Terahertz Spectroscopic Breath Analysis as a Viable Analytical Chemical Sensing Technique

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TERAHERTZ SPECTROSCOPIC BREATH ANALYSIS AS A Viable ANALYTICAL CHEMICAL SENSING TECHNIQUE

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

By

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B.S. in Physics, Wright State University, 2001

2016
Wright State University
I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Robert Schueler ENTITLED Terahertz Spectroscopic Breath Analysis As A Viable Analytical Chemical Sensing Technique BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science.

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Abstract

Schueler, Robert M.S. Department of Physics, Wright State University 2016. Terahertz Spectroscopic Breath Analysis as a Viable Analytical Chemical Sensing Technique.

The ability to quantify trace chemicals in human breath enables the possibility of identifying breath biomarkers to aid in diagnosis. The vast majority of the studies in the analytical breath analysis rely on GC-MS techniques for quantification of the human breath composition\(^1,2,3,4\).

THz spectroscopy of breath is rapid, sensitive, and highly specific molecular identification in complex mixtures containing 10-100 analytes with near ‘absolute’ specificity.

THz spectroscopic breath analyzers require chemical preconcentration. A newly developed custom preconcentrator was constructed and compared in its performance to a commercial system. Unlike the commercial counterpart, the new system does not require cryogenic liquids, is compact, and offers significant advantages in terms of ease of operation and facilitates further development of THz breath sensors. Its preconcentration efficiency was assessed. The THz spectrometer coupled with the custom preconcentrator demonstrated first THz detection of breath isoprene, a chemical not detected with the commercial device.
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1) Introduction:

As many as 3500 trace gases have been reported in human breath\(^1\). The vast majority of the studies in the analytical breath analysis rely on GC-MS techniques for quantification of the human breath composition\(^2,3,4\). Variability in retention time in GC column from one chemical to another improves limited selectivity of MS in complex mixtures. However, the limited number of resolution channels, ambiguity in MS fragmentation, and accidental similarities in retention times can lead to erroneous GC-MS results. Sensitivity of GC-MS instrumentation is such that detection at parts per billion (ppb) and parts per trillion (ppt) levels usually require processing of a relatively large number of breath samples by means of cryogenic or sorbent traps. This limitation highlights the challenge of real-time volatile organic compounds (VOC) biomarker identification. The study by Philips et al.\(^5\) States that GC-MS results represents only a “tentative identification of the analytes present in normal alveolar breath”\(^5\) due to the necessity to calibrate each combination of trapping-desorption-GC-MS hardware for every species on the detected list\(^3\). Furthermore, GC-MS results are reported as Tentatively Identified Compounds (TICs).

THz spectroscopy of breath is rapid, sensitive, and highly specific molecular identification in complex mixtures\(^6\). This technology has been vetted in a 15 million dollar program funded by DARPA\(^7\) (Mission-Adaptable Chemical Sensor [MACS]), and resulted in a successful development of a packaged chemical sensor that had absolute specificity (probability of false alarm \(< \times 10^{-10}\)) stemming from detection based on highly specific THz spectroscopic molecular signatures. THz spectroscopy and the MACS program provides a major advantage
over competing technologies, such as GC-MS. The sensor demonstrated detection limit of 10 parts per trillion dilution (comparable to GC-MS state of the art limits) with a total sampling volume of 1 L and a total analysis time (including sampling) of ~10 minutes. It identified all chemicals in a mixture of 32 gases, demonstrating absolute specificity.

THz spectroscopy unambiguously (probability of false alarm is negligibly small) determines presence of each of the chemicals and calculates the uncertainty of the calculated dilution based on spectroscopic signal to noise ratios. The GC-MS results presented in Section 4.2.3 are calculated by comparing the peak area counts of the internal calibration standard (TO-15 mixture), (of which the mass injected into the GC-MS instrument is known) against the area count of the target analytes. Since the internal standards are not the actual compounds being quantified, there’s no easy way of determining the uncertainty in concentration estimation.

This thesis uses samples collected by and data taken for a study entitled “The Effects of Modafinil and OTC Stimulants on Physical and Cognitive Performance” performed by Naval Medical Research Unit at Dayton (NAMRU-D) sleep lab and “Terahertz Spectroscopic Chemical Sensor for Analysis of Fatigued Human Breath” (FA8650-15M-6590) performed by Advratech LLC and Wright State University. Any opinions, findings and conclusions or recommendations expressed in this material are those of the author and do not necessarily reflect the views of the US Air Force Research Laboratory. These studies collected breath from multiple subjects and performed GC-MS and THz analysis on similar breath samples allowing for direct comparison of the two methods. This work focused on acetone, acetaldehyde and ethanol. These three chemicals are associated with biological processes and are found in breath. Additionally, these three chemicals have the most overlap between the GC-MS data set and the THz data set. Future efforts will expand the THz system calibration to a wide range of chemicals.
In addition to GC-MS and THz breath analysis comparison, a custom preconcentrator was developed and tested. Previously, an Entech 7100A commercial preconcentrator was the standard tool we used to remove atmospheric gases from breath sample. The custom preconcentration system enabled the detection of chemicals not previously detected with the Entech 7100A without the use of liquid nitrogen and provided a path to future miniaturization. This custom preconcentrator demonstrated THz spectroscopic detection of Isoprene, an analyte not previously detected when preconcentrating with the Entech 7100A.

The custom preconcentrator and the Entech 7100A were calibrated using known dilutions of Acetone, Ethanol and Acetaldehyde injected into a Tedlar bag. Once calibrated, the breath analysis results from these preconcentrators coupled to the THz spectrometer are shown to be in agreement for acetone and ethanol through the testing of the same breath samples on both systems. Acetaldehyde was not shown to be in agreement. This could be caused by a small amount of unaccounted for contamination in the custom preconcentrator or the calibration factor could be incorrect due to the small number of data points taken.
2) Background:

2.1) Acetone, Ethanol and Acetaldehyde:

Acetone.

Acetone is a common VOC found in breath due to lipped metabolism of fatty acids by β-oxidation and production of ketone bodies. The ketone body acetoacetate gradually breaks down to acetone and CO₂. This is the impetus investigating acetone in breath as a marker for blood glucose levels as ketone body production is sensitive to insulin therapy. Acetone is readily detected and is linked to glucose and lipid metabolism and exercise.

Ethanol and Acetaldehyde

Ethanol and acetaldehyde will be discussed in tandem as acetaldehyde is formed as a result of alcohol dehydrogenase enzymes acting on ethanol in the liver. The profile of ethanol and acetaldehyde is nearly identical in the first two test subjects. However, test subjects had no access to alcoholic beverages during the study.

Even without consuming alcoholic beverages, levels of ethanol and acetaldehyde in breath fluctuate. In performing oral glucose challenges, Galassetti and colleagues demonstrated a dramatic rise in exhaled ethanol, along with plasma glucose, following consumption of drink spiked with 75 g glucose. This spike is most likely produced by the gut flora, and not related to plasma glucose. Thus, ingestion of carbohydrates in general may produce ethanol and acetaldehyde in breath, but at much lower levels compared to ingestion of
alcoholic beverages\[8\]. However, a link has been observed between pathological fatigue (such as chronic fatigue syndrome) and gut microbiota disorders, suggesting ethanol and acetaldehyde may be appropriate breath diagnostics for other conditions\[11\].

2.2) THz Spectroscopic Sensor:

The stepping–sweeping spectrometer used for this study is shown in Figure 1. In its current configuration it can scan between 210 GHz and 270GHz and spectroscopically interrogate the 0.875 liter cell (top) or the 14 liter cell (bottom). This spectrometer tunes to a center frequency then sweeps ± 2.5 MHz around that frequency. These frequencies are chosen to be in the center strong absorption lines of the analytes. Care is taken to choose absorption lines in locations that do not have overlapping absorption lines from other analytes. Each of these 5 MHz wide sequences take approximately 3 seconds to acquire and are referred to as snippets. Typically, 5 snippets are chosen for each analyte. Having multiple snippets for each analyte removes the possibility that any one accidental overlapping absorption line will be confused with signal. Parts of the spectrum that lie in-between the chosen snippets are not acquired to reduce the time required to collect data.

Figure 1: THz Spectrometer with Entech 7100 and the custom built preconcentrators.
As the frequency sweeps across a 5 MHz snippet it is frequency modulated (FM) at a rate of 15 kHz with a 400 kHz depth of modulation. A SRS SR530 lock-in amplifier provides a voltage that is proportional to the first or second derivatives of the change in absorption. If the absorption feature is expected to be wider than 5 MHz wide, three 5 MHz snippets are located side by side providing a 15 MHz wide window to record the feature (such as the HCN feature accompanied by nitrogen quadrupole satellites).

The THz signal is produced by VDI multiplier chains which are driven by a microwave signal. That microwave signal is produced by an Agilent E8257D microwave synthesizer coupled to a custom microwave sweep circuit. The VDI multiplier chains multiply the frequency of the microwave signal by a factor of 24. The microwave synthesizer steps through the snippet central frequencies divided by 24 and FM modulates the signal. This microwave signal is split. One branch drives a heterodyne detector. The other drives the custom microwave sweep circuit which in turn drives the source multiplier chain with a signal that has a 2.4 GHz offset form the signal driving the heterodyne detector.

The THz signal from the source multiplier chain is collimated and passed through the absorption cell, then focused into the heterodyne detector. The heterodyne detector multiplies its driving microwave signal by 24 and mixes it with the source signal. The resultant difference signal is at the intermediate frequency of 2.4 GHz. This signal is amplified then converted to a DC signal proportional to its amplitude by a Hetotek DHMA18AB zero-bias schottky diode detector. That signal is sent to a SRS sr560 preamplifier and DC coupled for DC Baseline scans and AC coupled for Chemical Baseline and Snippet Scans.
A DC Baseline scan measures the THz power for each snippet. To perform this scan, the FM modulation is turned off and the signal is acquired after a DC coupled preamplifier. This scan is necessary to normalize the Chemical Baseline and the Snippet Scan for power.

Snippet scans are performed on an absorption cell containing a captured breath. Snippet Scans are AC couple the DC signal from the diode into a pre-amplifier with a band pass filter centered on the FM modulation frequency (15 kHz). This amplified AC coupled signal is fed into a lock-in amplifier. The lock-in amplifier rejects noise occurring at frequencies other than the modulation frequency. The phase of the lock-in amplifier is adjusted to maximize the signal on one channel. It takes the first or second derivative of the signal and outputs the signals that are in-phase with the FM modulation and a portion that is out of phase with the FM modulation. Both phases are digitized and recorded. The in-phase signal is used when calculating dilution.

Chemical baseline scans differ from Snippet Scans because they are performed on a closed absorption cell under vacuum. This Chemical Baseline scan measures the chemical contamination of the absorption cell. This contamination is subtracted from the Snippet Scan measurements when calculating how much of a particular analyte is in a sample.

2.3) Vacuum Requirements:

The high specificity of THz spectroscopy is at least in part due to the narrow line widths of rotational spectra. Line widths are determined by the natural width\textsuperscript{12}, Doppler broadening from molecular movement, pressure broadening due to interactions between molecules of the same or different species and other effects such as Stark, Zeeman, instrumental effects, etc. Typically, line width are approximately 1 MHz wide at pressures on the order of 10 mTorr. Pressure broadening starts to affect THz spectral resolution at pressures around 100 mTorr (\textasciitilde10^{-5} atm). Thus, to achieve best spectral resolution in the THz range a sensor must operate in
this pressure regime and requires a pumping system. To avoid excessive pressure broadening in a breath, major atmospheric gases such as O2, N2, H2O, CO2, and Ar must be removed while retaining the target analytes. This necessitates preconcentration. Effective preconcentration simultaneously requires retention of analytes and removal of atmospheric gas.

The ENTECH 7100A we use is an example of a turnkey commercially available preconcentrator (Figure 2). The Entech 7100 is typically used as a preconcentrator of volatile organic compounds for gas chromatography (GC) and gas chromatography mass spectroscopy (GC-MS). Entech 7100’s typically have three traps. The first is a glass bead filled cryogenic trap. It traps both water and the VOCs form the sample. The second trap contains Tenax TA sorbent. Sample is passed from the first to the second trap by increasing the temperature of the first trap and flowing N2 through the two traps. This process transfers the VOC’s from the first trap to the second leaving behind water. The last trap is a “focusing” trap for GC applications. This trap is not needed for THz spectroscopy and has been removed from the ENTECH 7100A used in this work.

*Figure 2* Entech Preconcentrator and schematic. In its current configuration the first trap uses Tenax Ta, the second trap is a cryogenic trap, the third focusing trap has been removed.
2.4) Data Analysis:

The data is recorded in 5 MHz wide snippets. The central frequencies of these snippets were chosen to coincide with the strong absorption lines of particular molecules and not to have known overlapping absorption lines from other molecules. In addition to strategic selection of snippets, multiple snippets for each chemical are recorded. This prevents any one unknown overlapping absorption band from being confused with absorption from the intended molecule.

Once collected, the snippets are compared with spectral libraries. Spectral libraries are produced from pure samples with well-defined pressures, usually 2 mTorr, 5 mTorr and 10 mTorr. Because it is a pure sample of known temperature, volume and pressure, the number of absorbers is known for each library. Each absorption line for a given chemical and pressure might have a slightly different line width due to differences in pressure broadening for each molecular species. Figure 3 shows an example of an overview spectra and the chosen snippets for the 1 mTorr libraries for cis-1,2-dichlorethane. This particular over view spectra and related snippets is for the frequency rage 290 GHz through 330 GHz. Similar library data between 210 GHz and 270 GHz exists for each targeted chemical we can identify at multiple pressures. The libraries’ number density inside the chamber is calculated from the temperature, volume and pressure using the using the ideal gas approximation.
Figure 3 Overview spectra and snippets of Chloroethane and cis-1,2-Dichloroethane. Potential library snippets are shown below.

For each breath sample, data is collected on the same snippets and are then fitted to library spectra. Figure 4 shows the raw data collected on a typical breath sample. The red line shows is the DC Baseline scan and proportional to THz power. The black line is the Chemical Baseline scan which shows the chemical contamination of the cell. The blue line in Figure 4 shows the raw data from a sample scan.

Figure 4 Data collected from 500 ml of breath on a typical sample. The red line is the DC scan and it is proportional to THz power. The black line is the chemical baseline scan. The blue line shows is the snippet scan performed on a breath sample.
The snippet and baseline scans can be scaled by the gain setting on the system when they were taken and power available at that frequency measured with the DC scan. The data is then fitted using Igor Pro to a library with a known number density. The number density of the sample is divided by the number density of ambient air to give the volumetric dilution of the chemical in the sample.

Volumetric dilution for each AC coupled data set is calculated using the equation 1. If the system is performing linearly independent of sample volume then the volumetric dilution will be the same for all sample volumes. The decrease in sample volume is expected to be compensated for by a decrease in partial pressure of the analyte. When the custom preconcentrator is used and there are more than one desorption cycle performed on the sorbent, the results of the runs are added together. In equation 1, γ is the preconcentration efficiency.

\[
\text{Volumetric Dilution} = \frac{\text{Partial Pressure of Analyte}}{\text{Atmospheric Pressure}} \times \frac{\text{Cell Volume}}{\text{Sample Volume}} \times \frac{\text{Sample Temperature}}{\text{Cell Temperature}} \times \gamma \ [1]
\]
3) Experimental

3.1) Novel Sorbent Tube Preconcentrator:

The custom preconcentrator was constructed to add greater flexibility in sorbent selection and processing; to remove the need for liquid N\textsubscript{2} cold traps and to allow for the detection of previously undetected chemicals such as Isoprene. Despite the fact that isoprene is one of the more common chemicals in breath and is in sufficient concentrations for THZ spectroscopy to detect, isoprene was never detected using the THz spectrometer coupled to the Entech 7100A preconcentration system. It is our belief that this Entech system is removing isoprene from the sample when it is removing water from the sample. Thus it does not deliver isoprene to the THz spectrometer efficiently.

The new preconcentration system is built around the use of commercially available sorbent tubes. Different sorbent materials trap various chemicals with differing efficiencies. The sorbents require heat to release the stored chemicals. Heating the sorbents to release a trapped chemical sample is known as thermal desorption. After the sorbents have trapped and released a chemical, the sorbent must be reconditioned to prepare it to trap the next round of chemicals. Reconditioning is done by flowing an inert gas, usually helium, through the sorbent at elevated temperatures.

The Markes International\textsuperscript{14} C3-AXXX-5266 stainless steel thermal desorption tubes were chosen for their ability to collect a wide range of compounds. This is a multiple bed sorbent tube that contains three sorbents, Tenax TA, Carbograph 1TD and Carboxen 1003. Combined,
these three sorbent are capable of effectively collecting organic compounds with 2 to 30 carbon atoms and are hydrophobic so they will collect little water. Too much water present in the sample will lead to pressure broadening thus it is important to minimize the amount water that is collected with the organic chemicals when storing a breath sample.

Trapping a breath sample, releasing a breath sample and reconditioning the sorbent tube are 3 modes of operation that are automated by the custom preconcentrator. Tenax TA is designed to trap VOCs with 7 to 30 long carbon chains, Carbograph 1TD traps VOC’s with 5 to 14 long carbon chains, and Carboxen 1003 traps VOC’s with 1 to 6 long carbon chains. When storing a breath Tenax TA removes the larger VOCs with carbon chains greater 14 that can damage Carbograph 1TD and Carboxen 1003. Thus, when desorbing or reconditioning a tube, it is important that the flow is reversed so the larger VOCs released from the Tenax TA do not pass through the Carbograph 1TD and Carboxen 1003. The direction of flow during these functions is important to protect Carbograph 1TD and Carboxen 1003 from damage. Figure 5 shows a schematic of the three modes of operation. The hollow arrow on the tube denotes the direction of flow that is required when storing a breath. During reconditioning and sample desorption the flow is in the direction opposite of the arrow printed on the sorbent tube. Thus, larger trapped VOC’s do not flow through the Carbograph 1TD and Carboxen 1003 during desorption.
Figure 5: Schematic of the three processes necessary for sorbent tube based preconcentration. The circles with Xs’ signify closed valves. The large arrow on the sorbent tube signifies the orientation of the sorbent tube.

To store a breath in the sorbent tube, we connected a Tedlar bag full of a subject’s breath to the breath port near valve 5 in Figure 6. Figure 6 shows a schematic of the custom preconcentrator attached to the absorption cell. While the sorbent tube is at room temperature, valve 5 between the Tedlar bag and the sorbent tube and valve 1 between the flow-mass controller the sorbent tube is opened. The flow mass controller will allow 0.5 liters of breath to pass through the sorbent tube at a rate of 0.5 liters/minute. The flow mass controller can be calibrated for many different chemicals. The calibration factor for Air and humid air is the same as the calibration factor for nitrogen 1.0000. The sorbent tube will remove the VOCs that make exhaled breath different than humid air making 1.0000 the appropriate calibration factor for this situation.
In our experiment, the sorbent tube is typically thermally desorbed within minutes after storing a breath. When desorbing a sorbent tube, it is put under vacuum before heat is applied to remove gases that are not trapped by the sorbent. This is accomplished by opening valve 4 between the sorbent tube and the absorption cell while valve 6 between the chamber and the high-vacuum pump is open. This removes the molecules that are not bound to the sorbent including nitrogen, argon and helium. Once the pressure reaches equilibrium valve 6 between the sample chamber and the vacuum pump is closed and the sorbent tube is heated to thermally desorb the sample.

The sample trapped within the sorbent tube is desorbed in three stages, a 120°C stage, a 200°C stage and a 310°C stage. This keeps pressure inside the test chamber below 20 mTorr. Approximately 1/3rd of the VOCs stored in the sorbent tube are released during each stage. In the first desorption stage, once valve 6 between the pump and the sample chamber is closed, the sorbent tube is heated to 120°C at a rate of 50°C per minute. It is then held at 120° for 2 minutes to allow time for the VOCs to desorb and defuse into the absorption cell before valve 4.

Figure 6: Custom preconcentrator schematic.
between the absorption cell and the sorbent tube is closed trapping the sample. Data acquisition then begins and the sorbent tube is allowed to cool.

Once data is acquired on the first desorption stage is completed, valve 6 between the absorption cell and the vacuum pump is opened to release the trapped sample. Valve 6 is held open for enough time for the vacuum pressure to reach equilibrium before it is closed. After valve 6 is closed, valve 4 between the sorbent tube and the absorption cell is opened and the sorbent tube is heated to 200°C. Controlling the valves in this sequence prevents loss of sample before it is measured. The sorbent tube is held at 200°C for 2 minutes and the trapped chemicals diffuses into the absorption chamber. Then valve 4 between the sorbent tube and sample chamber is closed. Data collection begins and the sorbent tube is again allowed to cool.

The third and final desorption stage is very much like the second desorption. The valves are controlled with the same sequence to prevent loss of sample. During the third desorption stage the sorbent tube is heated to 310°C and that temperature is held for 2 minutes. After the sample has evolved into the sample chamber and valve 4 is closed, the sorbent tube is not allowed to cool. Instead, while the data is being collected on the third desorption, the sorbent tube is reconditioned. Reconditioning the tube before it is allowed to cool down after the third and final desorption saves time and reduces the number of thermal cycles the tube will undergo.

Reconditioning the sorbent tube requires Inert gas (helium or nitrogen) to flow through the tube in the direction opposite of the arrow at a temperature of 335°C for 10 minutes. The custom preconcentrator opens valves 2 and 3 to allow helium to flow through the sorbent tube while heating sorbent tube. The rate of heating of the tube must be controlled or the sorbent may be damaged. The tube is heated at a rate of 50° per minute until the reconditioning
temperature of 335°C is reached. Usually reconditioning a tube begins right after the 310°C desorption to reduce thermal cycling while the THz spectrometer is interrogating the highest-temperature sample. The flowing helium at elevated temperatures removes the VOCs left in the sorbent after the last desorption. After 10 minutes at 335°C with flowing helium, valves 2 and 3 are closed and the sorbent tube is allowed to cool to room temperature. Once at room temperature the sorbent tube is ready to store the next breath sample. Each sample takes approximately 1.5 hours. This time can be significantly reduced by optimizing the absorption cell and geometry to allow for fewer thermal desorption cycles. Strategic selection of snippets will reduce the time required for each desorption.

3.2) Breath Samples

NAMRU-D Cognitive Readiness and Resilience (CRR) Laboratory as part of “The Effects of Modafinil and OTC Stimulants on Physical and Cognitive Performance” (NAMRU-D IRB# NAMRUD.2013.003)” collected breath samples for GC-MS and THz spectroscopic analysis. This thesis contains data from breath samples from three subjects labeled here as Subject 1, Subject 2 and Subject 3. Subjects 1 and 2 underwent commercial GC-MS analysis at the highest level of sensitivity provided by ALS Environmental (“All TICs (up to 200) - TO-15 Modified”), while Subject 3’s breath was analyzed using “Top 50 TICs - TO-15 Modified” method. TIC is a n abbreviation for Tentatively Identified Compounds. We believe that GC-MS results for Subject 3 show significant inconsistencies (last three points of acetone are the same, and acetaldehyde not identified), thus the “Top 50 TICs - TO-15 Modified” data is not used.

The breath samples were taken at 5 predetermined times over a 40 hour sleep deprivation study. At each breath sample collection time, the subject sequentially filled two Tedlar bags with approximately 2 liters of breath. One bag was sent to ALS Environmental for
GC-MS analysis the other was analyzed using THz spectroscopy. THz spectroscopy using the Entech 7100A preconcentrator was performed on all fifteen samples. Subject 3’s samples were run on both the THz spectrometer coupled to the Entech 7100A preconcentrator and the same THz spectrometer coupled with the custom preconcentrator. The datasets from Subject 1 and 2’s breath allow for direct comparison of GC-MS and THz spectroscopy techniques. Subject 3’s breath data set allows for the direct comparison of the Entech 7100A and the in house custom preconcentrator.

3.4) Calibration Mixture:

To measure the preconcentration efficiency of the preconcentrators Tedlar bags with known quantities of N₂ water, ethanol, acetone and acetaldehyde were prepared and analyzed with the THz spectrometer and the results compared to the expected dilutions. Preparing Tedlar bags with known quantities required injecting a Tedlar bag with a solution that contains known concentrations of water, ethanol, acetone and acetaldehyde. A Hamilton microliter model 7000.5 syringe was used to inject 0.5 µl of solution into the Tedlar bag. After the needle punctured the Tedlar bag and the plunger depressed a droplet formed on the end of the needle. The needle was then made to touch the bag on the side opposite to the puncture to leave behind the droplet. Figure 7 shows one such droplet on the inside of a Tedlar bag with a dime to show the scale. Visual confirmation of the droplet inside the bag was required before removing the syringe.
The target dilutions were below 500 ppb for ethanol and acetone, and below 100 ppb for acetaldehyde to match concentrations found in breath and to prevent saturation of the sorbents. A typical solution required approximately 25 g of water, 0.25 g of acetone 0.06 g of Ethanol and 0.035 g of acetaldehyde. The acetone and ethanol were weighed in syringes. The syringe was wetted with the chemical, then the scale was tared, then chemical was drawn into the syringe, weighed before being injected through paraffin film into a container with 23 g of water. Weighing small amounts of acetaldehyde was complicated by its low boiling point (20.2°C) thus, an intermediary dilution was used.

To make the intermediary solution of acetaldehyde, the syringe, containers, acetaldehyde and water were all chilled to prevent the acetaldehyde form boiling off. Approximately 0.2 g of acetaldehyde was weighed using the chilled syringe and injected through
a paraffin barrier into 10 g of chilled water then capped. The solution was gently mixed in so the solution would not come into contact with the paraffin. After 5 minutes, the cap was opened to remove the paraffin then replaced so the solution could be mixed more vigorously. Several grams of this intermediate solution was added to the previously prepared mixture of acetone, ethanol and water to produce a liquid calibration mixture ready to be injected into a Tedlar bag containing a known quantity of nitrogen.

Using a flow mass controller, a Tedlar bag is filled with a known quantity of nitrogen usually 4 liters. 4 liters is a convenient volume because many of the Tedlar bags used have a maximum capacity of 5 liters. Larger 10 liter Tedlar bags were also used. Once filled with nitrogen, 0.5 µl of calibration solution was injected into a Tedlar bag producing the resultant dilution. The error in the dilutions was calculated from the systematic errors in each measurement and the fractional uncertainties of those measurements.
4) Results

4.1) Spectrometer Sensitivity Improvement:

The THz spectrometer sensitivity was improved by having both the signal driving the source multiplier chain and the heterodyne detector multiplier chain FM modulated. This is the situation described in Section 2.2. The blue line in Figure 8 is representative of the five isoprene snippets when both signals are FM modulated during a chemical baseline scan (no sample in the absorption cell). In previous efforts, the signal driving the heterodyne receiver did not FM modulate while the signal driving the source multiplier chain was FM modulated. The red line in Figure 8 is representative of chemical baseline scans where the heterodyne receiver did not FM modulate. The peaks in the center of the snippets for the red trace are artifacts origination from mixing the FM modulated source signal with non-FM modulated signal to the heterodyne detector. When both signals are FM modulated, those artifacts are removed (blue line) thus, improving the detection limit.
Figure 8 Comparison of the System Spectral Response Before (red) and After (blue) the modification to the microwave signal driving the heterodyne detector. A clear reduction of system’s systematic noise is visible.

4.2) THz Spectrometer Coupled with Entech Preconcentrator and 14 Liter Absorption Cell

4.2.1) Linearity with Sample Volume

The linearity of the THz spectrometer coupled to the Entech preconcentrator was investigated by taking samples of various volumes from a Tedlar bag with prepared dilutions. That prepared Tedlar bag had 43.20± 0.87 ppb acetaldehyde, 186.1 ± 3.7 ppb acetone and 469.3 ±9.4 ppb ethanol. Using the Entech preconcentrator, a 100 ml, a 250 ml and three 500 ml samples were drawn from the bag and dilutions measured with the THz spectrometer. Ideally,
the larger the sample volume results in a higher number density of analytes in the absorption cell. This sample set is intended to show the linearity of the system as a function of sample volume. Smaller sample volumes resulted in lower signal levels which is reflected by the larger error bars for smaller sample volumes. The error bars in Figure 9 through Figure 11 reflect the uncertainty of the least squares fit. Random errors are accounted for in the calibration data in Section 4.2.2.

**Acetone**

Figure 9 below shows the measured dilution of acetone as a function of sample volume. Because this is a graph of the measured dilution vs. sample volume for the same sample the result is expected to be a horizontal line. If a graph of mass of analyte measured vs. sample volume, strait line with positive slope would be expected. The Tedlar bag in this experiment was prepared with 186.1 ± 3.7 ppb of acetone. The black line at the top of the graphs shows the prepared dilution. The blue dots show the dilution as measured before baseline absorption cell contamination is accounted for. The red dots show the dilution after absorption cell contamination (measured with the Chemical Baseline scan) is accounted for. A red dot on the solid black line would correspond to 100% preconcentration efficiency. The doted black line corresponds to 52.0 % efficiency (the number calculated in Section 4.2.2). While the amount of cell contamination varies run to run, the effects of cell contamination increase as the sample size decreases. Smaller sample volumes are expected to result in a larger variance. The error bars displayed in Figure 9 do not include variance because there was only one data point taken for the 100 ml and 250 ml sample volumes. If variance was accounted for, the 100 ml sample may have been in agreement. The 250 ml data point and the three 500 ml data points are
consistent with the system being linear with sample volume.

![Acetone Dilution vs Sample volume](image)

*Figure 9 Acetone Dilution vs Sample volume. Section 4.1 calculated the efficiency of the THz spectrometer coupled to the Entech preconcentrator to be 52.0% for Acetone.*

**Ethanol**

The Tedlar bag was prepared with 469.3 ±9.4 ppb of ethanol. And THz chemical analysis was conducted on 100 ml sample, 250 ml sample and 500 ml. Like acetone, a negligible amount of ethanol was measured in the 100 ml sample. This sample volume is insufficient for this dilution. The 250 ml sample and the 500 ml samples show the system is linear with sample size. The doted black line corresponds to 54.0 % efficiency (the number calculated in Section 4.2.2).
Figure 10: Ethanol dilution vs sample volume Section 4.2.2 calculated the efficiency of the THz spectrometer coupled to the Entech preconcentrator to be 54.0% for Ethanol.

Acetaldehyde

The prepared bag had a dilution of 43.20± 0.87 ppb. Figure 11 shows the measured dilution as a function of sample volume. The 100 ml sample seemed to work better with Acetaldehyde than it did with acetone or ethanol. Since 100 ml sample sizes are clearly too small for other chemicals of interest, 100 ml sample sizes are insufficient for our needs. If in the future, 100 ml sample sizes became of interest, more work would have to be done to show consistency at this sample volume. Acetaldehyde is linear with sample size. The doted black line corresponds to 64.4 % efficiency (the number calculated in Section 4.2.2).
4.2.2) Entech Preconcentration Efficiency

To measure the preconcentration efficiency, Tedlar bags filled with various known chemical concentrations were measured using the THz spectrometer with a 500 ml sample volume. These measured dilution values were plotted against the prepared dilution values and fitted to a straight line Figure 13-Figure 15. The slope of that line is the preconcentration efficiency of the Entech preconcentrator. The Entech preconcentrator was found to be 52±7.8% efficient at concentrating acetone, 63.4±9.5% efficient at concentrating acetaldehyde and 54.0±9.5% efficient at concentrating ethanol.
Acetone

During the course of the Entech preconcentration efficiency test we discovered acetone contamination not accounted for in our chemical baseline data. This contamination is in the sample transfer lines or in the Entech itself. Figure 12 shows the concentration of acetone as measured from seven tests with of pure N\textsubscript{2} drawn from a dedicated Tedlar bag that has only contained pure N\textsubscript{2}. The red circles show the amount of acetone measured before the contamination inside the cell is accounted for. The blue circles show the amount of acetone after the contamination within the cell is accounted for. This is the contamination that is coming from the preconcentrator. The average contamination in this data set is 23.3 ppb and the standard deviation is 9.7 ppb. To measure this contamination, a pure N\textsubscript{2} sample is run every day so that breath samples from that same day can be adjusted by the amount of contamination in the Entech preconcentrator.

![N\textsubscript{2} Sample Acetone Contamination](image)

*Figure 12 Acetone measured during 8 runs on pure N\textsubscript{2} sample. The blue dots show acetone contamination in the Entech preconcentrator.*
Figure 13 shows the acetone dilution as prepared vs. as measured as well as the dilution of acetone as measured from a Tedlar bag containing only N₂. The contamination on the inside of the chamber is accounted for in this data. The contamination seen when testing bags of pure N₂ (Blue points in Figure 13) could only come from the Entech preconcentrator or the transfer tubes as described above. The purple data points on the graph have been adjusted for all known contaminations. The black line is the linear fit of the dilutions. The slope of the black line is the preconcentration efficiency of the Entech preconcentrator for acetone. The Entech preconcentration efficiency for acetone is 56.2 ± 7.8 %. This is in agreement with the acetone preconcentration efficiency of 52.1 ± 6 % result reported by Fosnight, Moran and Medvedev¹⁶

![Acetone Measured vs. Prepared](image)

*Figure 13 Acetone dilution as prepared vs. as measured for the THz spectrometer coupled to the Entech preconcentrator. The Entech is 56.2% efficient when preconcentrating acetone.*

**Ethanol**

The preconcentration efficiency of ethanol was found to be 54.0 ± 9.5 %. The blue points on the graph (Figure 14) correspond to the ethanol found in N₂ bags ran on the same day
as the prepared dilutions. There is no significant ethanol contamination in the Entech or the transfer tubes. Contamination within the cell was small as compared to the size of the error bars. The purple dots on the graph have been adjusted for this small contamination. Fosnight, Moran and Medvedev\textsuperscript{16} reported ethanol efficiency of 13.1 ± 4\% with this system for ethanol. Each prepared dilution in this work had less than 0.5 ppm of Ethanol. Fosnight, Moran and Medvedev’s sample dilution was 13 ppm. It is possible that the Entech was saturated and lost sample during their measurement.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{ethanol_dilution.png}
\caption{THz Measured Ethanol Dilution vs prepared dilution for the THz spectrometer coupled to the Entech preconcentrator. The Entech is 54.0\% efficient when preconcentrating ethanol.}
\end{figure}

\textbf{Acetaldehyde}

The preconcentration efficiency of acetaldehyde was found to be 63.4 ± 9.5\%. The blue points on the graph correspond to the ethanol found in N\textsubscript{2} bags ran on the same day as the prepared dilutions. The average acetaldehyde contamination of the Entech preconcentrator is
negligible. The highest contamination measured was $4.7 \pm 1.2$ ppb. Unlike acetone, contamination is not seen in the entire dataset thus Acetaldehyde contamination of the Entech preconcentrator is not persistent. The purple dots on the graph have been adjusted for absorption cell contamination measured in the chemical baseline scan.

![Graph showing THz Measured Acetaldehyde dilution vs prepared dilution for the THz spectrometer coupled to the Entech preconcentrator. The Entech is $63.3 \pm 9.5$ % efficient when preconcentrating acetaldehyde.]

4.2.3) Comparison of Entech Coupled THz Sensor with GC-MS

Breath samples from Subject 1 and subject 2 were measured by both GC-MS and THz spectroscopy. At each sampling time, the subject sequentially filled 2 bags with breath. One sample was sent to ALS Environmental for GC-MS testing, other sample was tested using the THz spectrometer coupled with the Entech preconcentrator. ALS Environmental reported the dilutions in units of $\mu g/m^3$. The units were converted to volumetric dilution by dividing by the
analyze molar mass to get the number of analyte moles per cubic meter. The moles per cubic meter was then divided by the number of moles in a cubic meter of air room temperature and pressure 44.60 mol/m$^3$. That number was calculated by dividing the density of air 1292 g/m$^3$ by the molar mass of air 28.9645 g/mol. The GC-MS data was plotted against the THz data for each chemical. If the samples are exactly the same and both the GC-MS and the THz spectrometer are well calibrated, the data would fall on the x=y line where the slope is one.

Figure 16 shows Acetone as measured with GC-MS vs. the THz data. The data trending along the line $x=b\cdot y$ where $b=0.965 \pm 0.075$ with a linear correlation coefficient of 0.686401. Analysis of this data would benefit from the addition of error bars on the GC-MS data. Since the internal standards are not the actual compounds being quantified, there’s really no easy way of determining the uncertainty in concentration estimation. Without error bars, we can say that the general trend in the data, $b=0.965 \pm 0.075$ suggests the two data sets are in agreement.

![Acetone GC-MS vs. THz](image)

*Figure 16 Acetone as measured with GC-MS vs as measured with THz.*
The comparison between GC-MS and the Entech coupled spectrometer for ethanol is more complicated. Each sample was measured twice with the THz spectrometer and once with GC-MS. A linear fit that includes the points where the THz measurements were 820 ppb and GC-MS measurement for that sample was 1.56 ppm results in a poor fit with the rest of the data. This could be an indication that the GC-MS reached a saturation limit or the samples may not have been identical. The Tedlar bags of breath samples tested were filled consecutively, not simultaneously. The discrepancy between the GC-MS data and the THz data for the outlaying point may be a result of the two samples having different amounts of ethanol. For future data sets, the two bags will be filled at the same time using a T shaped tubing connector. This will ensure that both the GC-MS and THz spectrometer receives identical samples. It is also possible that the samples were degraded in transport. Samples were delivered overnight from Dayton Ohio to ALS Environmental in Simi Valley, California via FedEx.

The linear fit line to the entire data set in Figure 17 has a slope of 0.209 ± 0.017. When the two outlying points are omitted the slope of the fit line is 0.380 ± 0.028. The THz spectrometers measurements are 2.6 times higher for ethanol than the GC-MS.
There were three samples where GC-MS that did not detect acetaldehyde and the THz spectrometer did. In Figure 18 the blue points correspond to those samples. The red points in Figure 18 correspond to the samples where both GC-MS and the THz spectrometer detected Acetaldehyde. The blue points were not used when calculating the trend line. The slope of that trend line is $0.188 \pm 0.012$. THz spectroscopy is detected 5.3 times more acetaldehyde than GC-MS and can detect acetaldehyde in samples where GC-MS fails to detect acetaldehyde.
4.3) Custom Preconcentrator

One prepared Tedlar bag was used to calculate preliminary efficiencies for acetone, ethanol and acetaldehyde for the custom preconcentrator coupled with the THz spectrometer using the 0.875 liter (top) absorption cell. Future work will expand this calibration to include a larger number of data points acquired from multiple dilutions to show linearity as was done with the Entech coupled system. Figure 19 shows in blue the data points as measured. The red points are the chemicals measured when running a pure N₂ sample. Acetone and acetaldehyde contamination still exists in the system even after the bake out procedure is completed. Contamination in the preconcentrator and the absorption cell was removed from the data set before percent efficiency was calculated. Preliminary preconcentration efficiency calculated for acetaldehyde is 27.8 ± 9.3 %, ethanol is 4.4 ± 1.2 % and acetone is 13.6 ± 4.2 %.
The custom preconcentrator was found to concentrate acetaldehyde at 27.8 ± 9.3 % efficiency, Ethanol at 4.4 ± 1.2 % efficiency and acetone at 13.6 ± 4.2 % efficiency. The Entech calibration used various prepared chemical concentrations thus demonstrating both concentration efficiency and linearity. Calibration of the custom preconcentrator focused on one prepared dilution. Multiple measurements at the same concentration was performed to examine the spread of the data as quantified by the standard deviation.

Despite having lower preconcentration efficiencies, the THz spectrometer has more signal when coupled to the custom preconcentrator than when coupled with the Entech preconcentrator. Figure 20 shows the signal measured using the first (120° C) desorption cycle with the custom preconcentrator (Top) and the Entech preconcentrator (bottom) for the same breath sample. These two data sets are normalized for power and plotted on the same Y axis. The custom preconcentrator has more signal for methyl cyanide, chloromethane, acetone, HCN and methanol because it has a higher number density in the absorption cell. This higher number density is a result of having the sample delivered into a smaller 0.875 liter cell. The Entech can’t be coupled to the smaller absorption cell because the delivered analytes and atmospheric gases (not removed from the sample) would increase the pressure to an unacceptable level (greater
than 100 mTorr). The carbon monoxide and formaldehyde seen in the custom preconcentrator data come from sorbent thermal degradation and are not part of the breath sample.

In the current configuration the collimated THz beam is 2 inches in diameter for both the large (bottom) and small (top) absorption cells. The small absorption cell has a 1 inch aperture. Thus THz radiation outside this aperture is lost and transmitted power is reduced. Changing the optics to produce a 1 inch diameter collimated beam would increase the power transmitted through the small cell. This change would decrease the noise. It is likely that with the use of optimized optics the noise level in the measurements through the small absorption cell would be the same as the noise level in the larger absorption cell while the increased
number density in the small cell over the large cell would remain. Thus, the use of optics
optimized for the small cell would result in further signal to noise improvement.

4.4) Entech vs Custom Preconcentration

Subject 3’s breath was run with both custom and Entech preconcentrators cell allowing
for a direct comparison of the two systems. Each sample was run twice on the large absorption
cell with the Entech preconcentrator and once on the small absorption cell with the custom
preconcentrator. Each bottom cell data point was plotted against the top cell data point from
the same sample. This resulted in the top cell data being represented twice. Each data point
was adjusted for the measured preconcentration efficiency of the system. Data for Ethanol,
Acetone and Acetaldehyde were plotted with the custom preconcentrator coupled THz
spectrometer and the smaller absorption cell data on the Y axis and the Entech preconcentrator
coupled THz spectrometer and the larger absorption cell data on the X axis. Data where the top
and bottom cell that are in agreement will be on the line Y=X drawn in black in Figure 21-Figure
23. The dilutions of ethanol as measured by both the top and bottom cell for Subject 3 are in
good agreement Figure 21.
Figure 21 Ethanol Top Cell vs Bottom Cell for Subject 3. The black line shows the line x=y. The data is on this line within the limits of uncertainty showing the THz spectrometer coupled with the Entech preconcentrator is in agreement with the THz spectrometer coupled with the custom preconcentrator.

The dilution measurements for top and bottom cell for acetone within the limit of uncertainty are in agreement. However, all the data points for acetone are below the x=y line. This could be due to variations in the small data set, or a small portion of the acetone in the sample could have deposited itself on the surface of the Tedlar bag in the time between top cell and bottom cell measurements. The Entech preconcentrate data was taken a Wednesday, Thursday and Friday shortly after they arrived in the lab. All of subject 3’s custom preconcentrated data was taken on the following Saturday. During the time between scans, a small amount of sample could have been deposited on the walls of the Tedlar bag. Additionally,
Subject 3’s data set was taken approximately two months before the system was calibrated.

The sorbet tube’s acetone efficiency may have changed over the course of several months.

Subject 3 had very little acetaldehyde in his breath. The dilutions for each sample overlap each other shown in Figure 23. This could be caused by a small amount of unaccounted for contamination in the custom preconcentrator or the calibration factor could be incorrect due to the small number of data points taken.
4.5) Isoprene Detection

Using the system enhancements described in Section 2.2, isoprene was detected in subject 3’s third breath sample run with the custom preconcentrator on the top cell. To the author’s knowledge, this was the first time a statistically determined amount of isoprene was detected in a breath sample by THz spectroscopy. Figure 24 shows the raw data in red and the fit to the isoprene library in black for the first detection. Not only are the absorption lines in the correct location but the intensities of the lines relative to each other are correct as shown by the fact that the fit fits all five snippets. The dilution was calculated to have a concentration of $78\pm5$ ppb. That calculation assumes a preconcentration efficiency of 100%.
Figure 24 First THz detection of breath isoprene. The effective isoprene dilution in breath was calculated at 78±5 ppb. Red dots correspond to the breath spectrum, while black trace corresponds to the least square fit to the THz library of isoprene.

To find further evidence that we are indeed detecting isoprene, another sample was drawn from the Tedlar bag and was run with 15 isoprene snippets. Snippets for other chemicals were omitted from this run to decrease the time necessary to sweep across all the snippets. All the data reported thus far is based on an average from 4 passes across all the snippets. The reduced number of snippets enabled 10 passes to be averaged thus reducing random noise. The black sticks in Figure 25 denotes the center of each snippet where there is an isoprene absorption line. These absorption lines were identified from an overview spectrum taken with the THz spectrometer of a pure sample of isoprene in the absorption cell. The sticks line up with the absorption lines. This is further proof that isoprene is being detected. An experimental library for all these snippets is not currently available thus there is no fit to the data in Figure 25.
Increasing the quantity of sorbent that is capturing the isoprene may increase the sensitivity to isoprene thus making it easier to detect. Currently the custom preconcentrator uses a tribed sorbent tube that contains Tenax TA, Carbograph 1TD and Carboxen 1003. Isoprene desorbed from the tribed sorbent tube at a relatively low temperature. As previously explained in Section 3.1 the sorbent tube is desorbed in multiple stages with each stage having a higher temperature than the last. The data in Figure 24 was taken on the first desorption stage that had a maximum temperature of 150°C. The data displayed in Figure 25 was taken on a first desorption with a maximum temperature of 70°C. Tenax TA has the lowest minimum desorption temperature of the three sorbents in use. This suggests that the isoprene may be stored in the Tenax TA. Further testing is necessary to verify this hypothesis.
5) Conclusions

The THz spectrometer when coupled to the Entech preconcentrator was shown to have: A linear response as a function of breath sample size for sample volumes between 250 ml to 500ml for the ethanol, acetaldehyde and acetone; And an efficiency of 56.2±7.8% for acetone, 63.4±9.5% for acetaldehyde and 54.0±9.5% for ethanol. This system within the limit of uncertainty agrees with GCMS on the measured dilutions of Acetone, detects 5.3 times more acetaldehyde and 2.6 times more ethanol. There is no easy way of determining the uncertainty in concentration estimation of the ALS’s GC-MS data and all the THz measurements have estimations of the uncertainties.

When the THz spectrometer is coupled with the custom preconcentrator, the efficiency of acetaldehyde is 27.8 ±9.3%, ethanol is 4.4±1.2% and acetone is 13.6±4.2%. Sample may be lost during the initial pump down when the sorbent tube is opened to vacuum before the sorbent tube is heated up. It takes several minutes for the pressure to reach an equilibrium once the sample is open to vacuum. During this time any chemicals that detach from the sorbent will be lost to out the vacuum pump. Future efforts will test this theory by placing a cold trap between the cell and the sorbent tube. This cold trap will trap any VOCs that pass through it. Once the system is sufficiently pumped down the valve to the pump will be closed, then the cold trap will be heated up and the trapped VOCs will evolve into the vacuum of the cell for testing.

The THZ spectrometer when coupled with the custom preconcentrator and the smaller cell has more signal than when it is coupled to the commercial preconcentrator and the larger cell
despite having lower preconcentration efficiencies. Further improvement of signal to noise performance of the spectrometer when coupled with the custom preconcentrator and the smaller cell can be obtained by using a collimating optic with a 1 inch aperture to match the smaller absorption cell’s aperture.

Acetone and ethanol data taken with the small cell coupled with the custom preconcentrator are in agreement with data taken on the same samples with the large cell coupled with the Entech preconcentrator. Sample containing more acetaldehyde could be run on both top and bottom cells to verify agreement between top cell and bottom cell for acetaldehyde.

Utilizing THz spectrometer’s improvements that were described in Section 2.1, isoprene was detected at a concentration of 78 ± 5 ppb in Subject 3’s breath. To the author’s knowledge, this was the first time a statistically determined amount of isoprene was detected in a breath sample by THz spectroscopy. This detection was performed by the THz spectrometer coupled to the smaller absorption cell and the custom preconcentrator on the first and lowest temperature desorption. Of the three sorbents in the sorbent tube Tenax TA has the lowest desorption temperature associated with it. The low desorption temperatures that were necessary to desorb isoprene into the chamber suggests that the isoprene seen in Figure 24 and Figure 25 was trapped in the Tenax TA portion of the tribed sorbent tube. Increasing the volume of Tenax TA may increase the isoprene trapping efficiency thus improving isoprene detection. The volume of Tenax TA could be increased simply by replacing the tribed sorbent tube with one of the same volume but containing only Tenax TA.
Bibliography