ASSESSMENT OF THE APPLICABILITY OF TERAHERTZ SPECTROSCOPIC BREATH SENSING TOWARDS MONITORING TYPE 1 DIABETES MELLITUS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science

By

Jessica Rose Thomas
B.A. in Art History and East Asian Studies, Wittenberg University, 2011
Bachelor of Fine Arts, Wittenberg University, 2011

2015
Wright State University

Ivan R. Medvedev, Ph. D.  
Thesis Director

Chair, Department of Physics

Committee on Final Examination

Ivan Medvedev Ph.D.

Jason Deibel Ph.D.

Brent Foy Ph.D.

Robert E. W. Fyffe, Ph.D.  
Vice President for Research and Dean of the Graduate School
ABSTRACT

Thomas, Jessica Rose. M.S. Department of Physics, Wright State University, 2015. *Assessment of the Applicability of Terahertz Spectroscopic Breath Sensing towards Monitoring Type 1 Diabetic Mellitus*

Type 1 diabetes is a condition that cumulatively costs around $14.9 billion in medical expenses every year in the United States[1]. Besides being costly, the monitoring of this disease is invasive, painful, and often embarrassing to the afflicted individual; blood and urine testing is currently the daily method of monitoring blood glucose and ketone levels in the body of type 1 diabetics. Though the use of these samples is standard, another avenue for possibly determining blood glucose has not been completely explored. With over 3000 chemicals reportedly found in exhaled human breath, biomarkers associated with this disorder and many of the complications caused by it may exist and could be utilized in lieu of blood and urine samples. To examine breath for these markers our lab employs Terahertz radiation. Unlike competing methods of gas testing, the high specificity, speed of testing, and small sample size that the Terahertz spectral range affords is ideal for the medical field. Presented here are both the spectral assignments and the most recent results of our investigation into the exhaled biomarkers of type 1 diabetics utilizing Terahertz spectroscopy.
## Contents

Chapter 1 ................................................................................................................................. 1

1.1 Introduction ....................................................................................................................... 1
1.2 Type 1 Diabetes Mellitus ................................................................................................. 4
1.3 Ketosis vs. Ketoacidosis ................................................................................................. 7
1.4 Terahertz and Rotational Spectroscopy .......................................................................... 8
1.5 Advantages of Rotational Spectroscopy ..................................................................... 10

Chapter 2 .................................................................................................................................. 12

2.1 The relevance of Urea ..................................................................................................... 12
2.2 The Molecule ................................................................................................................... 13
2.3 Spectral Procedures ....................................................................................................... 15
2.4 Results ............................................................................................................................ 16
2.4 Discussion ....................................................................................................................... 25

Chapter 3 .................................................................................................................................. 26

3.1 The Relevance of Methyl Nitrate .................................................................................. 26
3.2 The Molecule ................................................................................................................... 27
3.3 Synthesis and Spectral Procedures ............................................................................. 27
3.4 Results ............................................................................................................................ 31
3.4 Discussion ....................................................................................................................... 35

Chapter 4 .................................................................................................................................. 36

4.1 Fluctuations of Blood Glucose .................................................................................... 36
4.2 Blood Glucose verses Volumetric Dilutions in the breath of T1 Diabetic ..................... 40
4.3 Extended data set: 90 points ......................................................................................... 45
4.4 Discussion ....................................................................................................................... 48

Conclusion ............................................................................................................................... 49

Appendix ................................................................................................................................... 51

Bibliography ............................................................................................................................ 52
List of Figures

Figure 1.1: ........................................................................................................ 5
Figure 1.2: ...................................................................................................... 6
Figure 2.1: .................................................................................................... 16
Figure 2.2: ................................................................................................. 17
Figure 2.3: ................................................................................................. 19
Figure 2.4: ................................................................................................. 19
Figure 2.5 ..................................................................................................... 21
Figure 2.6 ..................................................................................................... 23
Figure 2.7 ..................................................................................................... 24
Figure 3.1 ..................................................................................................... 29
Figure 3.2 ..................................................................................................... 30
Figure 3.3 ..................................................................................................... 30
Figure 3.4 ..................................................................................................... 31
Figure 3.5 ..................................................................................................... 32
Figure 3.6 ..................................................................................................... 32
Figure 3.7 ..................................................................................................... 33
Figure 3.8 ..................................................................................................... 34
Figure 3.9 ..................................................................................................... 35
Figure 4.1 ..................................................................................................... 37
Figure 4.2 ..................................................................................................... 40
Figure 4.3 ..................................................................................................... 42
Figure 4.4 ..................................................................................................... 46
Figure 4.5 ..................................................................................................... 47
Figure A.1 .................................................................................................... 51
List of Tables

Table 2.1 ........................................................................................................21
Table 3.1 ........................................................................................................33
Table A.1 ......................................................................................................51
Chapter 1

1.1 Introduction

Doctors heavily rely on blood and urine samples taken from patients to diagnose, monitor, and even predict the future effects of different pathologies in the human body. After all, blood is a connective tissue. Blood unites all the cells of the body to one another and provides a route for waste products to be carried away. Contained within this waste are chemical byproducts of metabolism and other processes in the body. The kidneys filter this waste out of the blood and rid the body of it in the form of urine. That being said, it is quite obvious that both blood and urine samples provide an invaluable resource of information about the body and how it is functioning at any given time. However, though urine and blood samples are wonderful in their versatility and the information that they furnish, acquiring these samples are often invasive and/or uncomfortable for the patient.

Type 1 diabetic mellitus [T1DM] is one of many different pathologies that affect humans. It requires regular trips to the hospital and its management calls for expensive medical equipment. T1DM afflicted individuals require daily blood tests, ketone tests, insulin injections/medications, and frequent trips to endocrinologists and registered nurses: these individuals must closely monitor their disease or risk serious and potentially deadly complications. Though T1DM patients can self-administer many of these tests using commercially purchased blood meters and test strips, these supplies are expensive. Unfortunately, some type 1 [T1] diabetics cannot afford the health care needed to properly treat this disease and struggle economically due to the extra financial burden placed on them and their families.[2]

Blood and urine analysis are powerful tools in the medical field; however, the expense, invasiveness, and embarrassment associated with these methods of testing detour people from providing these samples. With emerging technological advances taking place in the medical field, more focus should be placed on the development of non-evasive and cost efficient technologies to diagnose and monitor different pathological disorders like T1DM in the human body. If a new
affordable means of monitoring diseases could be utilized that lacked these negative attributes, then individuals would be more inclined to take the initiative to control their disease. This advancement may alleviate the economic struggle that many people feel and promote a healthier approach to controlling disorders.

Though not entirely a new idea, breath analysis remains a vastly unexplored field in medicine. Current breath analysis technologies rely heavily on Gas Chromatography Mass Spectrometry [GC-MS]. Though considered a gold standard for gas analysis, GC-MS is time consuming and requires daily calibrations to analyze gaseous mixtures. One drawback to this approach for medical applications is the delayed feedback that is needed to test rapidly fluctuating values such as breath chemicals related to blood glucose or ketones in the body. However, rotational spectroscopy may be utilized to provide quick and accurate readings. This method of testing may be an alternative to the current means of measuring the biologically relevant Volatile Organic Compounds [VOCs]. These compounds are associated with disorders in the human body and can be isolated in breath. Furthermore, the application of THz spectroscopy does not have to be limited solely to breath analysis. The use of rotational spectroscopy in medical applications could afford the medical community its speed and accuracy across many different disciplines.

T1DM is a prime example of a disorder in need of renovating testing methods. Millions of people across the world struggle with this disease and its effects medically, economically, and mentally. By both assigning spectra of T1DM relevant VOCs and analyzing them in the breath of healthy and T1DM afflicted individuals with rotational spectroscopy, it is the intent of this thesis is to illustrate the application of rotational spectroscopy in the medical field. Moreover, further development and verification of rotational spectroscopy technology in breath analysis, could provide an alternative to blood and urine samples used in medicine today. This investigation will provide a basic foundation for further study in the field of breath analysis and the potential correlations with detection of medically relevant VOCs.
This study begins by looking closely at the spectral assignments of urea and methyl nitrate. Chapter 2 of this thesis will focus solely on urea, whereas chapter 3 will be dedicated to exploring the spectral assignment of methyl nitrate. These chapters will also discuss the significance of these molecules for astronomers. Though these molecules have yet to be observed in breath by a THz breath sensor, they are of particular interest to T1DM, which will be discussed later. While our laboratory is on the brink of detecting methyl nitrate, this is not the circumstance with urea. Unfortunately, urea possesses a very low vapor pressure at standard conditions, which means that it may be a challenge to detect in the gaseous phase in breath. Regardless, urea is still of interest to this study as heated samples do evolve molecules into gas phase and produce spectra. Urea is known to be is secreted through the skin[3]. Like the sweat tests done on children suspected of cystic fibrosis, technology could be developed to measure urea excretion from the skin. This molecule is important for the monitoring of T1DM, as will be discussed later.

Chapter 4 discusses breath samples from healthy and T1 diabetic volunteers will be examined. The breath samples taken and analyzed in our laboratory focused on acetone, ethanol, acetaldehyde, hydrogen cyanide [HCN], and methanol. Acetone was chosen because it is a byproduct of fatty acid metabolism, which will be discussed in section 1.2 and 1.3. It has been suggested that breath dilutions of acetone possesses a correlation with blood glucose[4] and blood ketones levels [5]. Likewise, ethanol in body is argued to lead to a rise in blood glucose concentrations [6]. Acetaldehyde is a product of ethanol catabolism and therefore a worthy component of our list. Methanol was added to the list because is a small alcohol that is easily detectable within the body. The addition of HCN was done due to the fact that it readily detectable in most samples processed in our laboratory.
1.2 Type 1 Diabetes Mellitus

Diabetes mellitus is a growing health concern in the United States, where roughly 21 million people have been diagnosed with some form of diabetes and an estimated 8.1 million people remain undiagnosed [7]. The American lifestyle has been used to explain much of this growth. After all, type 2 diabetes mellitus [T2DM]—the most prevalent kind of diabetes mellitus—is heavily influenced by diet and exercise. However, a smaller subset of this data is caused by T1DM. Where a resistance to the naturally occurring hormone insulin causes T2DM, T1DM is the results of an autoimmune event that destroys the insulin producing cells in pancreas. For T1DM this results in the body’s inability to produce insulin in vivo. Though this disease accounts for only a fraction of the total number of diabetics, it is a condition that is still prevalent in our society: T1DM afflicts roughly 1.25 million Americans [7]. Furthermore, it has been estimated that by the year 2050 the individuals diagnosed with T1DM will nearly increase threefold [8].

The inability to produce insulin in T1DM individuals has lasting effects on their health. The incapacity to maintain a homeostatic level of blood glucose within the body has been known to cause many other medical complications, such as cardiovascular disease, stroke, neuropathy, reduced blood flow in the legs, diabetic retinopathy, and kidney failure [9]. It is estimated that the T1DM life expectancy when compared with non-diabetic individual is shortened about 11 years for men and 13 years for women [10].

Due to the nature of T1DM, close monitoring of blood glucose must be done to stave off these common medical complications. In a healthy individual the equilibrium of blood glucose is maintained mainly utilizing two hormones: Insulin and glucagon (figure 1).
Figure 1.1: Blood glucose homeostasis illustration. The range illustrated in white is the typical range of blood glucose in a healthy individual. When blood glucose elevates higher than the homeostatic ideal, insulin is released into the blood to lower it. Likewise, when the blood glucose level deviates lower than the homeostatic ideal, glucagon is added to the blood to promote stored glucose to be released into the blood.

Insulin stimulates the tissues of the body to uptake blood glucose whereas glucagon communicates to the body to break down the stored energy within the body into glucose[11]. Though every individual is different, non-diabetic fasting blood glucose typically fluctuates between 70-120mg/dl. Even after the consumption of a meal, the blood glucose of a healthy person does not linger above 200mg/dl long. For Type 1 [T1] diabetics that lack the capacity to produce insulin, the body has no direct pathway to lower the amount of sugar in the blood. Because of this, T1DM individuals must continuous perform blood glucose tests and correct any deviations from normal with insulin injections. Daily blood tests and insulin injections are costly. T1DM cumulatively costs around $14.9 billion in medical expenses every year in the United States[1]. Expense aside, the monitoring of this disease is invasive and often painful to the afflicted individual.

As the cells of the body are essentially starved of fuel, they have to rely on different chemical or metabolic pathways in order to produce enough energy to maintain crucial functions needed for life. Energy is typically produced in the body by the breakdown of sugar within cells. The citric acid cycle is one component of energy metabolism. Glucose, a sugar, is broken down into Acetal-CoA, which then is able to enter the citric acid cycle within the mitochondria. Glucose is the primary

1 The citric acid cycle is a series of chemical reactions that produces a small amount of adenosine triphosphate [ATP]—the energy currency in the body—as well as the needed materials for the electron-transport chain within the inner surface of the mitochondrial membrane. The electron transport chain is a process that creates the bulk of energy needed by the body to maintain life.
material that is used in energy production. However, alternate pathways are available in dire situations. Fatty acids and amino acids (i.e., protein) can be integrated into the energy metabolic pathway to also provide a supply of Acetyl-CoA via fatty acid oxidation and protein metabolism, respectively. However, fatty acids and amino acids, when used as the main route to produce energy, are not ideal and leave residual chemicals in the bloodstream.

Protein metabolism is the breakdown of amino acids within the body. When an amino acid enters the chain, the reactive amine group undergoes a transamination and oxidative deamination that leaves a keto-acid and ammonia as the byproducts [11]. (Figure 2) Ammonia, another promising breath chemical, is toxic to the body and it is quickly converted to the waste product urea, which is then filtered out of the body by the kidneys. Because the kidneys readily filter out urea, the amount of urea found in the blood is often used in medicine to measure the function of the kidneys.²

When fatty acids are metabolized they undergo a beta-oxidation to create Acetyl-CoA needed for the citric acid cycle. However, when large amounts of fatty acids are utilized by the body, the breakdown of these molecules produce more Acetyl-CoA than can enter the citric acid cycle. The excess Acetyl-CoA undergoes ketogenesis to produce ketone bodies: acetoacetic acid, β-hydroxybutyric acid, and acetone [11]. (Figure 2) These ketones are then released in the blood. When large amount of ketones are found in the blood—a state called ketosis—the body is forced to eliminate them by depositing them in the urine and lungs[11].

---
² This test is called the BUN test (Blood Urea Nitrogen)
1.3 Ketosis vs. Ketoacidosis

The ketones produced during ketosis in a healthy individual are an important component of metabolism. The state of ketosis is, in fact, utilized as a treatment for several different disorders, such as intractable seizures [12]. Furthermore, ketogenic diets are becoming very popular as a means of maintaining lean muscles and losing weight [13]. This is not to be mistaken for the diabetic ketoacidosis.

Whereas ketosis (also called nutritional ketosis) in healthy individuals is a normal and even beneficial part of metabolism, in diabetic individuals ketosis is a dangerous medical condition. Due to the impaired metabolic pathway in T1DM and T2DM individuals, the body cannot control ketosis. The out of control ketone production in the body causes the blood pH to shift acidic, which is lethal if not treated. This condition is called ketoacidosis. Ketoacidosis may occur when a T1DM individual suffers a biological stress event (e.g., sepsis, heart attack, infection) or low insulin.

There are tests that must be periodically done to test ketones in the blood of diabetic individuals. These tests are conducted by calculating the amount of a single molecule dissolved in the blood. This molecule is $\beta$-hydroxybutyrate—the anion for $\beta$-hydroxybutyric acid and one of the ketones produced during fatty acid metabolism [5]. Like blood glucose, there is a range of acceptable values for the amount of ketones that are dissolved in the blood of the human body. According to Diabetes.co.uk the range of ketone values in the blood are as follows:

- **Under 0.6 mmol/L** - a normal blood ketone value
- **0.6 to 1.5 mmol/L** - indicates that more ketones are being produced than normal, test again later to see if the value has lowered
- **1.6 to 3.0 mmol/L** - a high level of ketones and could present a risk of ketoacidosis. It is advisable to contact your healthcare team for advice.
- **Above 3.0 mmol/L** - a dangerous level of ketones, which will require immediate medical care [14].

Individuals that fall above the 1.6 mmol/L range of ketones present in the body are utilizing fatty acids as a primary means of creating energy for the body. Fatty acid metabolism would only dominate
the metabolic pathway for energy if there were insufficient glucose molecules within the cells of the body. For T1DM individuals, this would allude to poorly controlled blood glucose and insulin levels in the body. Like the daily blood tests that T1DM individuals must perform daily upon themselves, the tests for ketones in the body should also be performed on a regular basis. Blood meter and urinalysis strips that test for ketones can be purchased over the counter. The costs of these tests, alongside the blood tests and insulin injections are a large part of the healthcare costs that plague T1DM individuals.

Though this use of blood and urine is standard for T1DM individuals, another avenue for possibly determining blood glucose has not been completely explored. With over 3000 chemicals reportedly found in exhaled human breath, biomarkers for blood glucose levels in the human body may exist and could be utilized in lieu of blood samples. Breath analysis could replace the invasive procedures incorporated in the daily life of T1DM people, and, if the right technology could utilized, cut the healthcare costs.

1.4 Terahertz and Rotational Spectroscopy

Terahertz [THz] radiation occupies the space between the infrared and microwave frequencies on the electromagnetic spectrum and shares some characteristics with both. This kind of radiation is non-ionizing, which means that it carries only enough energy to rotationally excite the molecule in gas phase. It can penetrate a wide variety of non-conducting materials, such as paper, wood, and plastics, thus making its application in very versatile in a variety of different fields. Some commonly known applications of THz radiation include technologies in medical imaging, security, communication, and, most importantly for breath analysis[15], high-resolution rotational spectroscopy[16].

The fundamental mechanism behind rotational spectroscopy is based on the fact that Terahertz radiation interacts strongly with quantized rotational energy states of polar molecules, and thus provides a route for chemical sensing utilizing molecular rotational transitions. Rotational spectra are only produced when THz radiation interacts with a molecule with a permanent magnetic
or electric dipole moment. Therefore, not every molecule produces a spectrum because not every molecule possesses a distinct dipole moment. However, rotational spectra are very sensitive to molecular structures. Because every molecule is unique, the rotational spectrum produced by THz radiation interacting with a specific molecule is distinct.

This spectral uniqueness can be understood by introducing the rotational constants that define the Hamiltonian in when solving the time independent Schrodinger’s equation for a heteronuclear, linear molecule. To consider this, let’s look at the rotation of a rigid linear molecule in isotropic space. The kinetic energy of this can be expressed as:

\[ T = \frac{1}{2} I_x \omega_x^2 + \frac{1}{2} I_y \omega_y^2 + \frac{1}{2} I_z \omega_z^2 \]

\[ = \frac{1}{2} I_x \omega_x^2 + \frac{1}{2} I_y \omega_y^2 \]

\[ = \frac{J_x^2}{2I_x} + \frac{J_y^2}{2I_y} \quad \text{(Eq. 1.1)} \]

In this equation \( I \) represents the moment of inertia along each axes and \( \omega \) represents angular velocity. \( J \) is the symbol used to represent the total angular momentum of this system. Where the z component would vanish due to the linearity of the molecule (\( I_z = 0 \)). Because of this, \( I_x = I_y = I \) for a linear molecule. The Hamiltonian operator for a linear rigid molecule is:

\[ \hat{H} = \frac{J^2}{2l} \quad \text{(Eq. 1.3)} \]

When plugging this operator into the Schrodinger’s equation the solution\(^3\) becomes apparent. Namely:

\[ \frac{J^2 \psi}{2l} = E \psi \quad \text{(Eq. 1.4)} \]

\[ E = \frac{J(J+1)\hbar^2}{2l} \quad \text{(Eq. 1.4)} \]

\(^3\) This is because \( \psi_m = Y_{jm} \) due to the fact that \( \psi \) must be one of the spherical harmonics.
From this it is possible to find the energy eigenvalue right out of the equation as being equal to 

\[ F(J) = BJ(J+1) \]

where B is a rotational constant that is equal to \( \frac{\hbar^2}{2I} \). It is now possible to solve for the separation between adjacent J transitions. This is demonstrated in equation 1.6:

\[
\nu_{J+1-J} = F(J') - F(J'') = B(J + 1)(J + 2) - BJ(J + 1) = 2B(J + 1)
\]

(Eq. 1.6)

This result directly shows that the 2B is the separation between adjacent J transitions for a linear ridged molecule.\(^4\) In reality this is not always the case. Not all molecules are linear or rigid: \( I_z \) isn’t always zero and potential energy must be accounted for in the Hamiltonian operator. With the added complexity of these new values to account for, the rotational constant B is joined by rotational constants A and C. In addition, when fitting molecular spectra to the parameters of an effective Hamiltonian, these constants alongside a set of other parameters allow for the introduction of the potential energy operator. The A, B, and C constants are very useful in understanding a molecule, as they are dependent on the specific molecular geometry, and are inversely proportional to the moment of inertia along its axis. With these constants it is possible to create predictions of what the molecule’s rotational spectra looks like along the electromagnetic spectrum.

### 1.5 Advantages of Rotational Spectroscopy

The focus of the experiments conducted during the course of our investigation into T1DM was on a selection of VOCs. These VOC’s were selected for both for the information they could indicate about the processes in the human body as well as the feasibility of detecting them in the current settings of our THz spectrometer. After all, rotational spectroscopy requires polar molecules in a gaseous phase. Further, molecules with high symmetry, such as CO\(_2\), do not produce spectra due to the lack of a net dipole moment. Liquids and/or solids that do not possess high vapor pressures in most cases cannot be detected without high resolution.

\(^4\) This is due to the selection rule \( \Delta J = \pm 1 \)
For example, a molecule such as glucose would be hard to detect in breath. Glucose is a solid at standard conditions and is non-volatile. Further, there is no documentation that it can pass through the alveolar membranes of the lungs. In order to understand glucose within the body—a crucial task if trying to understand T1DM—then the focus of the investigation must be done on the chemicals produced by the breakdown of this simple sugar; Even though it cannot be seen in breath, the breakdown of glucose in the body may yield chemicals of interest that do possess the capability and volatility needed to be detected with THz rotational spectroscopy.

THz spectroscopy is versatile and sensitive to many VOCs. Unlike competing methods of gas testing like GC-MS, the high specificity, speed of testing, and small sample size that the Terahertz spectral range affords is ideal for the medical field. The total amount of sample needed to use this kind of spectroscopy for analysis is small. Breath is composed of thousands of volatile chemicals in trace amounts—typically on the order of parts per billion or smaller. Even these tiny amounts are detectable with this technology. Because of these advantages, the application of THz spectroscopy is a suitable choice when looking for the composition and dilutions of complex gaseous mixtures, such as breath.
Chapter 2

2.1 The relevance of Urea

Because poorly maintained T1DM individuals heavily utilize different metabolic pathways in order to sustain enough energy for vital functions in the body, urea is an important molecule in understanding and monitoring this disease. As mentioned before, as proteins are broken down to create fuel for the body urea is produced. This molecule then enters the bloodstream and is eventually filtered out of the body by the kidneys. By knowing the amount of urea present within the blood or urine of a particular individual, qualitative statements on the function of the kidneys and the metabolic pathway being utilized can be made. Urea is already used as a standard medicine in regards to the urinary system: its detection is the foundation of the Blood Urea Nitrogen [BUN] test that is performed in hospitals when a patient is suspected of having compromised kidney function.

Kidney disease is a common complication in T1 diabetics. It is estimated that diabetic individuals account for nearly 44% of people diagnosed with kidney failure, and that around 30% of T1DM individuals will eventually develop kidney failure during the course of their lives [17]. T1DM individuals suffer from kidney problems due to the imbalance of dissolved molecules—such as sugar—in their blood. This imbalance leads to a cascade of effects that forces your kidneys to work harder even though they are being damaged in turn [18]. Because of the large amount of diabetics that are afflicted with kidney problems due to their disease being able to monitor kidney function in afflicted individuals could lead to better preventative and protective measures taken to protect the kidneys: the ability to trace the amount of urea in the T1DM individuals would aid in the monitoring of one of the large complications that afflict them, and therefore assistance in understanding the progression of the disease on a case by case level. As mentioned before, this molecule might be difficult to detect in breath due to low vapor pressure at standard conditions. This fact should not deter the investigation of this molecule using THz spectroscopy. Sweat concentrations of urea have been documented to be 3.6 times greater than that of serum levels [3]. Heating a sample of sweat and then processing the vapors it produces with THz spectroscopy is a real possibility that will allow for
many of the benefits associated with this technology to be applied to a different method of monitoring this disease.

2.2 The Molecule
Urea has a rich history in science and is a molecule that is still prevalent in many projects carried out today. It was the first organic molecule synthesized outside of the body which subsequently disproved the vitalist theories in chemistry [19]. This molecule has a broad presence in both biology and astrophysics. Other than being a metabolite of protein metabolism, it is hypothesized to be a crucial part in the prebiotic synthesis of pyrimidines. Thus, it is seen as one of the precursors for the development of terrestrial life [20]. Because urea has been found in carbonaceous meteorites such as Murchison, it is hypothesized that large deposits of this chemical were delivered to Earth throughout its history [20]. Furthermore, the detection of urea is an expected result when analyzing composition of interstellar medium, where it shares many chemical attributes and structural similarities of molecules widely accepted to be found in interstellar space. One example is isocyanic acid. This molecule is, in fact, a thermal decomposition product of urea. Line frequencies believed to be that of urea have been observed in the hot molecular core of Sgr. B2 (N-LMH) using CARMA and IRAM [21], and tentative identification of this molecule has been made in its solid state using infrared spectra [22].

Definitive detection of urea in interstellar bodies may furnish insight into not only the mechanisms governing star formation, but even pre-life processes. However, with nearly 200 organic species documented in interstellar gas and dust clouds a need for the precise frequencies of spectra transitions of each constituent, including urea, has arisen to alleviate spectral congestion and to aid in molecular identification. Previous microwave studies of urea have yielded a handful of line transitions of the ground state of urea in the range of 5-50 GHz [19]. Recently a spectral assignment has been conducted up to 254 GHz [21]. This initial list of low J transitions—23 and under—needs to be extended alongside the growing technological advances in submillimeter telescopes, which can
be seen in projects such as ALMA, HERSCHEL, and SOFIA. With the aid of these astrophysics projects and an extended list of spectral transitions, the detection of interstellar molecular spectra can be achieved at unprecedented spatial and spectral resolutions and definitive identification urea, and other chemicals, can be made.

Though urea is similar to species already identified in interstellar medium, unique chemical attributes distinguish urea from many other organic compounds. Urea exists as a solid, white, crystalline substance at room temperature. This molecule possessing a melting point of 132.7°C, and at standard conditions has negligible vapor pressure. The major constituent of urea’s dipole moment lies along the carbonyl group, and has been found to be 4.56 Debye. The synthesis of Urea in interstellar space has been proposed to be able to be carried out by the Wöhler’s synthesis, Eq. 2.1, as well as an acid/base interaction, Eq. 2.2 [20].

\[
\begin{align*}
NH_4^+ + OCN^- & \rightarrow NH_2CONH_2 \\
NH_4^+ + OCN^- & \Leftrightarrow NH_3 + HNCO \\
NH_3 + HNCO & \rightarrow NH_2CONH_2
\end{align*}
\]

This is not to detract from the relevance of urea detection. The detection of this molecule could have many practical applications in the medical field. Accurate assessments of urea in the body could potentially provide a means of diagnostics and monitoring different pathologies, such as heart, kidney, and liver disease [23]. Expanding spectral libraries of this chemical, and other chemicals biomarkers, would prompt the development of sensors that would provide less invasive techniques in medicine, such as breath or sweat analysis. For T1DM individuals new sensor advances could alleviate many of the discomfort associated with monitoring their condition and prompt healthier practices when treating the effects and complications associated with their disorder.
2.3 Spectral Procedures

The experimental design of the spectrometer was adopted from previous research [15]. The source and the detector were driven by a custom build microwave synthesizer. This synthesizer derives its frequency from a 10 MHz rubidium clock. A schematic layout of the system can be seen in the appendix. The setup of this spectrometer incorporates a heterodyne system that utilizes continuous wave harmonic multipliers made by Virginia Diodes. A 2-meter long (14 liter in volume) absorption cell was treated with a SilcoNert 2000 coating [24] to improve degassing characteristics of the cell inner surfaces and thus reduce the probability of contamination effecting the spectrum of the urea obtained during the scan. The pressure in the absorption cell was monitored using an MKS Instruments 120AA Baratron powered by a MKS Instruments 600 series pressure controller [25].

A commercial pure sample of urea was purchased from Sigma Aldridge and was used without additional purification. Due to negligible vapor pressure at room temperature modifications of the delivery system into the absorption cell were made that promoted the formation of gas phase urea. This was done by heating the sample in a vacuum tight round bottom flask with a basket heater. Both the sample and the absorption cell were heated and maintained at 120°C. The spectrum was taken while allowing a continuous flow of the sample through the absorption cell to reduce the decomposition of urea into its derivatives as well as minimize the probability of gas phase urea transitioning to a solid state. The complete spectrum was taken in 2 distinctive segments. The first segment of data recorded the millimeter wave transitions in the frequencies ranging from 207 GHz - 270 GHz. For this range a heterodyne detector that incorporates continuous wave harmonic multipliers made by Virginia Diodes [26] was utilized to increase sensitivity and reduce signal to noise. The second part of spectrum was taken between 300 GHz to 500 GHz. For this range the detector employed was a liquid helium cooled InSb bolometer. Because of the lower signal to noise ratio associated with higher band, the flow rate of sample through the absorption cell was reduced to promote a buildup of around 10 mtorr of sample at any given time. This increased the number of urea
molecules present at any given moment, but also increased the time the sample remained in the cell. This extra time allowed for more decomposition products of urea to evolve.

The spectrum was power normalized in Igor Pro by dividing spectrum by DC baseline. DC baseline is a measure of the power of the spectrometer at a given frequency. The division of the spectrum by DC baseline removes the effects of any systematic fluctuation of power in the spectrum and relative intensities of each line transition in the spectrum can be trusted. Using CAAARS software [27], the assigned lines were fitted using spfit/spcat software package [28]. This was done in an iterative process, which began with previously assigned microwave lines transitions. Figure 2.1 illustrates the completed normalized spectrum obtained from the spectrometer.

![Normalized Spectrum](image)

**Figure 2.1:** Overview Spectrum of urea between 210-270 GHz and 300-500 GHz. The y-axis is the intensity in arbitrary units. The spectrum shown is the second derivative absorption spectra. The intensity of the peaks is based on both the number of molecules absorbers and the strength of the absorption of the molecule at that particular frequency.

### 2.4 Results

Understanding the physical conformation of a urea is crucial when tackling the assignment of its ground and excited states. This molecule is considered near oblate asymmetric top. It receives this designation due to how the principle axes are assigned to the molecule. The selection of the principle axes is a standard convention. The z-axis is always chosen to have the highest order of rotational symmetry, whereas the x-axis is chosen to be out of the plane for planar molecules. This is not to be confused with the a, b, and c axes, which are chosen to always make $I_a \leq I_b \leq I_c$ a true statement. For near oblate asymmetric tops the moments of inertia along the three principle axes falling into the
order $I_a \approx I_b < I_c$. The quantum number $J$ and two pseudo-quantum numbers, $K_a$ and $K_c$, characterize its rotational levels.

For asymmetric tops the allowed transitions all obey selection rules $\Delta J = 0, \pm 1$ and the components of its dipole moment dictate the transitions between $K_a$ and $K_c$. In general, any given molecule possesses three components to its dipole moment along its principle axes in a standard basis: $\mu_a$, $\mu_b$, and $\mu_c$. Each non-vanishing component of the dipole moment allows particular transitions to occur, and are governed by a set of selection rules. In the case presented here, urea relies on $b$-type transitions due to the fact that the major nonzero component of its dipole moment lies along the $b$ axis. Figure 2.2 shows the assignments for the axes of urea. The major contributor to the dipole moment in this molecule is the carbonyl group, which lines up with the $b$-axis. $b$-type transitions are governed by the selection rules:

\[
\Delta K_a = \pm 1(\pm 3, \pm 5 \ldots)
\]

\[
\Delta K_c = \pm 1(\pm 3, \pm 5 \ldots)
\]

This implies that, for urea, the transition between quantum numbers would allow a transition $1_{11}-0_{10}$. (In this notation, the first number represents the total angular momentum quantum number $J$. The two subscript values are $K_a$ and $K_c$, respectively.) A transition such as $1_{11}-0_{10}$ is not as it would require components of the dipole moment to be along the $a$-axes.

![Figure 2.2: The axes and planarity of urea. In this figure the atoms of the molecules are color-coded: the red represents oxygen, blue represents nitrogen, black represents carbon, and white represent hydrogen. The small dots in this figure stands for electrons.](image-url)
Figure 2.2 also illustrates the predicted planarity of this molecule. Notice that each orbital of the backbone of urea are \( \text{SP}^2 \) hybridized. This hybridization is due to the conjugation of the lone pair of electrons on each nitrogen group with the carbonyl group. Because of this conjugation the carbon-nitrogen bonds share some double bond characteristics and forces the planarity of this molecule. An understanding of the planarity of this molecule can be made by directly comparing the moments of inertia along each of the principle axes. Inertial defect \( \Delta \) can be taken as direct evidence towards non-planarity. It is defined as:

\[
\Delta = I_C - I_A - I_B \quad \text{(Eq. 2.3)}
\]

For a perfectly planer molecule \( \Delta \) would be equal to 0. There is a direct relationship between the moments of inertial along a principle axes and the rotational constants. The rotational constants for an asymmetric top are defined as:

\[
\begin{align*}
A &= \frac{\hbar^2}{2I_A} \\
B &= \frac{\hbar^2}{2I_B} \\
C &= \frac{\hbar^2}{2I_C} \quad \text{(Eq. 2.4)}
\end{align*}
\]

The fit initially was based on previous microwave data [19], and then was iteratively updated throughout the assignment. Figure 2.3 shows a sample of the spectrum being overlaid by the predictions produced by SPCAT software [28]. The most prominent clusters of lines pictured are P-branches\(^5\) and R-branches.\(^6\) The line transitions that lie between these dominate clusters are called Q branches\(^7\). The overall fit of the predictions coincides nicely with the spectra obtained from the spectrometer. However there existed a handful of lines that appeared to fall out of the periodic pattern created from different J transitions. Upon further investigation these lines were deemed to be a product of the decomposition of urea. Urea decomposes following equation 2.5 [29]:

\[
\text{NH}_2\text{CONH}_2 + \text{heat} \rightarrow \text{NH}_4^+\text{NCO}^- \rightarrow \text{HNCO} + \text{NH}_3 \quad \text{(Eq. 2.5)}
\]

\(^5\) Defined as \( \Delta J=+1 \)
\(^6\) Defined as \( \Delta J=-1 \)
\(^7\) Defined as \( \Delta J=0 \)
Figure 2.4 shows spectrum overlaid with predictions obtained from this study for the ground state of urea. These predictions are the blue trace. Added to this graph are also the predictions for isocyanic acid. These predictions were obtained from the Jet Propulsion Laboratory [JPL] line catalog [30]. These lines—highlighted in purple—show that the unpredicted lines that occur in our spectrum are, in fact, isocyanic acid, and that during the course of recording the spectrum urea was readily decomposing. This fact also can explain why the contamination lines appear stronger in the 300 GHz to 500 GHz stretch of data (Figure 2.1). The flow rate of latter band of data was altered to allow a buildup of sample in the cell. Due to the heat added to the sample to help it maintain gas phase and the prolonged time spent in the cell, the probability of the decomposition of the molecule increase. Thus this yielded a more contaminated spectra than the 207-210 GHz stretch.

![Figure 2.3: Spectrum overlaid with ground state predictions](image)

![Figure 2.4: Isocyanic acid [HNCO] contamination caused by the decomposition of urea.](image)

The ground state and the first three excited vibrational states of urea were assigned during the course of the investigation. Though portions of the ground state has been assigned within the microwave domain [19], and more recently in portions up to 254 GHz [21], the data presented here is
the most expansive examination of the ground state and the first assignments of the excited states of this molecule. Table 2.1 shows the constants that were obtained, moments of inertia along each principle axes, deviation from planarity, inertial defect, and the root mean square (rms) of the fit. Figure 2.5 shows a sample of the spectrum with the individual state transitions labeled. In this image, blue points represent the ground state transitions, green represents the first excited vibrational state, purple the second excited vibrational state, and orange the third excited vibrational state.

Both the ground state and the first vibrational state were rather straightforward to assign. The ground state assignment is a global fit with the early microwave data. 47 line transitions were taken from Brown, Godfrey, and Storey’s article published in the *Journal of Molecular Spectroscopy* in 1975 [19] and through an iterative process, 991 additional lines were assigned. The ground state assignment consists of a total of 1038 lines. The first vibrational state has 942 lines. Despite the large number of lines each of these states, the root mean square [rms] error of the fit was found to be only 48.84 kHz and 47.268 kHz, respectively. For the ground state the rotational constants obtained from the fit coincided very well with previous microwave data [19]— column 2 in the table.

The second and third vibrational states assignments were not as direct as the ground state and first vibrational state assignment. The vibrational energy levels of these two states are very close to one another, and because of these, they perturbed each other. In order to assign these states, the spectra was charged to Dr. Zbigniew Kisiel at the Polish Academy of Sciences in Warszawa Poland, who utilized AABS software package to assign the two states [31]. In his fitting routine several coupling schemes were explored. It was found that an $a$-axis Coriolis interaction best described the perturbation. The constants found by Dr. Kisiel are presented in the last two columns of table 2.1. The second vibrational state assignment consists of 522 lines and has a calculated rms error of 48.5 kHz, whereas the third vibrational state assignment has 518 lines and an rms error of 43.7 kHz.
Table 2.1: Constants obtained from the fitting of the ground state, first excited, second excited and third excited vibrational states. (a) Previous ground state values are based on fit by Brown, Godfrey, and Storey [19]. The second and third vibrational states were assigned by Dr. Zbigniew Kisiel at the Polish Academy of Sciences in Warszawa Poland. These fits were done with a III, A reduction.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Current Ground State Values</th>
<th>Previous Ground State Values (a)</th>
<th>First Vibrational State Values</th>
<th>Second Vibrational State Values</th>
<th>Third Vibrational State Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (MHz)</td>
<td>11233.30379 (15)</td>
<td>11233.3161 (87)</td>
<td>11208.96617 (51)</td>
<td>11234.92764 (56)</td>
<td>11253.91029 (51)</td>
</tr>
<tr>
<td>B (MHz)</td>
<td>10369.39594 (43)</td>
<td>10369.4074 (88)</td>
<td>10361.07866 (47)</td>
<td>10346.42813 (49)</td>
<td>10339.90938 (55)</td>
</tr>
<tr>
<td>C (MHz)</td>
<td>5416.62847 (51)</td>
<td>5416.6392 (86)</td>
<td>5419.07181 (53)</td>
<td>5416.84950 (29)</td>
<td>5412.65058 (27)</td>
</tr>
<tr>
<td>Δv / kHz</td>
<td>10.7773 (68)</td>
<td>10.58 (37)</td>
<td>10.72512 (59)</td>
<td>10.57105 (69)</td>
<td>10.62861 (74)</td>
</tr>
<tr>
<td>ΔK / kHz</td>
<td>21.02649 (259)</td>
<td>20.982 (33)</td>
<td>20.88652 (197)</td>
<td>20.4613 (31)</td>
<td>20.7024 (34)</td>
</tr>
<tr>
<td>ΔS / kHz</td>
<td>10.97581 (212)</td>
<td>10.809 (29)</td>
<td>10.89551 (161)</td>
<td>10.6174 (25)</td>
<td>10.7906 (28)</td>
</tr>
<tr>
<td>δv / kHz</td>
<td>0.21800 (34)</td>
<td>0.2208 (43)</td>
<td>0.168023 (248)</td>
<td>0.12515 (47)</td>
<td>0.20127 (66)</td>
</tr>
<tr>
<td>f / Hz</td>
<td>0.015656 (147)</td>
<td>0.015510 (151)</td>
<td>0.01570 (11)</td>
<td>0.1561 (13)</td>
<td></td>
</tr>
<tr>
<td>fR / Hz</td>
<td>0.118911 (182)</td>
<td>0.114563 (222)</td>
<td>0.10914 (24)</td>
<td>0.10984 (43)</td>
<td></td>
</tr>
<tr>
<td>fυ / Hz</td>
<td>0.265200 (273)</td>
<td>0.26253 (32)</td>
<td>0.23976 (42)</td>
<td>0.24001 (65)</td>
<td></td>
</tr>
<tr>
<td>fυ / Hz</td>
<td>0.161865 (183)</td>
<td>0.163278 (202)</td>
<td>0.16328 (27)</td>
<td>0.14638 (27)</td>
<td>0.14592 (36)</td>
</tr>
<tr>
<td>ΔE / MHz</td>
<td>-43408.094 (82)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ia / amu A^2</td>
<td>45.002878</td>
<td>45.0028268</td>
<td>45.1009111</td>
<td>45.03645958</td>
<td>44.92047537</td>
</tr>
<tr>
<td>Ia / amu A^2</td>
<td>48.75221304</td>
<td>48.75215916</td>
<td>48.79134853</td>
<td>48.86043702</td>
<td>48.8912086</td>
</tr>
<tr>
<td>Ia / amu A^2</td>
<td>93.32945813</td>
<td>93.32927225</td>
<td>93.28737794</td>
<td>93.32564999</td>
<td>93.39804284</td>
</tr>
<tr>
<td>Δ</td>
<td>-0.425632913</td>
<td>-0.425714597</td>
<td>-0.6045617</td>
<td>-0.571246702</td>
<td>-0.413667989</td>
</tr>
<tr>
<td>N lines</td>
<td>1038</td>
<td>942</td>
<td>522</td>
<td>518</td>
<td></td>
</tr>
<tr>
<td>Avg. / kHz</td>
<td>4.523</td>
<td>3.969</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rms / kHz</td>
<td>48.84</td>
<td>47.268</td>
<td>48.5</td>
<td>43.7</td>
<td></td>
</tr>
</tbody>
</table>

Figure 2.5: Sample of the assignment of the different states of urea. The second and third vibrational states were assigned by Dr. Zbigniew Kisiel at the Polish Academy of Sciences in Warszawa Poland.

This study of urea also investigated the Ray’s asymmetry parameter [κ] of each state of urea.

This parameter calculates the degree of asymmetry for the molecule. It is defined as:

$$\kappa = \frac{2B-A-C}{A-C} \quad (\text{Eq} \ 2.6)$$
For a molecule that is taller than it is wide, called a near prolate top, this number would return a negative value within the range of -1 to 0. If a positive value from 0 to 1 is calculated then the molecule is known to be near oblate. An initial guess from looking at the principle axes and understanding bond lengths of within a molecule can be made before an assignment of a molecule. The value obtained from this calculation reaffirms the shape of the molecule that is being investigated. Urea’s states consistently return a value right around .70, proving the shape of urea to be a near oblate top.

An inquiry was also made regarding the separation of energy between vibrational states. The energy differences between the vibrational states based on the experimental intensity of spectra can be determined utilizing the eq 2.7.

$$\alpha_{l\rightarrow u}(T) = \nu \left(1 - e^{-\frac{h\nu}{kT}}\right) \frac{B_l^{\nu^2}}{3cR} \sum_{i=x,y,z} |\mu_{l\rightarrow u}|^2 \frac{g_l e^{-E_l/kT}}{Q}$$  (Eq 2.7)

Where $\alpha$ is the intensity, $\nu$ is line frequency, $T$ is temperature, $\sum_{i=x,y,z} |\mu_{l\rightarrow u}|^2$ is the square of the dipole matrix elements, $g_l$ is the degeneracy of the lower level, $E_l$ is the lower state energy, and $Q$ is the partition function. A ratio between line transitions with the same quantum numbers in two different states can be used to determine the separation of energy between them. In order to create this ratio, firstly the assigned lines for the ground state was matched to the assigned lines of the first, second, and third vibrational states. The intensity of each line was determined experimentally from the spectra produce. The ground state energies were taken from the predictions calculated by SPCAT, and the equation was solved for the energy value of the excited state. After this, the predicted vibrational energy was added and the predicted ground state was subtracted from this value. The final equation was:

$$\Delta E = \ln \left(\frac{\alpha_{gr} \nu_{gs}}{\alpha_{vs} \nu_{vs}} \left(\frac{1-e^{-\frac{h\nu_{gs}}{kT}}}{1-e^{-\frac{h\nu_{vs}}{kT}}}\right)\right) kT + E_{gs} - E_{vs}$$  (Eq 2.8)
In this equation $E_{gs}$ represents the predicted ground state energy and $E_{vs}$ represents the predicted vibrational energy. The result of this equation, $\Delta E$, is the difference in energy between the two different states. Note that in this equation the partition function and dipole moment matrix elements cancel out, simplifying the calculation. This could only be done under the assumption that the dipole matrix elements were the same in both states. Figure 2.6 elucidates the relationship between vibrational and rotational states that we are exploiting to find the separation between the two states. Figure 2.6 shows that by understanding the separation between the same line transition in two different states, the relationship between the states themselves can be determined.

**Separation between Ro-Vibrational Energy States**

![Diagram](image)

Figure 2.6: The separation of energy in two different states.

Each pair of lines produced a calculated separation of energy between that and the ground state. Next, a gaussian curve was fitted to a histogram of the calculated energy obtained for each state from these calculation. Only lines that fell within one standard deviation before or after the gaussian was used in the final energy calculations. This was done because urea possesses a transition rich spectrum and accidental overlaps with the excited states does occur. The addition of these accidental overlaps could skew the results. Figure 2.7 shows the values obtained from fitting the Gaussian to the histogram of each state. The separation from the ground state was found to be 52.152 cm$^{-1}$ for the first
vibrational state. The second and third vibrational state was found to be 264.98 cm$^{-1}$ and 262.93 cm$^{-1}$, respectively. Notice that the separation between the second and third energy states is very small. This is why these states were perturbed. These states likely interact with each other due to the close proximity they have in energy. The number obtained from this calculation coincides shows a $-2.05$ cm$^{-1}$ difference between these two states. This number coincides well with Dr. Kisiel value of $-43409.094$ MHz, or $-1.448$ cm$^{-1}$.

Figure 2.7: Histogram of the separation of energy levels based on experimental intensities
2.4 Discussion
This spectral assignment of urea conducted during the refines previously published rotational constants for the vibrational ground state. Further, for the first time the assignments of three excited states have been made. Calculations based on experimental data has found the separation of first, second, and third excited states to be 52.2, 265.0 and 262.9 cm\(^{-1}\), respectively.

These assignments of urea could have many different practical applications. With the refined rotational constants and line lists furnished by this investigation seeking urea and understanding its role in interstellar or biological bodies and systems is possible using rotational spectroscopy. Though this molecule has low vapor pressure, there still exists many possibilities for it in the medical field. With the development of breath, skin, and sweat sensors, T1DM individuals’ could apply this assignment in new technologies to aid in diagnosing and monitoring some of the complications associated with their disease in a less invasive manor.
Chapter 3

3.1 The Relevance of Methyl Nitrate

Methyl nitrate [CH$_3$ONO$_2$] is of great interest when looking for new methods of monitoring T1DM; it has already been successfully exploited in studies to reverse engineer blood glucose measurements in regards to T1DM individuals. In a study published in the *American Journal of Physiology-Endocrinology and Metabolism*, methyl nitrate was used as one of a small handful of chemicals to predict the plasma glucose concentrations in healthy and T1DM individuals [32]. In addition, recent studies conducted on T1DM children have shown that breath concentrations of methyl nitrate are strongly correlated with the acute spontaneous hyperglycemic events [33]. It is believed that this molecule is a byproduct of oxidative stress in the body and has been shown to rise after ingestion of fatty meals [33]. Because of these studies linking methyl nitrate with blood glucose and fat ingestion, it became a natural molecule to investigate during the course of this study. After all, T1DM individuals are dependent on daily invasive and expensive blood glucose tests. Even further, the breakdown of fat as an alternative energy pathway is what produces the dangerous ketones within the T1 diabetic body. Understanding the rotational spectrum of this molecule brings us closer to uncovering it in breath and applying it towards monitoring T1DM.

Though these studies suggest a direct correlation between blood glucose and methyl nitrate, there is still a dearth in the understanding of this molecule and the best approach to detecting it in breath. The studies mentioned above relied on GC-MS analysis to quantify the amount of sample found in exhaled breath. Because of the importance of this molecule in the development of a quick, cost efficient technology a complete study of this chemical needs to be done utilizing THz/rotational spectroscopy.

The current hardware of Wright State’s THz spectrometer lacks the sensitivity to detect the amount of methyl nitrate found in breath (around 5 parts-per-trillion [33]). Our current system setup can detect this molecule on the order of 3 parts per billion. This does not mean that this chemical should not be investigated. There have been constant improvements towards increasing the sensitivity
of the spectrometer. These improvements include a new absorption cell and a novel versatile preconcentration system. Our laboratory is constantly working towards this chemical’s detection because of the importance it may play in our study of T1DM. By understanding it and how it interacts with THz radiation, an important step towards applying it to breath analysis will be made.

3.2 The Molecule

At standard conditions methyl nitrate is a colorless liquid with a melting point of -82.3°C. It has a dipole moment along its a-axis of 3.07 Debye and .23 Debye along its b-axis [34]. This molecule is highly volatile. Methyl nitrate also is challenging to store for extended periods of time because it undergoes hydrolysis. It is considered an explosive, and has a heat of the explosion of 6748 kJ/kg from its liquid state and possesses a detonation velocity of 6300 m/s [35]. The powerful explosive qualities of this chemical led it to be utilized by the Germans in land mines during WWII [36]. Methyl nitrate has been found to be relatively hard to detonate. Therefore it is not typically incorporated in modern day weaponry. Even so, its spectral signature could aid in identifying possible compositions of explosives.

This molecule may have other practical applications. It has been suggested that it also could be used as an indicator of urban air quality due to its high photo dissociation lifetime [37]. It is also notable that all of the compounds needed for the synthesis of methyl nitrate are found in interstellar medium; therefore, methyl nitrate should be added to the list of chemicals that astronomers are currently trying to locate in space.

3.3 Synthesis and Spectral Procedures

Methyl nitrate is not available for purchase commercially due to the highly volatile and explosive nature. In order to conduct a spectral analysis of methyl nitrate, an in-house synthesis of this molecule was conducted with the aid of Dr. David Dolson of the Wright State Chemistry Department. Though there are two different methods of synthesizing methyl nitrate [35], it was decided that the procedures published in Organic Syntheses [38] would be used to obtain the desired
product. The synthesis was scaled down from the published amount to yield roughly 3-4 ml of CH$_3$ONO$_2$. Equation 3.1 shows the overall desired reaction.

$$CH_3 OH \xrightarrow{HNO_3/H_2SO_4} CH_3ONO_2$$  \hspace{1cm} \text{Eq. 3.1}

This reaction was done by the nitration$^8$ of methanol with a mixture of sulfuric and nitric acid. This is a mixed-acid synthesis. In this nitration reaction sulfuric acts as a catalyst and absorbent for water.

In order to prepare for the synthesis of methyl nitrate, two different solutions were initially made and placed in an ice bath for future use. The first solution contained 10.69g NaOH added to 50mL H$_2$O, thus providing a roughly 5M solution of NaOH. The second solution was made with 5.60g NaCl dissolved in 20 mL of H$_2$O, which yielded about a 22% NaCl mixture.

Next, 10mL cold of H$_2$SO$_4$ with 10mL of cold HNO$_3$ were mixed in a 50mL Erlenmeyer flask and was placed in the ice bath.$^9$ In a separate Erlenmeyer flask 1.7mL of cold H$_2$SO$_4$ was mixed with 5mL cold CH$_3$OH and placed in an ice bath to cool. After allowing both of these solutions to cool, they were added together and swirled to mix. Product formation was observed immediately as two different liquid layers. After 2 minutes of mixing this solution was placed in the ice bath for about 15 minutes.

After the ice bath, the solution was placed in a large separatory funnel and the denser acid level was drained off. The product was then drained into a small separatory funnel where 1ml of the NaCl solution was added to provide a wash. In belief that the salt mixture was denser, 1 ml was drained off. This was repeated again, but due to uncertainty in the separation of the fluids, a closer examination on the density of each solution was done. It was discovered that the density of a 22% NaCl was around 1.16g/ml, whereas the density of methyl nitrate is around 1.2 g/ml. Armed with this knowledge all liquids (product and wash fluids) were added back into a large separatory funnel along

---

$^8$ Nitration is a general class of chemical process for the introduction of a nitro group into an organic chemical compound.

$^9$ These chemicals were initially cooled in an ice bath.
with 1mL of water. This was added to dilute the NaCl solution and lower the density of it relative to MeONO₂. After mixing, the product was deemed to be the lower level and drained off. 5 drops of NaOH mixture was added to find an alkaline pH. This product was then washed twice with 1mL of water and placed in a 50mL round bottom flask. The drying set up, seen in Figure 3.1, was used to rid the mixture of any excess water. Anhydrous CaCl₂ was used as the drying agent.

![Figure 3.1: Drying setup for the synthesis of methyl nitrate](image)

After allowing several days for the mixture to dry, the product of this reaction was placed in a vacuum flask. The sample was frozen with liquid nitrogen and connected to a vacuum line. This was done to evacuate any residual air present in the flask. The spectrum was taken with the same spectrometer used for the urea. Like urea, the data was taken in two distinct ranges: 210 GHz -270 GHz and 325 GHz -500 GHz. Instead of the liquid helium cooled InSb bolometer for the later band, a commercial heterodyne receiver manufactured by Virgina Diodes was used. Unlike urea, which had a continuous flow of sample flowing through the absorption cell, the spectrum of methyl nitrate was taken with a trapped sample of roughly 10 mtorr. The complete spectrum obtained can be seen in figure 3.2. A section of this spectrum is shown in figure 3.3.
Because this was an in-house synthesis, the purity of the sample was unknown. Furthermore, because there was uncertainty during the synthesis of this molecule there was some question if lingering contamination of the reactants and washes would be in our product. It was decided to test the purity of our sample qualitatively by utilizing the spectrum of the possible contaminants. This was done by consulting the JPL line catalogs and pulling the spectra of the reactants/catalysts of this reaction (H$_2$SO$_4$, HNO$_3$, methanol) and overlaying them with the spectrum recorded. Figure 3.4 shows the results of this. Notice that the spectrum recorded is very distinct from the spectral lines from JPL. Even more impressive is the fact that none of the spectral patterns of the methanol, H$_2$SO$_4$, or HNO$_3$ appear to contribute to the spectrum recorded. Because of this fact, there was confidence that this was a relatively pure sample of methyl nitrate.
3.4 Results
The physical conformation of methyl can be seen in figure 3.5. On this diagram it is possible to see the conjugation of the lone pair of electrons on the center nitrogen atom with of the oxygen atoms (Shown by the dotted line). This image also has the principle axes labeled according to standard convention. Methyl nitrate is considered prolate asymmetric top. The major component of methyl nitrate’s dipole moment lies along the a-axis. The selection rules for a-type transitions are:

\[ \Delta K_a = 0(\pm 2, \pm 4 \ldots) \quad \text{Eq.3.2} \]
\[ \Delta K_c = 1(\pm 3, \pm 5 \ldots) \]

Unlike urea, methyl nitrate also has another non-zero component to its dipole. It possesses a component along the b-axis. However dipole moment component along the a-axis is strongest, and therefore the lines in the spectra created by these a-transitions will be stronger.
The previous microwave research conducted on this molecule has yielded a handful of line transitions ranging in J values of 0-9[39] [34]. The paper by Ebright Wilson and William Dixen also furnished A, B, and C rotational constants. It is from these constants and these lines transitions that the initial prediction for our assignment of methyl nitrate’s spectrum was created. In figure 3.6, a sample of the spectrum is shown being overlaid by the predictions produced by SPCAT software [28].

The ground state assignment for this experiment consists of 1218 line transitions ranging in J values of 24-72. The constants that were obtained from this assignment are presented in Table 3.1. A sample of the assigned line transitions can be seen in figure 3.6. This is a global fit that includes the microwave line transitions assigned by Ebright Wilson and Dixon [39]. This fit used 12 parameters and returned a rms of 39.452 kHz. The second column shows the rotational constants of the
microwave assignment of methyl nitrate [39]. Notice that the values that were acquired from this study coincide very closely with the previously published data. The $\kappa$ value found in this experiment is a negative value, which supports the fact that this molecule is a near prolate asymmetrical top. It is also interesting to note that the error margin for our A constant is greater than for B.

This can be explained by the fact that the lines assigned are a-type transitions. THz radiation interacting with the dipole moment along the a-axis would cause rotation to occur mainly around the b and c axes. This causes the A constant to be less defined than the other two constants. Though this molecule does possess a component of its dipole moment along the b-axes, these transitions are weak. This is to be expected, as the dipole moment along the a-axis is more than thirteen times greater than that of along the b-axis, meaning that b-type transitions are $13^2$ times weaker. The S/N ratio for one of the strongest lines in our spectrum (shown in figure 3.5) is 131.1. Because of this, we know that our b-type transitions are in our noise floor.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Current Value</th>
<th>Previous Value ($a$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A$ / MHz</td>
<td>11795.18929(205)</td>
<td>11795.06</td>
</tr>
<tr>
<td>$B$ / MHz</td>
<td>4707.568413(176)</td>
<td>4707.52</td>
</tr>
<tr>
<td>$C$ / MHz</td>
<td>3438.236313(209)</td>
<td>3438.29</td>
</tr>
<tr>
<td>$\Delta_A$ / kHz</td>
<td>0.971321(52)</td>
<td></td>
</tr>
<tr>
<td>$\Delta_B$ / kHz</td>
<td>4.312252(199)</td>
<td></td>
</tr>
<tr>
<td>$\Delta_C$ / kHz</td>
<td>4.8366(129)</td>
<td></td>
</tr>
<tr>
<td>$\delta_1$ / kHz</td>
<td>0.2493936(169)</td>
<td></td>
</tr>
<tr>
<td>$\delta_2$ / kHz</td>
<td>2.7070(79)</td>
<td></td>
</tr>
<tr>
<td>$\phi_1$/Hz</td>
<td>0.2619 (64E-03)</td>
<td></td>
</tr>
<tr>
<td>$\phi_2$/Hz</td>
<td>5.126e(194E-03)</td>
<td></td>
</tr>
<tr>
<td>$\phi_3$/Hz</td>
<td>0.01727(66)</td>
<td></td>
</tr>
<tr>
<td>$\phi_4$/Hz</td>
<td>0.0641(257)</td>
<td></td>
</tr>
<tr>
<td>$I_a$/amu$A^2$</td>
<td>42.85908327</td>
<td>42.8596</td>
</tr>
<tr>
<td>$I_b$/amu$A^2$</td>
<td>107.3868621</td>
<td>107.388</td>
</tr>
<tr>
<td>$I_c$/amu$A^2$</td>
<td>147.0320693</td>
<td>147.03</td>
</tr>
<tr>
<td>$I_+ I_-$ / $I_c$</td>
<td>3.231876029</td>
<td>3.2176</td>
</tr>
<tr>
<td>$\kappa$</td>
<td>-0.696221313</td>
<td>-0.696239</td>
</tr>
<tr>
<td>Avg. / kHz</td>
<td>-0.732</td>
<td></td>
</tr>
<tr>
<td>rms / kHz</td>
<td>39.452</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.1 Constants obtained from fitting the ground state
Compared with previously known values

Methyl nitrate does have a very visible excited vibrational state, as seen in figure 3.8. An attempt was made at assigning this state, although this was eventually abandoned due to a strong
perturbation that complicated the assignment. The effects of the perturbation on the first vibrational state can be seen in figure 3.9. The predicted line transitions do not seem to match the spectrum. The problems with the assignment of this state has been discussed in previously published research, where it was hypothesized that the excited state actually arises from combination of the methyl group’s torsion and the NO$_2$ torsion of the first vibrational state, thus explains the difficulties associated with its analysis [39].

Figure 3.8: The two visible states of methyl nitrate
3.4 Discussion

The spectral analysis of this molecule between 210GHz -270GHz and 325GHz -500GHz will aid in the future of breath analysis in the course of the studies conducted at Wright State University’s THz laboratory. Constant improvements in the system should allow for this molecule to be detectable in breath in the near future. Being armed with the knowledge obtained during this study and the ability to detect this chemical will likely lead to many new and exciting discoveries about this molecule and its workings within the body. Methyl nitrate is an important chemical to watch for in breath, especially when trying to analyze the progression of sugar disorders—such as T1DM—and how fat ingestion affects the metabolism of the body. Because methyl nitrate is thought to be a product of oxidative stress, it could even be possible to employ this chemical in tests to quantify the amount of free radicals, oxidants, and/or antioxidants within the body.
Chapter 4

4.1 Fluctuations of Blood Glucose

The data that has been presented thus far in our investigation of T1DM had consisted of the spectral assignments of urea and methyl nitrate. The THz spectroscopy laboratory at Wright State University is avidly working toward the ability to detect them in breath samples from volunteers. These spectral assignments were done to aid in the pursuit of the detection of these molecules once hardware improvements have taken place to allow their discovery in samples. However, they are not the only chemicals that have vital importance to the examination T1DM via breath analysis. Acetone, ethanol, and acetaldehyde are imperative to research when monitoring breath sample of T1 diabetics. By seeking these chemicals in breath, the hope is to elucidate any possible correlations that they may share to this disease. In addition, chemicals that are known to fluctuate in the body—such as HCN and methanol—should also be explored in hopes of revealing critical relationships unbeknownst to the scientific community.

It is the inability of T1 diabetics to produce insulin in vivo that effectuates the cascade of health complications that afflict them. Maintaining normal blood glucose levels within the body is essential to prevent these complications from occurring. Because of this, the most important way of monitoring this disease is to monitor blood glucose levels. If THz based breath analysis can be used solely to monitor T1DM, then it must possess ability to recreate blood glucose values based on breath chemical concentrations.

In healthy individuals the typical fluctuation of blood glucose values falls somewhere within 60-120 mg/dl at any given moment in a day. The major variations of blood glucose in the human body occur after the consumption of a meal. After eating, glucose is absorbed into the blood. The increased dilution of this molecule triggers the pancreas to release insulin into the blood stream. This is done to return the body to a more homeostatic value, as illustrated by figure 1.1. The insulin causes the cells of the body to uptake the plasma glucose. As the amount of glucose dissolved in the blood falls,
insulin values decrease. Figure 4.1 illustrates the effects that a meal has on the plasma concentrations of insulin and glucose. Both insulin and glucose values along the y-axes show the rise-fall-rise trends based on an article published in *Nature Precedings* [40]. Though this figure does describe the typical oscillation of blood glucose and insulin over the course of a few hours, it also allows another important inference to be made. That is, different kinds of carbohydrates can alter the response. The dotted line shown in figure 4.1 is how this pattern changes upon the consumption of a sucrose\textsuperscript{10} rich meal. Notice that the sucrose consumption both increases the amount of dissolved glucose in the blood, and in turn, the response from the pancreas. With the knowledge gained from this figure, a series of experiments were devised to investigate the chemical concentrations of acetone, methanol, ethanol, acetaldehyde, and HCN in breath and their potential correlations to that of blood glucose.

**Figure 4.1:** The typical fluctuation of blood glucose after the consumption of meals. The solid line represents a typical meal, whereas the dotted line is a meal that contains high amounts of sucrose.

---

\textsuperscript{10} Sucrose is a simple disaccharide that is made up of a glucose and fructose molecule. Typical starch rich foods would be comprised of polysaccharides.
The first experiment was developed both to confirm the characteristic rise-fall-rise pattern in blood glucose values suggested by figure 4.1, as well as to test if any of the chemicals mentioned above formed a direct correlation to blood glucose. A non-diabetic, 27 year old volunteer was selected for this study. This volunteer has no family history of diabetes and was in general good health during the time of this study. The experiment conducted on her was similar in fashion to the Oral Glucose Tolerance Tests [OGTT] that are incorporated in medicine today to test for diabetes.

The volunteer was asked to fast the night before testing. When she arrived at the laboratory a baseline sample of breath was collected from her. At the same time, her blood glucose value was checked with a commercially available One Touch® UltraMini blood glucose meter. After 48 minutes had passed the volunteer was asked to consume 100g of table sugar dissolved in a glass of warm water. This was done over the course of two minutes. Seventeen minutes later another breath sample was donated and blood glucose values recorded. Blood glucose values and breath samples were taken five additional times over the course of the next two hours. These were taken at roughly 20-30 minute time intervals. This yielded a set of seven breath samples and blood glucose values.

The breath was processed using the THz breath spectrometer (published previously, [15]) to calculate the volumetric dilution of each chemical being investigated. The results obtained from this study are shown in figure 4.2. In this figure, a column contains a graph of blood glucose values and nine chemicals’ breath dilutions graphed against the time that each breath sample was taken. The red line that crosses each chemical trace marks the time that the sugar water was consumed. Inset-a is the predicted shape that blood glucose values and insulin values have graphed against time during and after the consumption of sucrose. This predicted shape of this inset is taken from the article published in Nature Precedings mentioned above [40].

Potential relationships do seem to appear when visually looking at the graphs in figure 4.2. A rise-fall-rise pattern similar to that predicted by 4.1 does seem to emerge: Acetone, ethanol, methanol, acetaldehyde, and HCN each seem to possess this similar rise-fall-rise pattern. Though this pattern
emerges in the chemical traces, the blood glucose meter did not identify this predicted rise-fall-rise pattern. To test how well these trends actually fit with the recorded blood glucose results, Pearson’s r correlation values were calculated. This single correlation value shows the degree of a linear relationship between any two data sets. The equation used to find this value is Eq. 4.1:

\[ r = \frac{\sum (X_n - \bar{X})(Y_n - \bar{Y})}{\sqrt{\sum (X_n - \bar{X})^2 \sum (Y_n - \bar{Y})^2}} \]  
Eq. 4.1

The values of this coefficient can range from -1 to 1. A value of 1 implies a perfect positive linear relationship, whereas a value of -1 signifies a perfectly negative linear relationship. 0 indicates that the data has no linear relationships at all. The values of Pearson’s r are shown alongside each chemical trace in figure 4.2. The Pearson’s r-values that are obtained from acetone, ethanol, acetaldehyde, and HCN are around or greater than .5. Though imperfect, this is a promising result from this investigation, as it shows that a minor correlation may exist between these chemicals and blood glucose.
Figure 4.2: The experimentally determined fluctuation of blood glucose and breath concentration of chemicals after the consumption of 100 g sugar in a healthy volunteer. Breath concentrations [parts-per-million] are shown graphed against time [minutes]. The red line crossing the each chemical trace represents the time that sucrose was consumed. Insert-a is predicted shape that blood glucose values and insulin values have graphed against time during and after the consumption of sucrose.

4.2 Blood Glucose verses Volumetric Dilutions in the breath of T1 Diabetic

An experiment was designed to obtain and analyze the breath of a T1 diabetic. It was suspected that, because T1DM afflicted individuals do not have the capacity to produce insulin, the amount of glucose dissolved within the blood would only rise after the consumption of a meal as a T1
diabetic’s body has no means of quickly lowering blood sugar. By observing how chemical dilutions in the breath of T1DM afflicted individuals varied from that of healthy individuals, the experiment would illustrate differences between the two as well as observe any linear correlations that may exist.

Two T1 diabetics volunteered for this experiment. Both of these volunteers were minors at or under the age of thirteen. Conducting an OGTT like test on a T1 diabetic without insulin and/or medication is dangerous, as high blood glucose values lead to many other health concerns. This is especially true for children. Because of all the health conditions associated with high blood sugar, it was crucial to ensure that this experiment was done under the care of a physician with a stringent IRB in place. The breath collection was carried out by our partners at the University of Dallas and their affiliated hospital. The experiments conducted on these two volunteers were similar in fashion as to the test conducted on the healthy volunteer in section 4.1. However, due to the initially high blood glucose values for each of these volunteers, before the start of the experiment insulin was taken. Further, a mixed meal was used in lieu of pure sugar consumption. This meal most likely contained both long-lasting carbohydrates as well as sucrose. Blood glucose values were taken every fifteen minutes over the course of two hours. The breath samples were collected at the same time blood glucose values were recorded. These samples were shipped overnight in Tedlar bags to our laboratory to be analyzed by our spectrometer. Tedlar bags are an EPA standard of gas collection. Based on previous tests conducted in our laboratory, the longevity of samples stored in these bags is estimated to be about 72 hours. The results from both of these volunteers can be seen in figure 4.3. The red traces of the chemicals are the volumetric dilution values that were found during the course of this study. The blue dotted line trace represents the chemical baseline of the absorption cell. The chemical baseline is a scan of an empty absorption cell that is taken before a sample is put inside. It is taken to measure any contamination present in the cell. This value is typically subtracted from the data. The Pearson’s r-value that appears beside every graph are calculated by the volumetric dilution (red line trace) minus the chemical baseline (blue dotted-line trace).
Figure 4.3: The experimentally determined fluctuation of blood glucose and breath dilutions of chemicals in 2 T1 diabetic volunteers.

Column-a on this figure shows the results obtained from the analysis of the first T1 volunteer’s breath, whereas column-c shows the results from the second T1 volunteer. Column-b of figure 4.3 is a zoomed in image of the acetone graph obtained for the first subject. For the first volunteer’s data set, a sample of Dallas’ laboratory air, a nitrogen bag, and a pre-day sample were also collected. The pre-day sample was taken the day before the experiment was conducted. The lab
air collected during the course of this study shows high readings on several of the different chemicals, such as ethanol and acetaldehyde. This is an odd finding for room air, and we hypothesize that the pump that was used to fill the Tedlar bag contaminated this sample. A nitrogen bag was processed before of the breath samples from the volunteer. This bag was analyzed to determine the level of contamination from the preconcentration procedures. This is in addition to the baseline scans that focus solely on the contamination in the absorption cell. The results obtained from calculating the pre-day sample shows an unusually high level of acetone. One possible explanation for this value is that this volunteer was in a state of pre-ketosis when he gave this sample.

Acetone—one of the ketones produced by the body—is a documented product of fatty acid metabolism. In section 1.3 the discussion of the dangers associated with T1DM afflicted individuals and the ketones, and ketoacidosis was explained. A high level of ketones in the body for T1 diabetics is a very serious health concern. Currently, blood or urine tests are used to monitor ketones in the body of T1DM afflicted individuals. That being said, the applicability of utilizing breath acetone dilutions medically as an indicator of ketones in the body is a very intriguing idea that has sparked numerous studies. One study recently published in the *Journal of Breath Research* conducted a regression analysis on hydroxybutyric acid vs. breath acetone dilutions of 111 breath samples [5]. The authors’ results showed a near linear relationship for lower values of blood ketones versus breath acetone. Unfortunately, this study lacked the high values of blood ketones that would allow the authors to clearly identify the relationship between these two quantities for all potential points. This study, though incomplete due to the lack of these points, supports the claim that it is possible to use breath acetone to recover the plasma concentrations of ketones.

The volunteers of this study fasted the night before. No medication or insulin was taken. In the morning of the experiment, each volunteer possessed a high blood glucose reading and took insulin before the experiment was conducted. That being stated, without insulin it is very likely that the first T1 volunteer’s body was relying on other metabolic pathways to produce energy. The
an abnormally high level of acetone could be an indicator of fatty acid metabolism. Though there is no means of being 100% certain about the pre-day dilution of acetone and the relationship with blood ketones values, this explanation is very plausible.

When looking at the data sets and the linear coefficient values calculated for each chemical trace, two noteworthy results become apparent. Firstly, the HCN chemical dilution trace in the second T1 diabetic volunteer has a very high Pearson’s r value—0.968. The HCN found in breath samples is likely the product of oral bacterial and enzymatic activity [41]. It is not an endogenous product the human body’s metabolism. According to an article published in Ieee Engineering in Medicine and Biology Magazine, blood glucose levels and saliva glucose levels are correlated fasting diabetic individuals [42]. Furthermore, it has been stated that oral bacteria thrive on glucose[43]. Because of this, diabetic individuals are known to suffer from mouth infections and periodontitis due to poorly controlled blood glucose [43]. The sudden rise in blood glucose concentrations would, in turn, increase the concentrations of saliva glucose. This could feed the HCN-producing bacteria and promote the production of HCN in breath. This result does suggest that, for individuals that possess these bacteria in their oral cavities, the HCN dilution levels from breath exhaled from the mouth could be potentially used as a means of monitoring blood glucose levels. With that being said, good oral hygiene would prevent the abundance of oral bacteria in the mouth.

Secondly, several molecules do show potential for use in monitoring blood glucose values. For instance, acetaldehyde has a slightly higher Pearson’s r correlation values in both subjects. Further, the correlation value is .55 in that of the healthy volunteer in section 4.1. This may imply that there is a slightly linear relationship with acetaldehyde and blood glucose. Better yet, this molecule may be utilized in conjugation with others, such as ethanol, acetone, and methanol to uncover an algorithm that could potentially recreate plasma glucose dilutions from concentrations of chemicals in breath.
4.3 Extended data set: 90 points

The tests presented in this chapter were all conducted with stringent time, medication, and food controls set in place. These controls were in place to minimize the parameters that could affect the results of the experiments. The results of those tests allowed the observation of insulin (or lack thereof) and the fluctuation of blood glucose caused by the consumption of carbohydrates over time. Those experiments also illustrated the volumetric dilutions of select chemicals in breath over the same time scale, thus, allowing the observation of how they behaved in breath with a rise in plasma glucose. The daily life of T1 diabetics are not as meticulous organized as these experiments. To treat their diabetes they do take insulin and other medications. They eat a variety of different foods at different times of the day.

The next study was conducted to monitor the breath composition of a typical T1 diabetic. This experiment was taken over the course of a year and consists of 90 breath samples and blood glucose readings taken from 6 different T1DM afflicted volunteers. These samples were donated at the convenience of the volunteers, and range in time from 4:45am to 9:00pm throughout the day. For most of these points other parameters were also traced. These include the volunteer’s Body Mass Index [BMI], time, insulin intake, carbohydrate intake, insulin pump functionality, basal rates, and medications. Insulin and carbohydrate intake was recorded up to 6 hours before the sample was given. A complete list of the information on each volunteer can be found in the Appendix.

The volumetric dilutions for each chemical examined in this study were calculated for each of these samples. The results of this calculation graphed against their respective blood glucose levels can be seen in figure 4.4. No apparent patterns in any chemical dilution graph in this figure seem to emerge visually, and therefore no conclusions about the relationship these chemicals have with blood glucose can be made at this time. It is important to remember that this data includes many different factors that are not accounted for in these graphs, such as insulin and carbohydrate intake. In the future this data and these unaccounted for parameters will be utilized to create an algorithm that
would allow for the determination of blood glucose values from breath concentrations of these chemicals.

Figure 4.4: 90 breath samples of 6 T1DM individuals taken over the course of a year

A hypothesis concerning the acetone dilution levels and ketosis can be made from this collection of data points. Figure 4.4 is an enlarged graph of the acetone dilutions graphed against
blood glucose values. Unlike above, this graph is separated by volunteer. Though there were six volunteers that contributed to this data set, the bulk of the data actually comes from just three: subject 3, subject 4, and subject 5. Some subsets of the data from this experiment are also graphed. Points in which the volunteers were fasting are circled, whereas points in which insulin pumps were malfunctioning are squared. Even with this extra information, no apparent correlation between fasting and/or pump malfunction seem to explain the breath acetone readings. In section 1.6, a discussion about normal blood ketone levels was presented. It was determined there that 1.6 mmol/L was the threshold between acceptable and unacceptable values for ketones found in blood. This correlates to about 1ppm of acetone. A dotted line has been placed horizontally at this volumetric dilution to illustrate this separation. A further division of the data points was made based on average versus high blood glucose values. For this discussion this division was placed a little above 200 mg/dl. With these divisions made this graph now possesses three different regions. The bottom region is being hypothesized to have acceptable levels of ketones in the body. The top region with low blood glucose values would signify a nutritional pre-ketosis. The last area on the graph would signify pre-ketosis due to insulin deficiency: the high blood glucose would signify that there is enough sugar in the blood to initiate carbohydrate metabolism, instead the cells of the body are utilizing fatty acids as an energy source.

![Graph showing breath acetone vs. blood glucose measurements of T1DM individuals](image)

*Figure 4.5: 90 breath acetone vs. blood glucose measurements of T1DM individuals*
4.4 Discussion

The results obtained from directly analyzing the composition of T1 diabetic breath is interesting, albeit incomplete. The OGTT based experiments for both the healthy volunteer as well as the T1 diabetic individuals’ needs to be repeated multiple times on different volunteers. With the addition of this data, more concrete trends in the volumetric dilutions of chemicals may become more apparent. The results from the calculations obtained from section 4.3 with the 90 data points need to be further analyzed with the aid of machine learning software to include the parameters unaccounted for in graphs. It is our hope that this data set will furnish an algorithm that will be able to predict the blood glucose values of an individual based solely on these exhaled VOCs.

Based on the fact that concentrations of breath acetone have been shown to correlate with blood ketone levels [5], and that this technology possesses the ability to quickly measure acetone, than this technology is a reasonable next step in monitoring ketosis. If this technology was developed further it could replace modern day testing of blood or urine ketone tests. This is very applicable to the younger T1DM community, whom, more so than adults, are likely to suffer from sudden hyperglycemic events. This could offer early detection of high ketones in the body and promote quicker responses to treat these occasions.
**Conclusion**

T1DM afflicted individuals require constant and costly monitoring of their condition or risk serious and potentially deadly complications. This is done through costly medical supplies that inflict their users with pain and/or discomfort. Further, the extra financial burdens for healthcare on these individuals are great, and cause many to struggle economically to properly treat this disease. Current means of testing need to be revolutionized to alleviate these negative attributes. The results here demonstrate a potential use of THz spectroscopy in monitoring of this disease. Utilizing this technology for breath analysis *in lieu* of current methods to maintain the health of T1DM could alleviate the pain and discomfort felt by the millions and would cut down healthcare costs.

It has been shown in this thesis that many of the known VOC’s associated with this disorder are readily detectable with this technology. Though no direct relation has been established that can reverse engineer blood glucose values based solely on breath dilutions of these VOCs, many promising molecules have been detected. With improvements to the system, such a smaller absorption cell and a custom built preconcentration system, crucial chemicals such as methyl nitrate and urea might become detectable in samples in the near future. Methyl nitrate has already been shown to be a major component of an algorithm to find plasma concentrations from breath dilutions of chemicals [32]. With this chemical added to the list of detectable molecules in breath using THz radiation, it is likely that an algorithm can be created that relies solely on THz detectable dilutions.

This technology also has been shown to be competent in monitoring some of the main complications associated with this T1DM. The future of this experiment should concretely correlate ketones in the blood with breath dilutions of acetone. THz spectroscopy also could be applicable to monitoring kidney function based solely on urea in heated sweat samples. This study has provided the foundation for the detection of both of these molecules. In the near future, the results of the spectral assignments of methyl nitrate and urea will be exploited in the investigation into this disease.

THz spectroscopy is a versatile technology suitable for the medical field. Eventually, this technology will have the capacity provide timely results for the monitoring of T1DM. That being
said, this technology is not limited to this disease. With the correct selection of VOCs in breath, THz spectroscopy will likely have the capability to monitor a vast array of different disorders or exposures in the body. It is this wide range of applications that this technology can afford that makes it an attractive choice for further development and integration into medicine.
Appendix

Figure A.1: The schematic layout of the THz Spectrometer at Wright State University. Discussion of the system has been previously published. [15] [44]

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Gender</th>
<th>BMI</th>
<th>Insulin pump</th>
<th>Number of points</th>
<th>Dates volunteered</th>
<th>Basal Rates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject 3</td>
<td>18</td>
<td>Female</td>
<td>22</td>
<td>Yes</td>
<td>23</td>
<td>9/2014-1/2015</td>
<td>12:00am - 8:00am</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8:00am - 2:00pm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2:00am - 6:00pm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6:00pm - 9:00pm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>9:00pm - 12:00pm</td>
</tr>
<tr>
<td>Subject 4</td>
<td>24</td>
<td>Female</td>
<td>28.7</td>
<td>Yes</td>
<td>37</td>
<td>9/2014-3/2015</td>
<td>12:00am - 4:00am</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4:00am - 12:00am</td>
</tr>
<tr>
<td>Subject 5</td>
<td>18</td>
<td>Male</td>
<td>21.7</td>
<td>No</td>
<td>24</td>
<td>9/2014-3/2015</td>
<td>N/A</td>
</tr>
<tr>
<td>Subject 6</td>
<td>-</td>
<td>Female</td>
<td>-</td>
<td>No</td>
<td>1</td>
<td>10/17/2014</td>
<td>N/A</td>
</tr>
<tr>
<td>Subject 7</td>
<td>-</td>
<td>Male</td>
<td>-</td>
<td>No</td>
<td>3</td>
<td>2/16/2015</td>
<td>N/A</td>
</tr>
<tr>
<td>Subject 8</td>
<td>30</td>
<td>Female</td>
<td>30.3</td>
<td>Yes</td>
<td>2</td>
<td>3/2015</td>
<td>12:00am - 2:00am</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2:00am - 6:00am</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6:00am - 1:00pm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1:00pm - 12:00am</td>
</tr>
</tbody>
</table>

Table A.1: Volunteers of the uncontrolled 90-points data set
Bibliography


