2.1 Line Selection

As mentioned above, previous researchers have used the ammonia absorption lines near 1046.4 cm\textsuperscript{-1} and 967.35 cm\textsuperscript{-1}. Recent advances in QCL technology have led to the development of a laser near 1103.46 cm\textsuperscript{-1}, which was recommended for improved sensitivity \cite{7}. These lines have been used in a laser-based sensor for atmospheric monitoring \cite{9} but not yet for breath sensing. Figure 3.1 shows the simulation demonstrating less interference from CO\textsubscript{2} and H\textsubscript{2}O for this line.

![Graphs showing ammonia absorption lines at different frequencies](image)

(a) Ammonia peak centered at 967.35 cm\textsuperscript{-1}  
(b) Ammonia peak centered at 1046.4 cm\textsuperscript{-1}  
(c) Ammonia peak centered at 1103.46 cm\textsuperscript{-1}

Figure 3.1: Comparing potential interference from other breath constituents
3.2.2 Path Length

The absorbance of all species increase proportionally to the optical path length because there are more molecules for the photons to interact with for a given mole fraction. In order to measure low concentrations, the absorbance can be increased simply by increasing the path length; however, the absorbance of all interfering species will also increase. Large path lengths are generally achieved by using multi-pass cells. Limitations include the availability of cells with the desired path length and the loss of signal strength due to losses from reflections. For this application, the volume of breath available to study is limited so a cell with a large volume would also limit the measurement pressure. Simulations were used to determine the maximum absorbance for a simulated mixture with 50 ppb NH$_3$ at atmospheric pressure. A cell with a pathlength of 76 m was chosen for this sensor to increase the absorbance as seen in Table 3.2. Cells with longer path length were available but their larger volume made them unsuitable for this application.

Table 3.2: Maximum absorbance at various optical path lengths for 760 torr and 50 ppb NH$_3$

<table>
<thead>
<tr>
<th>Path Length</th>
<th>Max Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 cm</td>
<td>$6.4 \times 10^{-5}$</td>
</tr>
<tr>
<td>7.2 m</td>
<td>$2.3 \times 10^{-3}$</td>
</tr>
<tr>
<td>36 m</td>
<td>$1.15 \times 10^{-2}$</td>
</tr>
<tr>
<td>76 m</td>
<td>$2.42 \times 10^{-2}$</td>
</tr>
</tbody>
</table>
3.2.3 Pressure

Pressure also has the affect of increasing the peak absorbance, but it also leads to broadening of absorption lines. Since the potentially interfering species have absorption peaks near the selected ammonia peak the increase in pressure will increase the interference. Simulations for pressures between 50 torr and 760 torr can be seen in Figure 3.2. The ideal pressure for the measurement is selected by compromising the affects of pressure on maximum absorbance and relative interference. The results of these simulations, shown in Table 3.3, led to the decision to perform measurements in the pressure range between 100 and 200 torr.

Table 3.3: Maximum absorbance and interference ratios at different pressures for a path length of 76 m and 50 ppb NH₃, 6% H₂O, and 6% CO₂

<table>
<thead>
<tr>
<th>Pressure (torr)</th>
<th>Max Absorbance (%)</th>
<th>$\frac{\text{Abs}_{\text{NH}<em>3}}{\text{Abs}</em>{\text{H}_2\text{O}}}$ at peak</th>
<th>$\frac{\text{Abs}_{\text{NH}<em>3}}{\text{Abs}</em>{\text{CO}_2}}$ at peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>760</td>
<td>2.42</td>
<td>5.2</td>
<td>9.5</td>
</tr>
<tr>
<td>300</td>
<td>1.55</td>
<td>25.8</td>
<td>36.7</td>
</tr>
<tr>
<td>200</td>
<td>1.28</td>
<td>49.3</td>
<td>76.6</td>
</tr>
<tr>
<td>150</td>
<td>1.15</td>
<td>79.4</td>
<td>128.3</td>
</tr>
<tr>
<td>100</td>
<td>0.99</td>
<td>155.3</td>
<td>250.2</td>
</tr>
<tr>
<td>50</td>
<td>0.75</td>
<td>468.4</td>
<td>734.9</td>
</tr>
</tbody>
</table>
Figure 3.2: Comparing absorbance and interference at different pressures
3.2.4 Strategy Choice

There are many measurement strategies based on laser spectroscopy which can be employed to study gases and design sensors. The simplest is direct absorption spectroscopy, but other strategies increase the sensitivity with the cost of increased complexity in the theory and experimental setup. Direct absorption spectroscopy is ideally suited for measuring lines with peak absorbance between 0.2 and 2.0. It can be used for lines with lower absorbance but the sensitivity and accuracy decrease with absorbance. Since the expected absorbance this sensor is designed to measure is on the order of 0.01 an improved strategy should be employed. WMS-2f/1f was chosen because it has higher sensitivity than direct absorption measurements \[5\]. This signal can also be simulated at various conditions to check for interference. Due to the nature of the technique, the signal is more sensitive to curvature than magnitude. This means that the water vapor absorption, which was a more significant source of interference than the carbon dioxide absorption in direct absorption spectroscopy, has a negligible effect since it is relatively constant. However, this also means that the carbon dioxide interference, while low in magnitude, will have a noticeable affect on the signal. As shown in Figure \[3.3\] this interference has an affect only for low concentrations of ammonia.
Figure 3.3: Comparing interference from CO₂ on the WMS signal at pressures of 100 and 200 torr and concentrations of 200 ppb and 1 ppm
Chapter 4

Ammonia Line Parameter Tests

4.1 Motivation

In order to implement the \textit{WMS-2f/1f} strategy for this sensor, the line parameters must first be measured. Of the gas parameters that affect the WMS signal, the pressure and temperature can be measured during the measurement and the mole fraction is the quantity of interest. Therefore, in order to determine the mole fraction, the linestrength and lineshape must be known. The ammonia feature selected for this sensor is made up of six individual lines, these properties must be determined for each of the six lines since the peak has contributions from each line as seen in Figure 4.1. The lineshape can be calculated if the collisional broadening coefficients are known, and these are specific to the bath gas. So the preliminary goal for the development of this sensor is the measurement of $S$, $2\gamma_{\text{NH}_3-N_2}$, $2\gamma_{\text{NH}_3-O_2}$, $2\gamma_{\text{NH}_3-CO_2}$, and $2\gamma_{\text{NH}_3-H_2O}$ for each of the six ammonia lines. The self broadening coefficient, $2\gamma_{\text{NH}_3-NH_3}$ is not necessary since the ammonia concentration is much lower than the others.
4.2 Practical Implementation of Direct Absorption Spectroscopy

Direct Absorption Spectroscopy (DAS), as described in Chapter 2, is practically implemented by experimental measurements followed by analysis of the data. Refer to Figure 4.2 for the experimental setup used for DAS. The experiment begins with determining the ideal laser settings. QCLs are designed for a limited wavelength range, but they can be tuned within that range by adjusting the laser’s temperature and input current. The input current and temperature are set so the laser is centered on the absorption peak. The laser wavelength is then scanned by modulating the current with a ramp of a specific amplitude and frequency. The scanning range is a function of these parameters. The goal is to have a single scan capture the entire absorption feature including some of the baseline so that the resulting signal can easily
Figure 4.2: Direct Absorption Spectroscopy experimental setup

by analyzed and the desired parameters determined.

The next step is to record this scan with an evacuated cell and an etalon in the optical path. An etalon is a solid cylinder made of optical material with reflective faces. Since the index of refraction is a function of the wavelength, the reflections will be in phase for some wavelengths and out of phase for others, leading to peaks within the detected laser intensity. The distance between the peaks is called the free spectral range (FSR)

\[
FSR = \frac{1}{2n_\nu d}
\]  

(4.1)

where \(n_\nu\) is the index of refraction and \(d\) is the distance between the reflective faces. The FSR has units of cm\(^{-1}\) and so it is used to convert the time domain, which the measurement is taken in, to the frequency (in wavenumber units) domain, which the analysis is performed in. For this research a germanium etalon was used with an FSR of 0.016426 cm\(^{-1}\).

The next step is to record the detected intensity for an evacuated cell to be used as the reference intensity, \(I_o\). The gas mixture is then added to the cell to a specific
pressure and the detected intensity with absorption, $I$, is recorded. This last step is repeated for many pressures.

The first step of the analysis is to develop a calibration to convert the time domain to the frequency domain using the signal recorded with the etalon. The peaks are identified as seen in Figure 4.3 and then the relative wavenumber units between the peaks are plotted against the peak location as seen in Figure 4.4. A polynomial fit is found from this which can be used as the calibration to convert the time domain into the frequency domain.

![Etalon Peaks](image)

**Figure 4.3: Peak locations from the etalon signal**

The next step in the analysis is to calculate the absorbance from the ratio of laser intensity with and without absorption, which can be seen in Figure 4.5. First, however, it is necessary to adjust the measured intensity to account for the detector offset, since

$$\alpha_{\nu} = - \ln \left( \frac{I}{I_o} \right) \neq - \ln \left( \frac{I + \text{Detector Offset}}{I_o + \text{Detector Offset}} \right)$$

(4.2)
Figure 4.4: Calibration curve to convert time domain to frequency domain

where $I$ and $I_o$ are the actual laser intensities after removing the detector offset. Once the absorbance is calculated the etalon calibration is used so the domain is in relative wavenumber units. This data is then fit with a Voigt lineshape profile as seen in Figure 4.6 and described by Equation 2.10. Guessed values for the line center, line width, and peak height are also used to converge to a solution. The Voigt fit gives as an output the integrated area of the lineshape function and, since the doppler line width is known from the temperature, molar mass and linecenter according to Equation 2.9, the collisional line width.
Figure 4.5: Measured intensity with and without absorption used to calculate the absorbance

Figure 4.6: Measured Absorbance and the Voigt fit
The linestrength can be determined from a single measurement, but the accuracy is higher when it is determined from many measurements over a range of pressures by plotting the integrated area for various pressures at known path lengths and mole fractions and calculating the slope of the best fit line as seen in Figure 4.7. This slope can then be used to calculate the linestrength.

\[
S = \frac{\text{Area}}{PX_iL} = \frac{d(Area)}{dP} \frac{1}{X_iL} \tag{4.3}
\]

Figure 4.7: Integrated area plotted for various pressures to determine the slope

The collisional broadening coefficient can be determined from the collisional line width, defined in Equation 2.6. For a two gas mixture the collisional line width is

\[
\Delta \nu_c = P (X^2 \gamma_{self} + (1 - X)^2 \gamma_{bathgas}) \tag{4.4}
\]

which can be solved for the bath gas broadening coefficient. Higher accuracy is
achieved by using the slope of the best fit line of measured line widths at various pressures, as seen in Figure 4.8. The coefficient can be calculated from this slope.

\[
2\gamma_{bathgas} = \frac{1}{(1-X)} \left[ \frac{\Delta \nu_c}{P} - X^2 \gamma_{self} \right] = \frac{1}{(1-X)} \left[ \frac{d \Delta \nu_c}{dP} - X^2 \gamma_{self} \right]
\]

(4.5)

Figure 4.8: Collisional line width plotted for various pressures to determine the slope

4.3 Unique Strategy to Determine Line Parameters

The above described analysis strategy is used for normal conditions. There were two challenges that required a unique strategy to be used to determine the line parameters for the ammonia lines selected for the sensor.

The first challenge in determining the line parameters was that the six ammonia
lines being studied are very closely spaced. In order to determine the linestrength and broadening coefficients for each of the six lines a Voigt lineshape fitting function was used that combined six individual fits into a cumulative fit. This fit is more accurate when the six lines are more distinguishable. Since the lines are so close to one another their features overlap due to broadening. At low pressures the collisional broadening decreases and the lines can be distinguished. However, when the pressure is too low, the broadening is dominated by Doppler broadening so it is difficult to measure the collisional broadening coefficients. These two conflicting affects led to narrow pressure ranges where the line parameters could be determined. The mole fraction of ammonia in the mixture also had to be adjusted so the absorbance was in the ideal range for the required pressure. Figure 4.9 shows an example of the fit at the high ends of the pressure range for the ammonia mixture with water vapor. It can be seen that all of the lines are still overlapping but can be distinguished from one another. The tests were performed at the best possible conditions given their close spacing but the result was that the uncertainty in the parameters was larger than it would be for individual lines.

The other challenge was that due to the polar nature of ammonia, the molecules tend to adsorb to surfaces. The above analysis requires that the mole fraction of the species of interest be known and constant for all the measurements. In preparing mixtures of ammonia with the various bath gases some of the ammonia molecules would adsorb to the walls of the mixing vessel leading to the mole fraction being lower than expected. The absorption measurements take place in a gas test cell, so as the ammonia mixture enters the test cell, some of the molecules adsorb to the walls of the cell. The overall affect was that the mole fraction was not known with sufficient accuracy. A unique strategy was developed to determine the mole fraction at the time of the measurements. A nearby absorption line centered at 1104.3325 cm$^{-1}$, for which the linestrength is reported in the literature [19, 20], was used to determine
Figure 4.9: Voigt fit and residual of absorption measured for six lines at the high end of pressure range

the initial mole fraction and the change in the mole fraction over time. Initially, a test cell with metallic components was used, however, the adsorption had such a large effect that the ammonia mole fraction decreased exponentially with time. A new test cell made entirely of glass was used because the adsorption affects were slower and linear as seen in Figure 4.10. This is due to the difference in molecular structure of metals and glass, since glass has fewer sites for adsorption.

Using the glass test cell, the variation in the mole fraction over time was found to be linear. The mole fraction was determined using the nearby line over sufficient time to calculate a linear fit. The laser settings were then adjusted to scan over the six lines of interest. The linear approximation was extrapolate to determine the mole fraction at the time the measurements of the six lines were recorded. The reference intensity for the laser settings at the nearby line was recorded before adding the gas, then, after all the measurements were done, the cell was evacuated and the reference
Figure 4.10: Comparing the affects of adsorption for different test cells

Intensity for the laser settings at the six lines being studied was recorded. The cell was therefore filled and evacuated for each of the measurements as the pressure was varied. The affect this has on the analysis is that the mole fraction was not exactly the same for the measurements at each of the pressures.

To determine the linestrength, instead of using the slope from the plot of integrated area at various pressures, the integrated area was plotted at different partial pressures. The linestrength could similarly be found from the slope of this line.

\[ S = \frac{\text{Area}}{PX_i L} = \frac{d(\text{Area})}{dPX_i} \frac{1}{L} \]  \hspace{1cm} (4.6)

Figure 4.11 shows an example of this for the ammonia mixture with nitrogen.

The collisional broadening coefficient, while being a linear function of pressure, is not a linear function of partial pressure. Therefore the coefficient was calculated for
Figure 4.11: Finding the slope for each line from the integrated area as a function of partial pressure

each pressure and the average value was reported.

\[ 2\gamma_{bathgas} = \frac{1}{(1 - X)} \left[ \frac{\Delta \nu_c}{P} - X^2 \gamma_{self} \right] \]  

These tests were performed with mixtures of ammonia in nitrogen, oxygen, carbon dioxide and water vapor in order to determine the line strength and collisional broadening coefficients. The measurements of ammonia in nitrogen were performed at \( X_{NH_3} \approx 1.4\% \) and for pressures between 3 and 9 torr. The measurements of ammonia in oxygen were performed at \( X_{NH_3} \approx 0.8\% \) and for pressures between 3 and 21 torr. The measurements of ammonia in carbon dioxide were performed at \( X_{NH_3} \approx 0.7\% \) and for pressures between 2 and 6 torr. The measurements of ammonia in water vapor were performed at \( X_{NH_3} \approx 2.3\% \) and for pressures between 1 and 2.5 torr. These tests were repeated twice for each gas mixture over these pressure ranges in order to
verify the repeatability of the results.

4.4 Uncertainty Analysis

The goals of these tests were to determine values for the linestrength and broadening coefficients for the ammonia absorption lines. It is necessary to quantify the uncertainty in the final reported values. Since a unique method for determining these parameters was used it is important to account for the uncertainty at every step and propagate this uncertainty through the calculations to determine the actual uncertainty of the final values.

The method to analyze the uncertainty begins at the end result and then works backwards to determine various sources of uncertainty. The linestrength is determined according to Equation 4.3. It is a function of four input parameters, \( P \), \( L \), \( \text{Area} \), and \( X_i \), each of which is known with some uncertainty.

The pressure was determined using an MKS Baratron with a reported uncertainty of 0.12% of the reading.

The path length of the cell was determined by measuring the length of the cell with calipers. The cell is designed with the windows placed at opposite angles from one another in order to reduce reflections. This results in a variation in the path length depending on the laser path. The measurements gave the maximum value and the minimum value. The difference was much larger than the uncertainty of the calipers used to make the measurement so that uncertainty negligible. Since the laser was aligned to go through the center, the most probably length is the mean between the minimum and maximum. Assuming a Gaussian distribution of lengths and defining that 99.7% of the possible laser paths fall between the maximum and minimum path length, the minimum and maximum are each three standard deviations from the mean. As a result of this, the standard deviation of the path length is one sixth of
the difference between the minimum and maximum path length as seen in Figure 4.12 and Table 4.1.

Figure 4.12: Uncertainty in the path length.

Table 4.1: Path length uncertainty

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$L_{max}(cm)$</td>
<td>20.125</td>
</tr>
<tr>
<td>$L_{min}(cm)$</td>
<td>19.687</td>
</tr>
<tr>
<td>$L_{avg}(cm)$</td>
<td>19.906</td>
</tr>
<tr>
<td>$\sigma(cm)$</td>
<td>0.073</td>
</tr>
</tbody>
</table>

The integrated area was determined by fitting the recorded absorbance with a Voigt lineshape and calculating the integrated area using the analytical solution based on the Voigt parameters of maximum height, collisional width, and Doppler width. The uncertainty in the integrated area comes from an imperfect Voigt fit. The residual of the measured absorption and the Voigt fit is an indicator of the error in the fit. However, since the properties for all six lines were determined from a single fit, the residual just gives a measure of the accuracy of the fit to the absorbance data, not the accuracy of the output integrated area and collisional line width. Since the lines were so closely spaced the fit sometimes gave more weight to one line over another while still fitting the data well. To quantify the uncertainty in the integrated area a strategy was used that involved recording ten consecutive cycles of the input signal for every measurement condition. Each of these cycles was analyzed and fit with a
Voigt lineshape resulting in a value for the integrated area for each cycle and each line. For each line the average value was used as the area and the standard deviation was used as the uncertainty.

Determining the mole fraction of ammonia in the mixture was one of the main challenges for this study as described above. The mole fraction was determined by measuring the absorption of a nearby line and calculating the mole fraction from the integrated area of the Voigt fit and linestrength values from literature.

\[ X_i = \frac{\text{Area}}{PLS} \]  

(4.8)

The uncertainty in each of these properties is used to calculate the overall uncertainty in the mole fraction.

The pressure and path length have the same uncertainty as described above and the uncertainty in the integrated area was found using the same method as described above, although it was lower since there was a single line to fit rather than six. The linestrength was taken as the average between two values reported in the literature, listed in Table 4.2. The uncertainty used was the larger of the two. The Euclidean norm is used to determine the uncertainty of the mole fraction by combining the weighted uncertainties of each of the terms.

<table>
<thead>
<tr>
<th>Source</th>
<th>Strength (cm(^{-2})/atm)</th>
<th>Uncertainty (cm(^{-2})/atm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aroui et. al. [19]</td>
<td>0.466</td>
<td>0.017</td>
</tr>
<tr>
<td>Fabian and Yamada [20]</td>
<td>0.447</td>
<td>0.008</td>
</tr>
<tr>
<td>Average used</td>
<td>0.4565</td>
<td>0.017</td>
</tr>
</tbody>
</table>
For
\[ y = f(a, b, c); u_y = \sqrt{\left(\frac{dy}{da} u_a\right)^2 + \left(\frac{dy}{db} u_b\right)^2 + \left(\frac{dy}{dc} u_c\right)^2} \]  
(4.9)
so,
\[ u_X = \sqrt{\left(\frac{1}{\text{PL}^2} u_A\right)^2 + \left(\frac{A}{\text{S}^2\text{PL}} u_S\right)^2 + \left(\frac{A}{\text{SP}^2\text{L}} u_P\right)^2 + \left(\frac{A}{\text{S}^2\text{PL}^2} u_L\right)^2} \]  
(4.10)
or,
\[ \frac{u_X}{X} = \sqrt{\left(\frac{u_A}{A}\right)^2 + \left(\frac{u_S}{S}\right)^2 + \left(\frac{u_P}{P}\right)^2 + \left(\frac{u_L}{L}\right)^2} \]  
(4.11)

This gives the uncertainty in each of the measurements of the mole fraction. The mole fraction used in the calculation of the line strength of the lines being studied is extrapolated from the linear fit of these mole fraction measurements taken over time. It is necessary to include the uncertainty in that fit as well. This uncertainty is calculated as the root mean squared error of the linear fit. This can just be added as another term. Since the uncertainty in the measurement was calculated for each value, the overall uncertainty is calculated from the average uncertainty in the measurement and the uncertainty in the fit.

\[ u_x = \sqrt{(u_{x_{\text{meas}}})^2 + (u_{x_{\text{fit}}})^2} \]  
(4.12)

All of these uncertainties must now be used to determine the uncertainty in the calculated linestrength. Since the slope of the integrated area as function of partial pressure was used the uncertainty in the slope must be taken into account in addition to the uncertainty for each of the input parameters. Since the integrated area and the mole fraction were calculated for each of the measurements, the average uncertainty was used with the uncertainty in the pressure, length, and slope to determine the
overall uncertainty.

\[
\frac{u_S}{S} = \sqrt{\left(\frac{u_A}{A}\right)^2 + \left(\frac{u_X}{X}\right)^2 + \left(\frac{u_P}{P}\right)^2 + \left(\frac{u_L}{L}\right)^2 + \left(\frac{u_{\text{fit}}}{SL}\right)^2} \tag{4.13}
\]

The other parameters being determined were the collisional broadening coefficients for ammonia with each of the bath gases. The broadening coefficient for ammonia with the bath gas is given by Equation 4.7. The uncertainties of each of the terms as well as the standard deviation from the mean value are used to determine the overall uncertainty in the coefficients.

The uncertainty in the pressure and the mole fraction have already been discussed. Since the mixtures had very low concentrations the self broadening coefficient had a smaller effect than the other parameters. For this reason, they were not determined experimentally, but the values given in HITRAN were used along with a 10% uncertainty. The collisional width was determined from the Voigt fit, so the same method of determining the uncertainty of the integrated area was used to determine the uncertainty in the collisional width. The uncertainty in the calculated value of the collisional broadening coefficient was found to be

\[
u_{2\gamma_{\text{bath}}} = \left[ \left( \frac{1}{P(1-X)} u_{\Delta\nu_c} \right)^2 + \left( \frac{\Delta\nu_c}{P^2(1-X)} u_P \right)^2 \right. \\
\left. + \left( \frac{2X}{(1-X)} u_{\gamma_{\text{self}}} \right)^2 + \left( \frac{\Delta\nu_c}{P} - 2\gamma_{\text{self}} \right) \frac{1}{(1-X)^2} u_X \right]^{1/2} \tag{4.14}
\]

These values were then taken at various pressures and the results were averaged to determine the reported values. The standard deviation from the mean was used as the uncertainty for this calculation. In this case, since the uncertainty was calculated for each pressure, the average uncertainty from the calculations was used with the standard deviation in the mean to determine the overall uncertainty. The total uncertainty is then found to be
4.5 Results

The ammonia lines being studied are $R$-branch rotational transitions within the $\nu_2$ vibrational band. Ammonia is a symmetric top molecule and thus has two rotational quantum numbers. These transitions all have the same $J$ rotational quantum number, representing the total angular momentum, their lower state $J'' = 6$. Each transition, however, has a different $K$ rotational quantum number, representing the orientation and direction of the rotation from $K = 1$ to $K = 6$. These transitions can be represented by the notation $sR(6, K)$.

The line strength was calculated for each line for each of the gas mixtures. The results were consistent with each other as well as with reported values in the HITRAN Database [18]. The reported uncertainty in HITRAN for each of these lines is between 10 - 20%. As can be seen in Figure 4.13 and Table 4.3 the measured values for the line strength are within the uncertainty given in HITRAN and are measured here with uncertainties less than 10%. The transition $sR(6, 5)$ has the largest uncertainty due to its location between two stronger lines which resulted in a less accurate Voigt fit. The relative magnitudes of the line strengths agree with the theory in that the intensities for transitions with nuclear spin statistical weight $g_i = 2$ (for $K = 3n$, $n = 1, 2, \ldots$) are larger than those with $g_i = 1$. Also, as $K$ approaches $J$ the intensities slightly decrease [19].

The collisional broadening coefficients for all six lines were calculated for ammonia with each of the bath gases. Collisional broadening due to water vapor was the strongest, decreasing with carbon dioxide, nitrogen, and oxygen respectively as can be seen by Figure 4.14 and Tables 4.4, 4.5, 4.6, 4.7. The results for oxygen, nitrogen,
and carbon dioxide also agree with the theory that the broadening increases as the $K$ rotational quantum number increases [19]. The results for water vapor do not follow this theory, however. Since water vapor has the strongest affect on broadening, it is likely that the difference from changing the $K$ rotational quantum number is insignificant when compared to the uncertainty in the measurement.

![Figure 4.13: Comparing measured linestrength to linestrength given in HITRAN database](image-url)
Table 4.3: Measured linestrengths for ammonia transitions sR(6,K)

<table>
<thead>
<tr>
<th>$\nu_0$ (cm(^{-1}))</th>
<th>Transition</th>
<th>$S$ (cm(^{-2})/atm)</th>
<th>$u_s$ (%)</th>
<th>HITRAN $S$ (cm(^{-2})/atm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1103.430</td>
<td>sR(6,4)</td>
<td>1.906</td>
<td>0.120 (6.31%)</td>
<td>1.866</td>
</tr>
<tr>
<td>1103.434</td>
<td>sR(6,5)</td>
<td>1.508</td>
<td>0.147 (9.75%)</td>
<td>1.625</td>
</tr>
<tr>
<td>1103.441</td>
<td>sR(6,3)</td>
<td>3.909</td>
<td>0.251 (6.42%)</td>
<td>3.933</td>
</tr>
<tr>
<td>1103.470</td>
<td>sR(6,2)</td>
<td>1.996</td>
<td>0.127 (6.37%)</td>
<td>2.004</td>
</tr>
<tr>
<td>1103.480</td>
<td>sR(6,6)</td>
<td>2.180</td>
<td>0.137 (6.29%)</td>
<td>2.197</td>
</tr>
<tr>
<td>1103.486</td>
<td>sR(6,1)</td>
<td>1.193</td>
<td>0.151 (7.57%)</td>
<td>2.014</td>
</tr>
</tbody>
</table>
Figure 4.14: Collisional broadening coefficient for ammonia in bath gases found in breath
Table 4.4: Measured collisional broadening coefficients for ammonia transitions \( sR(6,K) \) with nitrogen

<table>
<thead>
<tr>
<th>( \nu_o ) (cm(^{-1}))</th>
<th>Transition</th>
<th>( 2\gamma_{\text{NH}_3-N_2} ) (cm(^{-1}/\text{atm}))</th>
<th>( u_{2\gamma_{\text{NH}_3-N_2}} ) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1103.430</td>
<td>( sR(6,4) )</td>
<td>0.171</td>
<td>0.012(7.02%)</td>
</tr>
<tr>
<td>1103.434</td>
<td>( sR(6,5) )</td>
<td>0.182</td>
<td>0.024(13.06%)</td>
</tr>
<tr>
<td>1103.441</td>
<td>( sR(6,3) )</td>
<td>0.152</td>
<td>0.007(4.27%)</td>
</tr>
<tr>
<td>1103.470</td>
<td>( sR(6,2) )</td>
<td>0.142</td>
<td>0.006(3.92%)</td>
</tr>
<tr>
<td>1103.480</td>
<td>( sR(6,6) )</td>
<td>0.197</td>
<td>0.008(3.91%)</td>
</tr>
<tr>
<td>1103.486</td>
<td>( sR(6,1) )</td>
<td>0.123</td>
<td>0.007(5.42%)</td>
</tr>
</tbody>
</table>

Table 4.5: Measured collisional broadening coefficients for ammonia transitions \( sR(6,K) \) with oxygen

<table>
<thead>
<tr>
<th>( \nu_o ) (cm(^{-1}))</th>
<th>Transition</th>
<th>( 2\gamma_{\text{NH}_3-O_2} ) (cm(^{-1}/\text{atm}))</th>
<th>( u_{2\gamma_{\text{NH}_3-O_2}} ) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1103.430</td>
<td>( sR(6,4) )</td>
<td>0.085</td>
<td>0.008(9.29%)</td>
</tr>
<tr>
<td>1103.434</td>
<td>( sR(6,5) )</td>
<td>0.093</td>
<td>0.014(15.05%)</td>
</tr>
<tr>
<td>1103.441</td>
<td>( sR(6,3) )</td>
<td>0.077</td>
<td>0.005(6.17%)</td>
</tr>
<tr>
<td>1103.470</td>
<td>( sR(6,2) )</td>
<td>0.075</td>
<td>0.005(7.21%)</td>
</tr>
<tr>
<td>1103.480</td>
<td>( sR(6,6) )</td>
<td>0.105</td>
<td>0.005(5.18%)</td>
</tr>
<tr>
<td>1103.486</td>
<td>( sR(6,1) )</td>
<td>0.065</td>
<td>0.004(6.57%)</td>
</tr>
</tbody>
</table>
Table 4.6: Measured collisional broadening coefficients for ammonia transitions \( sR(6,K) \) with carbon dioxide

<table>
<thead>
<tr>
<th>( \nu_o ) (cm(^{-1}))</th>
<th>Transition</th>
<th>( 2\gamma_{\text{NH}_3-\text{CO}_2} ) (cm(^{-1}/\text{atm}))</th>
<th>( u_{2\gamma_{\text{NH}_3-\text{CO}_2}} ) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1103.430</td>
<td>( sR(6,4) )</td>
<td>0.291</td>
<td>0.022 (7.49%)</td>
</tr>
<tr>
<td>1103.434</td>
<td>( sR(6,5) )</td>
<td>0.306</td>
<td>0.040 (13.18%)</td>
</tr>
<tr>
<td>1103.441</td>
<td>( sR(6,3) )</td>
<td>0.243</td>
<td>0.010 (3.93%)</td>
</tr>
<tr>
<td>1103.470</td>
<td>( sR(6,2) )</td>
<td>0.219</td>
<td>0.010 (4.74%)</td>
</tr>
<tr>
<td>1103.480</td>
<td>( sR(6,6) )</td>
<td>0.361</td>
<td>0.013 (3.71%)</td>
</tr>
<tr>
<td>1103.486</td>
<td>( sR(6,1) )</td>
<td>0.161</td>
<td>0.010 (6.25%)</td>
</tr>
</tbody>
</table>

Table 4.7: Measured collisional broadening coefficients for ammonia transitions \( sR(6,K) \) with water vapor

<table>
<thead>
<tr>
<th>( \nu_o ) (cm(^{-1}))</th>
<th>Transition</th>
<th>( 2\gamma_{\text{NH}_3-\text{H}_2\text{O}} ) (cm(^{-1}/\text{atm}))</th>
<th>( u_{2\gamma_{\text{NH}_3-\text{H}_2\text{O}}} ) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1103.430</td>
<td>( sR(6,4) )</td>
<td>0.568</td>
<td>0.036 (6.38%)</td>
</tr>
<tr>
<td>1103.434</td>
<td>( sR(6,5) )</td>
<td>0.576</td>
<td>0.071 (12.26%)</td>
</tr>
<tr>
<td>1103.441</td>
<td>( sR(6,3) )</td>
<td>0.552</td>
<td>0.022 (4.07%)</td>
</tr>
<tr>
<td>1103.470</td>
<td>( sR(6,2) )</td>
<td>0.581</td>
<td>0.020 (3.51%)</td>
</tr>
<tr>
<td>1103.480</td>
<td>( sR(6,6) )</td>
<td>0.592</td>
<td>0.028 (4.74%)</td>
</tr>
<tr>
<td>1103.486</td>
<td>( sR(6,1) )</td>
<td>0.567</td>
<td>0.024 (4.20%)</td>
</tr>
</tbody>
</table>
Chapter 5

Sensor Implementation

5.1 Gas Flow Through the Multi-pass Cell

In order to sense low concentrations of ammonia in breath this sensor utilizes a multi-pass cell with an effective path length of 76 m. This cell is from Aerodyne Research, which designs astigmatic mirror multi-pass absorption cells for long path length spectroscopy [21, 22]. When testing the cell for use with ammonia, it was discovered that the amount of ammonia in the cell decreased over time. This was to be expected since ammonia gas tends to adsorb to surfaces it comes in contact with. The larger surface area and non-glass components, like the mirrors, in this cell provide more adsorption sites than the all-glass cell which was used for the line parameter tests. Due to this phenomenon, the cell could not be used to study a static gas sample. Instead, a gas flow setup was used so the sensor measures the actual amount of ammonia in the gas sample. A more detailed explanation of the adsorption phenomenon and methods to eliminate the effects is described in Chapter 6. Initially, the cell is evacuated and then the inlet to the cell is opened while the outlet is still open to the vacuum pump. The pressure quickly rises to a steady state value as the gas flows into the cell and then out through the vacuum pump. The
measured amount of ammonia increases at a slower rate than the pressure since the ammonia is adsorbing to the surfaces of the cell; eventually all the adsorption sites fill and the amount of ammonia approaches the actual value in the gas sample. An example of these trends can be seen in Figure 5.1.

![Figure 5.1: Pressure and mole fraction in the cell during gas flow experiments](image)

### 5.2 WMS Laser Parameters

As described above, in order to use WMS to determine gas properties, the laser parameters must first be known. These are determined according to the method described by Li [23] in which the sinusoidally modulated laser intensity is compared with the laser frequency to determine the laser parameters \( a, i_o, i_2, \psi_1, \psi_2 \). The laser intensity is easily measured while the laser frequency is calculated by recording the laser intensity with an etalon in the laser path. The frequency between the peaks of the fringes in the signal is constant and equal to the \( FSR \) of the etalon. The peak
locations are fit with a sine wave to determine the modulation depth, $a$.

$$\nu(t) = \nu_0 + a \sin(\omega t + \phi_\nu)$$  \hspace{1cm} (5.1)$$

The intensity is fit with a sine wave of the same frequency to determine $i_o$ and $\psi_1$.

$$I(t) = I_0 (1 + i_o \sin(\omega t + \phi_I))$$  \hspace{1cm} (5.2)$$

The normalized amplitude of this fit is $i_o$ and $\psi_1$ is found by comparing the phase of the intensity to the phase of the laser frequency as seen in Figure 5.2. Since the wavelength modulation is defined as having zero phase offset and both the intensity and the wavelength have the same frequency, $\psi_1$ is just the difference between the phase of the intensity fit and frequency fit.

$$\psi_1 = \phi_I - \phi_\nu$$  \hspace{1cm} (5.3)$$

The difference between the measured intensity and the fit intensity, the residual, is then fit with a sine wave with twice the frequency of the modulation to determine $i_2$ and $\psi_2$.

$$I(t) = I_0 (i_2 \sin(2\omega t + \phi_R))$$  \hspace{1cm} (5.4)$$

The normalized amplitude of this fit is $i_2$ and $\psi_2$ is found by comparing the phase of the residual to the phase of the laser frequency as seen in Figure 5.3. Since the residual has twice the frequency as the laser frequency, $\psi_2$ is calculated by adjusting $\phi_\nu$ for the doubled frequency.

$$\psi_2 = \phi_R - 2\phi_\nu$$  \hspace{1cm} (5.5)$$
Figure 5.2: Comparing the laser intensity to the laser frequency to determine the linear laser parameters

Figure 5.3: Comparing the residual of the laser intensity fit to the laser frequency to determine the non-linear laser parameters
5.3 Experimental Procedure

WMS is implemented by comparing experimental measurements to simulated conditions. The experimental setup is shown in Figure 5.4. The laser is set to a fixed temperature and center current at the peak of the absorption, the current is then modulated with a high frequency, 10 kHz in this case, sine wave. The resulting laser intensity, after having passed through the multi-pass cell, is measured by the detector, this raw data is then processed to determine the $\frac{2f}{1f}$ signal. This measured value is compared to the simulated value determined by the theory described in Chapter 2 where the laser parameters are determined by the method described in the previous section and the temperature and pressure are measured during the experiment. A guessed value for the ammonia mole fraction is used to calculate the simulated $\frac{2f}{1f}$ signal. The WMS technique, although it modulates the laser across the absorption feature, only gives the value corresponding to the center wavelength. For this reason
an additional sinusoidal modulation with low frequency, 80 Hz in this case, is added to the high frequency modulation so that the $2f/1f$ signal for a range of wavelengths can be determined. The purpose of this scan is to make sure the peak $2f/1f$ signal is measured. The peak value can then be compared to the peak value of the simulation. There is a background signal in the absence of absorption in both the simulated and measured signals so this must be subtracted from each before they can be compared. The simulated background signal is found by running the simulation with an input ammonia concentration of zero. The experimental background signal is measured with pure nitrogen flowing through the cell. Finally the background subtracted $2f/1f$ measured signal, $C_{\text{meas}}$, can be compared to the simulated signal, $C_{\text{sim}}$ to calculate the measured ammonia mole fraction.

$$X_{\text{meas}} = X_{\text{guess}} \frac{C_{\text{meas}}}{C_{\text{sim}}}$$  \hfill (5.6)

Figure 5.5 shows the output from the simulation for the first and second harmonic signals, the background subtracted $2f/1f$ signal, and the region of the peak that is scanned during the measurement.

As mentioned above, the recorded laser intensity with absorption must be processed in order to determine the first and second harmonic components. This is performed using a digital lock-in filter where the raw signal is multiplied by $\sin(\omega_m t)$ and $\cos(\omega_m t)$ to get the first harmonic $X_{1f}$ and $Y_{1f}$ components respectively. The second harmonic $X_{2f}$ and $Y_{2f}$ components are calculated using $\sin(2\omega_m t)$ and $\cos(2\omega_m t)$ respectively. These components are then digitally filtered with a low pass Butterworth filter to remove the high frequency noise and isolate the desired harmonics [5]. The first and second harmonics are calculated from their respective components after being filtered.

$$R_{1f} = \sqrt{X_{1f}^2 + Y_{1f}^2}$$  \hfill (5.7)
Figure 5.5: Simulated WMS signals including the first and second harmonic signals as well as the background subtracted $2f/1f$ signal showing the region of the slow scan

\[ R_{2f} = \sqrt{X_{2f}^2 + Y_{2f}^2} \] (5.8)

The background signals are also processed in the same way. An example of the first and second harmonic compared to the background for five slow scan cycles is shown in Figures 5.6 and 5.7 for a measurement of ammonia at 9 ppm. As would be expected based on the simulated signal, as seen in Figure 5.5, the first harmonic has one peak for each slow scan cycle, while the second harmonic has two peaks for each slow scan cycle.
Figure 5.6: WMS 1f signal compared to the 1f background signal

Figure 5.7: WMS 2f signal compared to the 2f background signal
The actual signal of interest is the background subtracted $2f/1f$ signal which is calculated from the individual components

\[
C_{\text{meas}} = \sqrt{\left(\frac{X_{2f}}{R_{1f}} - \frac{X_{2f}^o}{R_{1f}^o}\right)^2 + \left(\frac{Y_{2f}}{R_{1f}} - \frac{Y_{2f}^o}{R_{1f}^o}\right)^2}\]

(5.9)

where $X_{2f}^o$, $Y_{2f}^o$, and $R_{1f}^o$ are the background components. This is calculated for many slow scan cycles from which the peak values can be determined. Figure 5.8 shows an example of the measured, background, and background subtracted $2f/1f$ signals. The peaks are found by taking the maximum value for each scan across the peak. These peaks are then compared to the simulated peaks as mentioned above.

Figure 5.8: WMS $2f/1f$ results with peak detection for $X_{NH_3} = 9$ ppm
5.4 WMS Simulation Sensitivity Analysis

Since the measured peak signal is compared to the simulated peak to infer the ammonia mole fraction, it is important to quantify the sensitivity of the simulated peak value to various input parameters. The two main categories of input parameters are the gas properties and the laser parameters. The gas properties include the pressure, the temperature, the mole fractions of the various species in the mixture, and the line parameters for ammonia used to determine the absorbance.

The line parameters will have the greatest affect since they have the largest uncertainty. Chapter 4 described the measurement of the line strength and broadening coefficients; these were measured with some uncertainty as listed in Tables 4.3, 4.4, 4.5, 4.6, and 4.7. In order to determine the affect these have on the simulated signal, simulations were performed in which one of the parameters was adjusted by its uncertainty while the others were maintained at the measured value. The resulting peak value was compared to the peak value for the simulation with all of the parameters at the measured value. Figure 5.9 shows the affect of the uncertainty of the line strength for each of the six ammonia lines where $S_1$ refers to the line at 1103.430 cm$^{-1}$ and consecutive numbers refer to lines located at increasing wavenumbers. Line three is the strongest and the main peak being measured so it has the largest resulting sensitivity leading to a change in the simulated peak of 2.31% with decreasing sensitivity as the lines are further from line three. Figure 5.10 shows the affect of the uncertainty of the collisional broadening coefficients for each of the bath gases. The peak is most sensitive to nitrogen since it is the most abundant bath gas in breath leading to a change in the simulated peak of 1.68%.
Figure 5.9: Comparison of simulated peak values to determine the sensitivity of the peak value to the uncertainty in the measured line strengths for each of the six ammonia lines.

Figure 5.10: Comparison of simulated peak values to determine the sensitivity of the peak value to the uncertainty in the measured collisional broadening coefficients for each of the bath gases.
Similar analysis can be done to determine the sensitivity of the peak to other unknowns. The relative concentrations of the other gases in breath is one such unknown, since they will not be simultaneously measured. Exhaled breath typically has 6% water vapor and between 3 - 6% carbon dioxide \cite{24}. These gases have potential interference because they absorb light near this wavelength region. As shown previously in Figure \ref{fig:interference}, the water vapor interference is negligible since it is constant and the carbon dioxide interference is significant for ammonia mole fractions less than 1 ppm. The relative amount of this interference will depend on the ammonia mole fraction as well as the carbon dioxide mole fraction, which is unknown. The interference can be included in the simulation so the only unknown affect is the relative difference in the interference due to variation in the carbon dioxide concentration. At a pressure of 100 torr and ammonia mole fraction of 200 ppb the difference in peak is 1% between carbon dioxide concentrations of 4% and 6%.

The uncertainty in the relative amounts of bath gases also affect the ammonia signal because they are included in the calculation of the collisional line width. Oxygen typically makes up 15% of breath while nitrogen accounts for the remainder. At a pressure of 100 torr, a change in the amount of water vapor by 1% results in a change in the peak value by 0.84%, while a change in the amount of carbon dioxide by 3% results in a change in the peak value by 0.62%, and a change in the amount of oxygen by 3% results in a change in the peak value by 0.43%. Since the nitrogen makes up the remainder of the mixture in the simulations, the affect of changing the amount of nitrogen is already accounted for.

The temperature of the gas in the cell is assumed to be room temperature which is measured occasionally and found to always be between 294 and 296 K. This 2 K difference, if not accounted for, leads to a change in the simulated peak of 0.38%. The pressure in the cell during experiments is measured with an MKS Baratron with a reported uncertainty of 0.12%. At the beginning of the measurement the pressure
rises quickly, in this region there is a larger uncertainty in the pressure. However, the pressure eventually reaches a steady state as the gas mixture flows through the cell; the mole fraction isn’t measured until this is achieved so the pressure uncertainty is only important in this region. There is still a slight variation in the pressure which could cause a larger uncertainty in the measured pressure since the baratron is measuring the pressure at the outlet of the cell. A 0.5% uncertainty in the pressure measurement leads to a change in the simulated peak of 0.31%. Another parameter for the simulation is the path length of the cell. The manufacturer reports a path length of 76.45 ±0.05 m which was confirmed within experimental error by measuring an ethylene absorption line. The uncertainty is due to uncertainty in the mirror radii of curvature. This uncertainty in the total path length leads to a change in the simulated peak of 0.07%.

The other major inputs to the simulation are the laser parameters. As the temperature of the laser or center current is changed the laser parameters change as well. Also, for the same settings on different days, the laser parameters change due to minor changes in the laser temperature within the setting. A similar analysis as described above was performed to investigate the affect of this change. The day to day changes led to peak differences between 2 - 3%, so the laser parameters were measured before the sensor was used each day. The laser parameters are measured while the laser is being modulated with the high frequency sinusoid, but without the slow scan. There is some variation in the laser parameters at different center currents, which is equivalent to the variation as the laser is scanned across the peak. It is important, therefore, to record the laser parameters for the laser settings corresponding to the peak, which is used to compare the measurement to the simulation. Since there is some uncertainty in the exact peak location it is important to quantify the sensitivity of the peak to the laser parameters at different center currents near the peak. Based on measurements without the scan, the peak was consistently within a
variation of 0.3 mA in the center current. Simulations using laser parameters in this range led to peak differences of 0.8%. There could also be some uncertainty in the laser parameters based on the analysis technique used to measure them. This was investigated by determining the laser parameters multiple times for the exact same laser settings over time. The resulting variation in the laser parameters led to peak differences between 0.6 - 0.8%. It is likely that the variation in the laser parameters at the different center current settings was due to measurement error rather than the small change in laser settings. Therefore, by measuring the laser parameters before using the sensor, the simulation has a sensitivity to the measured laser parameters of 0.8%.

Sensitivity analysis, shown in Figure 5.11, shows that the most significant input parameters to the simulation program are the line strength of line three and the collisional broadening coefficients for ammonia in nitrogen. Based on this analysis the uncertainty in the simulated peak value is found by combining the affects of the uncertainties in the inputs using the Euclidean norm. For ammonia mole fractions less than 1 ppm, when the affect of the carbon dioxide interference is included, the uncertainty in the simulated peak is found to be 4.05%. For levels above 1 ppm, the uncertainty is found to be 3.92%. Since the measured mole fraction is proportional to the simulated peak, the uncertainty in the peak can be directly translated to uncertainty in the measured mole fraction.
Figure 5.11: Affect of the uncertainty in various input parameters on the simulated WMS $2f/1f$ peak
5.5 WMS Sensor Characterization

In order to qualitatively verify that the WMS strategy was functioning, tests were performed to compare WMS to DAS. The amount of ammonia was measured in the cell for a flow experiment using WMS; once the amount of ammonia approached steady state, the laser settings and modulation was changed to measure the amount of ammonia using direct absorption. These tests were performed at pressures below 20 torr so that the six ammonia lines could be distinguished. Scanned direct absorption, where the whole absorption feature is fit with a Voigt profile to determine the ammonia mole fraction, as well as fixed point direct absorption, where the peak absorbance is compared to a simulated absorbance profile to determine the ammonia mole fraction, were both used to verify the WMS measurement. Results for a mixture of ammonia at 9 ppm are shown in Figure 5.12. The mole fraction is shown in the figure.

Figure 5.12: Verification of Wavelength Modulation Spectroscopy by comparison to Direct Absorption Spectroscopy
for three different direct absorption methods, one of which was fixed point using the peak of line three, another was scanned from line three, and the last was the average for scanned lines 3 - 6. The first two lines were not included in the average because their close proximity made them indistinguishable, and the resulting uncertainties were much larger than for the other lines. The measured ammonia mole fraction by WMS clearly falls within the experimental uncertainty of the mole fraction measured by direct absorption. Similar tests were performed for ammonia mixtures of 2 and 6 ppm with similar results.

It is also important to quantify the sensor’s detectability limit and sensitivity. To do this, a test was performed by tracking the measured ammonia mole fraction as a small amount of ammonia was added to nitrogen flowing through the cell at 1 liter per minute. Figure 5.13 shows the results for this test. Initially pure nitrogen was measured, then a 9 ppm ammonia in nitrogen mixture was added at a slowly increasing fractional flow rate, then the ammonia mixture was turned off so that

Figure 5.13: Characterization of the sensor using WMS to measure low levels of ammonia
just nitrogen flowed through the cell. It can be seen that the measured amount of ammonia quickly decreases with little residual ammonia as the cell is flushed with nitrogen. Figure 5.14 shows a more detailed analysis of the measurements as ammonia is first added, including the uncertainty in each of the measurements.

Figure 5.14: Characterization of the sensor using WMS to measure low levels of ammonia

The sensor measured an ammonia concentration of less than 10 ppb when pure nitrogen was in the cell; this erroneous measurement is due to fluctuations in the background signal. The background signal used for these tests was the average of 10 tests with pure nitrogen flowing through the cell. Figure 5.15 shows how the measurement of pure nitrogen gives a non-zero peak value after background subtraction. Some erroneous peaks were ignored by only recording the maximum value from each of the peak regions, shown as the shaded region. When the detectability limit is defined as when the ratio of measured peak to background peak is one, the detectability of the sensor is 10 ppb. This is essentially when the signal to noise ratio is 1.

After the addition of the ammonia mixture, peaks begin to become distinct from
the background signal, as seen in Figure 5.16. When the detectability limit is defined as when the ratio of measured peak to background peak is two, the detectability of

![Figure 5.15: WMS 2f/1f results for pure N$_2$, this noise results in measurement of 7.3 ±2.1 ppb](image)

![Figure 5.16: WMS 2f/1f results after initial addition of ammonia leading to a measurement of 18.3 ±3.3 ppb](image)
the sensor is 18 ppb. The peak value used to determine the ammonia mole fraction is the average of 15 peaks, fewer are shown for clarity. The measurement uncertainty is defined as the standard deviation in measured peak value. The uncertainty in the peak value at a measured ammonia mole fraction of 18.3 ppb is 18.2%. Combining this uncertainty with the uncertainty in the simulated peak using the euclidean norm leads to a total uncertainty of 18.6% or 3.4 ppb.

Since this sensor has been designed to measure the amount of ammonia in exhaled breath it is important to quantify the sensitivity near expected values in breath. Healthy patients are expected to have anywhere from 150 to 500 ppb ammonia, while patients with CKD are expected to have greater than 1 ppm ammonia. Figure 5.17 shows the signal for a measurement of 155 ppb, near the lowest required ammonia level to be measured. In this case the peaks are clearly distinguished from the background level. The measurement uncertainty is found to be 2.1% leading to a total uncertainty of 4.58% or 7.1 ppb.

Figure 5.17: WMS 2f/1f results after initial addition of ammonia leading to a measurement of 154.6 ± 3.3 ppb
Chapter 6

Adsorption

6.1 Theory

As mentioned previously, ammonia tends to adsorb to the surfaces of the cell, making it difficult to measure the amount of ammonia in the gas sample. A brief description of the adsorption phenomenon is given here to describe the challenge adsorption contributes. The Langmuir theory describes the adsorption process considering only monolayer adsorption as a reaction between a gas phase molecule, $A$, and a surface site, $S$, to form an occupied site, $AS$.

$$A + S \xrightarrow{k_a/k_d} AS$$ (6.1)

where $k_a$ is the rate of adsorption and $k_d$ is the rate of desorption. The rate of change of the occupied sites is then

$$\frac{d[AS]}{dt} = k_a[A][S] - k_d[AS]$$ (6.2)

and at equilibrium

$$[AS] = \frac{k_a}{k_d}[A][S]$$ (6.3)
If the fraction of surface sites occupied is defined as \( \theta \) \((0 < \theta < 1)\), \([AS]\) is proportional to \(\theta\) and \([S]\) is proportional to \((1 - \theta)\). Also, since \([A]\) is proportional to its partial pressure, \(\theta\) can be determined by

\[
\theta = \frac{bp}{1 + bp}
\]  
(6.4)

where \(p\) is the partial pressure of the molecule undergoing adsorption and \(b\), the equilibrium constant, is the ratio of the adsorption to desorption reaction rates. Equation 6.4 is known as the Langmuir Isotherm \[25\]. At low pressures the fraction of surface sites occupied increases with pressure, while at high pressures it is independent of pressure.

One of the assumptions in Langmuir theory is that only a monolayer of adsorbed molecules is formed. The BET (Brunauer Emmett Teller) theory extends the Langmuir theory to include the affects of additional layers

\[
A + AS \overset{k_{2a}}{\underset{k_{2d}}{\rightleftharpoons}} A_2S
\]

\[
A + A_2S \overset{k_{3a}}{\underset{k_{3d}}{\rightleftharpoons}} A_3S
\]

and so on. The main difference in BET theory is that the fraction of adsorbed molecules continues to increase with pressure rather than approaching a constant \[26\].

As this is an equilibrium process it is important to understand what changes the equilibrium. The position of the equilibrium depends on three factors, the pressure, the temperature, and the stability of the molecule. The dependence on pressure was already discussed above. The temperature of the surface and the gas change the equilibrium constant, since it depends on the heat of adsorption, \(\Delta H_{ad}\). The fraction of surface sites occupied at equilibrium decreases as temperature increases. The
equilibrium also depends on the molecule and surface which are interacting, since the relative stability of the molecule in the adsorbed and gas phase will affect the equilibrium constant.

### 6.2 Implications for Sensor Design

Since the goal of this sensor is to measure the mole fraction of ammonia in a gas sample, and adsorption is a process which changes the amount of ammonia in the gas phase, its affect needs to be studied and reduced, if not eliminated. Figure 4.10 showed how using an all glass cell rather than a metallic cell decreased the affect of adsorption, however, with the Aerodyne multi-pass cell, the non-glass surfaces can not be eliminated so other measures must be taken to deal with the adsorption issue.

A detailed investigation of the affects of ammonia adsorption was performed for the design of an ammonia sensor based on photoacoustic spectroscopy (PAS) [27]. Since the adsorption process is unique to the cell and the previous ammonia exposure, a closed cell will equilibrate to ammonia levels that are not reproducible and therefore cannot be corrected to calculate the actual value in the initial gas sample. In the case of a flow experiment, the adsorbed molecules can be replaced by new molecules entering the cell and molecules that desorb are carried out by the flow. Therefore, after a period to reach saturation, equilibrium conditions are reached and the effective adsorption rate decreases rapidly. The ammonia levels were found to be reproducible irrespective of previous cell conditions once the equilibrium was reached under continuous flow conditions. For the adsorption effect to be negligible, it was necessary to have a flow that was high enough for the equilibrium to be reached on a time scale shorter than the gas-exchange time. Reliable measurements can, therefore, only be performed under continuous flow conditions after the equilibrium is reached. The period to reach equilibrium can be reduced by increasing the flow rate or by
increasing the temperature of the surface and the gas which shifts the equilibrium to the side of desorption. The conclusion of this investigation was four recommendations in the design of sensors for strongly adsorbing molecules. The surface of the cell and tubes should be smooth and as small as possible, the material should be chemically inert, the experiment should utilize high flow velocities, and the cell should be able to be heated. These conclusions were specifically for sensors utilizing PAS, but similar principles can be used in the design of other types of sensors.

A major difference between PAS and the sensor designed in this research is the size of the cell. Typical PAS cells have a volume less than 20 cm$^3$ [8, 13] while the multi-pass cell used for this research has a volume of 500 cm$^3$. The larger volume, and thus surface area, may require higher flow rates to achieve negligible effects from adsorption. For sensors utilizing multi-pass cells, the flow rate is limited by the cell volume and pressure. The limited volume of the breath sample also limits the test time for a given flow rate. Conditions must be chosen carefully to reach equilibrium at the desired pressure and flow rate in the time available. Some previous ammonia breath sensors have used flow at low pressures [4, 7], while others heat some of the sensor components to around 40 °C in addition to flow [8, 11, 12].

6.3 Verification of Equilibrium for Flow Conditions

These trends were verified for this sensor by measuring the ammonia mole fraction in the cell as an ammonia-nitrogen mixture flowed continuously through the cell. Figure 6.1 shows the results for four different tests. The first test was performed after the cell was evacuated for 12 hours; since the cell was initially far from equilibrium, it approached the equilibrium slowly and continued to increase slowly, showing that it didn’t reach equilibrium during the test time. The second and third tests were performed afterwards with only 10 minutes of evacuating the cell. These approached
the equilibrium faster as the cell was already close to equilibrium. The final test was performed with the cell heated to 35 °C. The fast response was due to both the heating as well as being close to equilibrium initially due to the previous tests.

![Graph showing equilibrium X\(_{NH_3}\) for clean, saturated, and heated cell](image)

Figure 6.1: Comparison of equilibrium X\(_{NH_3}\) for clean, saturated, and heated cell

This verification was performed allowing a long time to reach equilibrium and using an ammonia-nitrogen mixture with a higher amount of ammonia than typical in breath. An additional test was therefore necessary with ammonia at typical amounts and with the limited volume allowed by the sample breath bag. Figure 6.2 shows the measured amount of ammonia during a continuous flow test of a mixture of ammonia in nitrogen, carbon dioxide, and oxygen from the sample breath bag as well as the pressure in the cell during the measurements. Around 85 seconds into the test the pressure drops suddenly because the bag is empty. The bag volume is 1 liter, so the flow rate is around 700 sccm (standard cubic centimeters per minute), this is significantly higher than the flow rate for the sensor designed based on the above
mentioned investigations, which was 300 sccm [13]. This flow rate was necessary, as was predicted, since the cell is much larger. This verification test showed that this flow rate and sample volume are adequate to reach the equilibrium flow conditions. The time required to reach the equilibrium in this case is shorter than in the previous calibration tests because the amount of ammonia is lower and therefore the disturbance from equilibrium is smaller. The equilibrium can be reached even faster by heating the cell and saturating the cell so it is close to equilibrium before the sample is measured.

Figure 6.2: Flow experiment for a mixture of ammonia in nitrogen, carbon dioxide, and oxygen from the sample breath bag

The same method was used for breath samples, however, the results were slightly different. As can be seen in Figure 6.3, the amount of ammonia in the cell decreases as it approaches equilibrium rather than increasing as in the previous case. The major difference in the ammonia mixture and the breath is that breath also contains water vapor. The significance of this is that water vapor is a molecule which also tends to
The water vapor adsorption reaction competes with the ammonia adsorption reaction.

\[
NH_3 + S \xrightleftharpoons[k_{1d}]{k_{1a}} NH_3S
\]  
\[
H_2O + S \xrightleftharpoons[k_{2d}]{k_{2a}} H_2OS
\]  

Initially, ammonia molecules are occupying the adsorption sites, but when the water vapor molecules are present they can replace the ammonia disturbing the equilibrium and causing a net desorption of ammonia. Over time, with continuous flow, the equilibrium is re-established and the ammonia level still approaches the amount in the incoming sample. Figure 6.4 shows the trends for breath samples with higher amounts of ammonia, in which the amount of ammonia initially decreases and then increases as it approaches the equilibrium. The odd behavior in the amount of ammonia in the first 10 seconds is due to large uncertainty in the pressure measurement as the pressure
rises quickly, after this the pressure is known within the reported uncertainty and the measured mole fraction can be trusted. Due to this disturbance from the equilibrium it takes longer to reach equilibrium so the breath samples are consumed before a completely steady equilibrium is achieved. Further tests will be done to investigate the effect of the water vapor adsorption and develop flow configurations to achieve equilibrium before the sample is consumed.

Figure 6.4: Flow experiment for breath samples with various amounts of ammonia
Chapter 7

Medical Applications of the Ammonia Breath Sensor

7.1 Previous Research

The medical significance of the presence of ammonia in breath has been studied previously, demonstrating the usefulness of an ammonia sensor to diagnose and monitor a variety of medical conditions.

For healthy individuals, ammonia is present in exhaled breath at typical levels of a few hundred ppb. Research has linked chronic kidney disease (CKD) to elevated levels, greater than one ppm \(^{[4]}\). In addition to the diagnosis of CKD, ammonia breath sensors have been used during dialysis to monitor the treatment. A study was done to measure the ammonia levels in breath of patients undergoing hemodialysis who had CKD. Before dialysis the ammonia levels were between 1.5 and 2.0 ppm. The levels dropped sharply during the treatment and eventually reached levels between 150 and 200 ppb. The study also compared the breath measurements to blood tests, correlating the breath levels to Blood Urea Nitrogen (BUN) and Creatinine. Breath measurements have an advantage over blood tests in that they can be performed
more frequently as well as during the treatment. Currently, blood tests are only done once a month and blood is only taken before and after the treatment. The study indicated that breath ammonia measurements could improve the quality of renal care by providing a fast, painless, cost-effective in situ monitor to measure dialysis progress in real time [1].

Breath ammonia measurements may also be a feasible diagnostic test for the Helicobacter Pylori Infection. A study was done in which subjects who tested positive for H. Pylori responded differently than subjects who tested negative for H. Pylori. Ammonia levels in breath were recorded while subjects breathed normally; the patients then ingested a Urea tablet and continued breathing. Positive subjects showed a rapid rise in ammonia during baseline and minimal change after taking the tablet, while negative subjects showed a rise in ammonia after taking the tablet [2].

Breath ammonia measurements have also been shown to have clinical significance for the diagnosis of encephalopathy associated with hyperammonemia. It was shown that patients with chronic liver disease had elevated breath ammonia levels when their blood ammonia concentrations increased above 90 µg/dl; the normal range is between 12-66 µg/dl. Patients who had relatively higher breath ammonia concentration when compared to blood ammonia concentration were found to have subclinical encephalopathy [3].

Based on the established link between ammonia breath concentration and adverse medical conditions, the development of accurate sensors can improve medical treatment. Laser based sensors show great potential as they can achieve high sensitivity, provide real-time analysis, and their size makes them portable. In this research, a novel laser-based sensor was designed utilizing a unique strategy to achieve improved sensitivity. It is now proposed to use this sensor to investigate the potential diagnostic and monitoring applications for the ammonia breath sensor. The research is divided into two phases, phase one focuses on the qualitative diagnostic potential of
the ammonia sensor, while phase two demonstrates the use as a monitoring tool.

## 7.2 Breath Testing Methods

The medical investigations were designed based on previous research and collaboration with doctors of Internal Medicine at the International Medical Center (IMC) in Jeddah, KSA. The research proposal was submitted to IMC and was approved by the internal review board.

### 7.2.1 Phase 1

The objective of phase one is to investigate the qualitative diagnostic potential of the ammonia breath sensor. As shown in previous research, ammonia levels have been used to diagnose CKD, liver disease, encephalopathy, and Helicobacter Pylori infection. In the first two conditions, the elevated ammonia level was linked to the medical condition. In the last two conditions, the ammonia breath level was used in conjunction with other tests or procedures to be linked to the medical condition. The primary focus of this phase is to determine if the ammonia level alone can be used to diagnose a medical condition. It will involve the comparison of ammonia levels in exhaled breath of volunteers who are healthy, diagnosed with CKD, and diagnosed with liver disease.

Expected results are that healthy volunteers will have significantly lower ammonia levels than patients with medical conditions. Quantities of interest would then be the ammonia levels for patients diagnosed with CKD or liver disease. One possible result may be that levels in both cases are elevated but fall in the same range. This would mean an ammonia breath diagnostic could be used to indicate an adverse medical condition but further tests would be needed to determine the specific medical condition. Another possible result may be that one or both of the cases would not
have elevated results to distinguish it from the healthy case. This would mean that normal levels do not necessarily mean a negative result for the diagnostic.

Doctors at IMC selected patients who are diagnosed with CKD and liver disease. The doctor facilitates the acquisition of the sample at the IMC and labels the sample with a generic code identifying either chronic kidney disease (CKD) or liver disease (LD), i.e. CKD1, CKD2, etc. Only the doctor has the information connecting the patient to the label, in order to protect the anonymity of the donor. The samples are then collected and tested to measure the level of ammonia in the breath sample.

There are twenty four donors for this phase, eight for each of the categories of healthy, CKD, and liver disease. This sample size is large enough to show a correlation for positive or negative results of the diagnostic test. The sample size was calculated using methods accounting for the expected difference in positive and negative levels, the standard deviation, statistical significance and power [28].

At the same time the patients give the breath sample they also have a blood sample taken by the medical staff at IMC. The ammonia level in the breath can then be compared to the BUN in the blood to develop a correlation. The sample size is not large enough to definitively develop a correlation but this will be used as a pilot study to determine if further tests can be done to potentially replace the blood test with the non-invasive breath test.

7.2.2 Phase 2

The objective of phase two is to investigate the potential of the ammonia breath sensor to be used as a tool to monitor dialysis treatment. The first goal is to determine how ammonia levels in exhaled breath change while a patient undergoes dialysis. To do this, ammonia breath measurements are taken during dialysis. Previous research suggests that ammonia levels will initially be high before dialysis and then drop during dialysis, eventually reaching a level similar to the healthy volunteers of phase one.
The second goal is to relate the ammonia levels in breath to the currently employed indicator of dialysis adequacy. Currently, dialysis treatment adequacy is determined through the use of the urea reduction ratio (URR) which compares the BUN before and after dialysis.

\[
URR = \left(\frac{\text{BUN}_{\text{Before Dialysis}} - \text{BUN}_{\text{After Dialysis}}}{\text{BUN}_{\text{Before Dialysis}}}\right) \cdot 100\% \tag{7.1}
\]

Breath samples taken before and after dialysis can then be compared to the BUN. The results can be used to determine if the breath ammonia tests would be a suitable replacement for the URR. The added benefit would then be that this would be a much easier way to track the adequacy of the dialysis in real time. The ammonia breath test is easier and gives more information because it can give immediate results and can be used during every dialysis rather than taking a day to get results and being administered only once a month, as is the case with the URR.

### 7.3 Breath Sample Bags

Breath samples are taken by the hospital staff at the IMC according to the operating instructions found in Appendix A. The SKC Exhaled Breath Sample Bags 239 Series are made of 4-ply low-background Flex Foil PLUS material. These bags are specifically designed for collecting and storing human breath samples. A study was done to investigate the suitability of these bags for storing ammonia for short periods of time; 100% of the ammonia was recovered after 2 hours and over 90% of the ammonia was recovered after 6 hours [29]. The study also recommended a procedure for cleaning the bags to make them suitable for reuse. The cleaning procedure involved emptying the bag, flushing it with room air, filling it with zero air for 24 hours, emptying it, then refilling it with zero air again to measure the residual gas concentrations. Following this procedure the bags were found to have less than 25 ppb residual ammonia.
Another study investigating the bags for breath research included heating to 45°C as part of the cleaning procedure [30].

Tests were performed to verify the use and reuse of the bags. A new bag was filled with nitrogen and then the amount of ammonia in the bag was measured. The bag was found to have over 30 ppb of ammonia so the bag was filled and evacuated with nitrogen four times to clean it. It was then refilled with nitrogen to measure the amount of ammonia remaining. Figure 7.1 shows the results of this test; after cleaning the bag the ammonia level was measured to be less than 10 ppb, the detectability limit of the sensor. After being used for breath samples the bags were filled with nitrogen and evacuated twice, then filled with nitrogen and stored at room temperature for a day. Before reusing them, the bags were evacuated and filled with nitrogen twice then tested to measure the residual ammonia. Figure 7.2 shows that for each bag, regardless of the amount of ammonia in the original breath sample, this cleaning

Figure 7.1: Residual ammonia in a new sample bag before and after flushing with nitrogen
procedure led to measured ammonia levels again less than 10 ppb and, therefore, verifies the reusability of the bags.

![Graph showing residual ammonia in a new sample bag before and after flushing with nitrogen.]

Figure 7.2: Residual ammonia in a new sample bag before and after flushing with nitrogen

Based upon the above study, the time between taking the breath sample and measuring the ammonia concentration must be as short as possible and standardized for all samples. The sample bags are reused throughout the various phases. Bags are flushed according to the protocol advised and the fittings are cleaned according to the manufacturer instructions before each use. The bag will only be reused by the original donor as an added precaution to protect the donor.

### 7.4 Sample Analysis

Since ammonia tends to adsorb to the walls of the cell, the breath samples will be analyzed by flowing the breath sample through the cell. Initially the cell is evacuated
and the bag is connected to the input line to the cell. The bag output septum valve is then opened and the sample begins to flow into the cell. The vacuum pump and input valves are set to regulate the flow for ideal measurement conditions. It takes around 30 seconds for the pressure in the cell to rise to near 100 torr, after which the pressure remains relatively constant for 45 seconds at which point the bag is empty and the pressure begins to drop. This is adequate time for the measured ammonia level to reach the steady state value equal to the amount in the sample. The cell is then flushed with nitrogen and evacuated to remove any trace ammonia before the next sample is measured.

7.5 Initial Results

Initial tests were performed to validate the sensor for breath measurements as well as check the expected trends in patients with CKD before and after dialysis. Breath samples were taken from five patients with CKD before and after their dialysis treatment. The results of these tests were not what was expected, but some conclusions can be drawn from them and future tests have been designed.

The reported ammonia level for the test is the average measured value for the last five measurements, taken over 10 seconds, before the pressure dropped due to the sample being consumed. The reported uncertainty combines the uncertainty from the standard deviation in these five measurements, the measurement uncertainty which comes from the standard deviation in WMS $2f/1f$ peak height over 15 scanned peaks, and the simulation uncertainty based on the uncertainties of the input parameters. Figure 7.3 shows the results for patient CKD3 including the reported ammonia level and uncertainty.

It was expected that the patients with CKD would all have ammonia levels above 1 ppm before dialysis and then below 500 ppb after dialysis. However, only patient
CKD2 had ammonia levels above 1 ppm before dialysis, patient CKD3 had ammonia levels near 500 ppb, the upper range expected for healthy patients, and the rest had ammonia levels within the expected healthy range. All of the patients had similar ammonia levels after dialysis, consistently around 100 ppb. Figure 7.4 shows a comparison of before and after dialysis ammonia levels for all five patients.

A Breath Ammonia Reduction Ratio (BARR) can be calculated to determine the percent decrease in breath ammonia.

$$BARR = \left( \frac{X_{\text{Before Dialysis}} - X_{\text{After Dialysis}}}{X_{\text{Before Dialysis}}} \right) \cdot 100\% \quad (7.2)$$

Figure 7.5 shows the BARR for each patient. All of the patients had a reduction in their breath ammonia level due to dialysis, but for some it was not very significant.
Figure 7.4: Results of before and after dialysis ammonia levels for all five patients

Figure 7.5: Breath Ammonia Reduction Ratio for all five patients
Blood tests were performed for patients CKD3 and CKD4, so a comparison between the BUN and the breath ammonia level was made. Figure 7.6 shows that for each patient a decrease in the BUN was accompanied by a decrease in the breath ammonia level, however the two patients responded in drastically different ways. Since the dialysis adequacy is measured by the URR a comparison between the URR and BARR can be made. Figure 7.7 shows this comparison for patients CKD3 and CKD4. Dialysis is considered successful when the URR is greater than 65% [1]. While the BARR and URR are not the same, they do give the same qualitative measure of adequacy.

Figure 7.6: Breath ammonia levels compared to BUN for patients CKD3 and CKD4

Since the results were not as expected it is important to determine if there were errors in the measurement procedure or if the initial assumptions need to be reassessed. With the exception of patient CKD2 all of the results were lower than expected. One potential source of error is the sample storage in the breath bags. Since the samples
were transported from the hospital to the research facility the samples were not measured until 2 - 3 hours after being taken. The study on the suitability of the sample bags for ammonia storage showed that ammonia levels do not change for the first 2 hours and then lose less than 10% after 6 hours. This would not account for the low values observed here, however further tests need to be done to verify this result.

Another potential source of error is the sensor itself. However, since the measured ammonia level for patient CKD2 before dialysis was so high it does not appear to be a systematic error. Further tests need to be performed to study known amounts of ammonia in breath mixtures to verify the sensor performance. There could also have been error in the sampling procedure for each patient. Lastly, the assumption that all patients with CKD will have elevated ammonia levels needs to be questioned and confirmed by more tests.
Chapter 8

Future Work and Summary

8.1 Future Research Work

8.1.1 Sensor Verification

Chapter 6 showed that the adsorption of ammonia in the cell is an important challenge to address and one that needs to be studied carefully. The current work showed that with continuous flow the measured ammonia level approaches an equilibrium. Further tests need to be performed to first show that the equilibrium reached in the limited time available for breath tests is in fact the final equilibrium and second to show that this equilibrium is in fact the ammonia level in the incoming sample.

Tests with larger volumes from multiple breaths can be performed to determine the relationship between the equilibrium level reached from a single breath and the final level reached after longer time. If there is a significant difference a correction factor can be developed or the flow conditions can be adjusted to reach equilibrium within a single breath sample. To verify that the equilibrium is the actual amount in the breath sample the repeatability of the measurement must be shown. Since there is some variation in consecutive breaths a mixture can be made in a larger volume of multiple breaths. Once the mixture is uniform it can be measured multiple times
under different flow rates and cell saturation conditions to verify that the equilibrium ammonia level is consistent.

The results of the breath tests in Chapter 7 suggested that the suitability of the bags to store ammonia for up to 3 hours needed to be verified. A similar large volume of breath can be made and then tested in the bag immediately and after various times to study this affect. Extra ammonia can be added to the breath mixture to study the affect for different amounts of ammonia.

8.1.2 Medical Tests

As discussed in the initial results of the medical tests in Chapter 7 there is some disagreement between the expected results and the initial results. If it is found that there is no significant source of error in the sensor itself, either the sampling method led to errors, or the assumptions about the expected ammonia levels in CKD patients is wrong. The first test to explore this will be to take two samples from each of the patients before dialysis again. Consecutive breaths should be quite similar so if there is large variation from one sample to the next there may be errors in the sample and measurement method. If the two samples for each patient agree, but similar variations in the amounts between patients, as found initially, are present, then it may be that not every patient with CKD shows the same elevated ammonia level in their exhaled breath. Measuring the ammonia in the exhaled breath may not be a feasible diagnostic in this case. Based on the initial results, however, the ammonia levels do decrease after dialysis so the sensor could be used as a monitoring tool, but it would need to be specific to each patient.

As outlined in Chapter 7, phase one and phase two of the medical tests still need to be performed to confirm or clarify the initial results. It would also be beneficial to develop another sampling method to eliminate the potential for losses to the sample bags and in order to perform online monitoring of breath.
8.2 Summary

8.2.1 Ammonia Sensor Design

Laser-based ammonia sensors have been designed for a variety of applications including environmental pollutant monitoring, atmospheric and combustion kinetic studies, and breath monitoring for medical applications. This sensor is specifically designed to measure ppb levels of ammonia in exhaled breath to be used as a medical diagnostic and monitoring tool. Previous sensors have been designed with the same application in mind, so the goal of this sensor is to demonstrate improved sensitivity using a novel measurement technique. HITRAN simulations of ammonia and interfering species were used to determine the sensor characteristics to achieve the optimized sensor performance. WMS-2f/1f normalization is employed to measure the ammonia concentration based on the six ammonia absorption lines near 1103.46 cm$^{-1}$ with a multipass cell of 76 m at measurement pressures near 100 torr.

8.2.2 Ammonia Line Parameter Tests

The development of a calibration-free sensor requires that the ammonia absorption line parameters be known. These parameters include the linestrengths for all six lines as well as the broadening coefficients for each line in each of the major bath gases present in exhaled breath: nitrogen, oxygen, water vapor, and carbon dioxide. Due to the close spacing of the lines and only recent development of tunable lasers in this region this is the first experimental study of the selected ammonia lines with absorption spectroscopy. They are included in the HITRAN [18] database with the linestrength based on theoretical calculations and measurements by Laser Stark Spectroscopy [31]. The linestrengths are listed as having uncertainties between 10 - 20%. In this work the linestrengths were measured in agreement with those given in HITRAN while having uncertainties less than 10%. This is also the first measurement of the collisional
broadening coefficients for the selected lines in nitrogen, oxygen, carbon dioxide, and water vapor, many of which were measured with uncertainties less than 5%. These are useful results because in addition to being required for the development of this sensor, they are fundamental properties of the lines and are therefore beneficial for the extension of this sensor to other applications.

8.2.3 Sensor Implementation

The sensor is used to determine the ammonia mole fraction in the gas sample by measuring the $WMS-2f/1f$ peak height and comparing it to the simulated peak height. WMS is implemented by modulating the laser wavelength sinusoidally at 10 kHz. The peak height is measured by scanning over the peak with a low frequency sinusoidal modulation of 80 Hz in addition to the high frequency modulation. The sensor is calibration-free because the simulated peak height can be determined based on the ammonia absorption line parameters and the laser parameters describing the wavelength and intensity modulation of the laser, which are measured and known in advance. The sensor uncertainty comes from two sources; the simulation and the measurement. Sensitivity analysis was used to determine the affect of the uncertainties of the input parameters on the simulated peak height, leading to an uncertainty of 4.05%. The uncertainty in the measurement comes from deviation in the measured peak height; this uncertainty decreases as the amount of ammonia in the gas increases. Pure nitrogen was measured to determine the detectability limit of the sensor, found to be 10 ppb. Small amounts of ammonia were added to the nitrogen to determine the detectability limit for a signal to background ratio of 2. Under these conditions the sensor was able to measure ammonia at $18.3 \pm 3.4$ ppb which included the uncertainty from the measurement and the simulation. At typical breath levels of 155 ppb, the total uncertainty was calculated to be 7.1 ppb or 4.58%.
8.2.4 Adsorption

The tendency of ammonia to adsorb to surfaces it comes in contact with makes it a difficult gas to study and measure practically. Based on previous research and experimentation with this sensor it was determined that the affects of adsorption can be neglected when the sensor measures a continuous flow of sample gas. Since adsorption is an equilibrium process, initial measurements after a change in the ammonia level will have errors due to the adsorption; however, with continuous flow, after equilibrium is established, the measured amount of ammonia is the actual amount in the incoming sample. The time it takes to reach equilibrium can be reduced by utilizing cell and sample lines which are chemically inert and as small as possible, increasing the flow rate, and heating the cell to shift the equilibrium towards desorption. The flow configurations for this sensor were such that the ammonia level approached equilibrium; however, the breath sample volume was not large enough for a high level of confidence that the measured amount had indeed reached equilibrium. Further tests are proposed to investigate this further.

8.2.5 Medical Application of the Ammonia Breath Sensor

A sensitive sensor to measure the amount of ammonia in exhaled breath has potential to be a useful diagnostic and monitoring tool since elevated levels of ammonia have been linked to various medical conditions. A medical research study was designed and proposed to the International Medical Center in Jeddah, KSA, to investigate these trends. The first phase of the research focused on comparing ammonia levels in the exhaled breath of healthy patients, patients diagnosed with liver disease, and patients diagnosed with chronic kidney disease (CKD) to explore the diagnostic potential of the ammonia sensor. The second phase of the research focused on measuring the ammonia levels from patients with CKD undergoing dialysis to investigate the use of the sensor as a monitoring tool. The breath ammonia levels before and after dialysis
can be compared to the blood tests currently used to measure the adequacy of the treatment. Initial results from patients with CKD before and after dialysis were not as expected but gave some indications for the development of future tests for further investigations. The initial tests demonstrate the proof of concept for the sensor to measure ppb levels of ammonia in exhaled breath. The medical tests proposed will be carried out as part of future work.
APPENDICES
Appendix A

Operating Instructions for Exhaled Breath Sample Bags
SKC Exhaled Breath Sample Bags are ideal for collecting and storing human breath samples. The samples are subsequently analyzed for volatile organic compounds (VOCs) or volatile sulfur compounds (VSCs). Exhaled Breath Sample Bags are constructed of 4-ply low-background FlexFoil® PLUS material. The special PVC exhaled breath fitting allows easy sample collection. The polypropylene sample removal fitting facilitates sample removal by gas-tight syringe for GC analysis or by a pump through a detector tube for immediate quantitative results.

Guidelines for Bag Sampling
1. Long-term storage of samples in bags is not recommended.
2. SKC sample bags are designed for sampling air at atmospheric pressure only. Attempting to pressurize the bag can result in bag rupture and sample loss. Do not ship bag samples by air freight in non-pressurized cargo cabin. Bags can burst under such conditions.
3. All federal and state packaging and transporting regulations apply.
4. Failure to follow warnings and cautions voids any warranty.
5. Bag fittings are durable, but not intended for use as handles or hanging devices. This type of handling may damage the seal causing leakage. It is considered misuse and will void any warranty.
6. Do NOT reuse sample bags.

Sampling

⚠️ Wear gloves when handling the bag and fittings (photos are shown without gloves for clarity).

⚠️ Before sampling, open and close exhaled breath fitting a couple of times to distribute fitting lubricant evenly.

1. Ensure exhaled breath fitting is in the closed position (hole on side of fitting visible).

2. Clean exhaled breath fitting with isopropyl alcohol and allow to dry completely before sampling.
3. Place bag on a flat surface. Place palm over exhaled breath fitting and press down firmly on fitting until it stops. The fitting is now open (hole on side of fitting not visible).

![Exhaled breath fitting (open position)](image)

⚠️ The sample removal fitting is shipped closed. It should remain closed until the sample is to be removed.

4. Have test subject place lips on the exhaled breath fitting and blow gently into the bag. Collect the desired volume of sample.

![Exhaled breath fitting (open position)](image)

⚠️ Do not fill bag more than 80% of its maximum volume.

![Incorrect Correct](image)

5. Immediately remove fitting from lips of test subject, quickly grip exhaled breath fitting at bottom of fitting (do not grip bag material), and twist and pull until hole in fitting appears above bottom part of fitting. The fitting is now closed (hole on side of fitting visible).

![Exhaled breath fitting (closed position)](image)

6. Replace bag(s) in original packaging and ship to a laboratory for analysis.
Removing the Sample from the Bag

The sample can be removed from the bag using either of the following procedures. Select the procedure that best fits your application.

**Procedure 1:** Using a gas-tight syringe and needle, carefully insert the needle into the septum port in the center of the brown cap on the fitting and pierce the septum. Use the syringe to withdraw the sample. Analyze sample.

⚠️ *Do not allow the needle to puncture the bag material when piercing the septum.*

**Procedure 2:** Open the sample removal fitting by gripping the side stem and turning the entire upper portion of the fitting (brown syringe port and white section) counterclockwise one revolution.

⚠️ *Do not turn side stem.*

Using a short length of tubing, connect the side stem on the sample removal fitting to the inlet of an appropriate detector tube and the outlet of the detector tube to a low flow sample pump. Pull the sample through the detector tube and read results on tube.

**Reference**

Ordering

<table>
<thead>
<tr>
<th>Description</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exhaled Breath Bags</td>
<td></td>
</tr>
<tr>
<td>1-liter maximum capacity, pk/3</td>
<td>239-01</td>
</tr>
<tr>
<td>3-liter maximum capacity, pk/3</td>
<td>239-03</td>
</tr>
</tbody>
</table>

Other SKC sample bags are available in FlexFoil PLUS, FluoroFilm, SamplePro®, FlexFilm, and Kynar® with fittings made of stainless steel, polypropylene, or PTFE in sizes up to 100 liters. SKC also manufactures custom sample bags. Contact SKC at 724-941-9701 or skctech@skcinc.com

For FlexFoil PLUS stability data, visit www.skcinc.com/instructions/1805.pdf.

For more product information or assistance with applications, contact SKC Technical Service at 724-941-9701 or skctech@skcinc.com.

SKC Limited Warranty and Return Policy

SKC products are subject to the SKC Limited Warranty and Return Policy, which provides SKC’s sole liability and the buyer’s exclusive remedy. To view the complete SKC Limited Warranty and Return Policy, go to http://www.skcinc.com/warranty.asp.
References


