Applications of Membrane Extraction with a Sorbent Interface

by

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AUTHOR’S DECLARATION

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

ABSTRACT

Membrane extraction with a sorbent interface (MESI) is a sample preparation technique with a rugged and simple design allowing for solvent-free, on-line performance. When coupled to gas chromatography (GC), MESI is an extremely promising tool for the analysis of volatile organic compounds (VOCs), as it is selective and sensitive for detecting trace levels of analytes. A new calibration method to be used with the MESI technique is presented herein. The aim of this project was to characterize and quantify the biomarker ethylene in human breath and plant emissions. The MESI-GC system was optimized, and an external calibration curve for ethylene standard was obtained. Qualitative measures were obtained from emissions of the higher plant *Arabidopsis thaliana*. The dominant calibration method was validated by examining changes in mass transfer trends when flow and temperature conditions were altered. Finally, the dominant calibration method was used to quantify ethylene in real human breath samples from non-smoking and smoking volunteers. Results were consistent with those reported in literature. These findings suggest that the dominant calibration technique is a useful tool for monitoring ethylene in human breath and Arabidopsis.

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DEDICATION

"When you feel like giving up, remember why you held on for so long in the first place." –Unknown author. [1]

Goals are dreams with deadlines. To those who have supported my goals throughout the years, thank you.

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ACC	1-aminocyclopropane-1-carboxylic acid
BAC	blood alcohol content
BVOCs	biogenic volatile organic compounds
FID	flame ionization detection
GC	gas chromatography
IR	infrared
MACC	malonyl-ACC
MESI	membrane extraction with a sorbent interface
MS	mass spectrometry
PLOT	porous layer open tubular
ppb	parts per billion
ppbv	parts per billion by volume
pptv	parts per trillion by volume
PGRs	plant growth regulatory substances
psig	pound per square inch gauge
RSD	relative standard deviation
SAM	*S*-adenosylmethionine
SPME	solid-phase microextraction
VOCs	volatile organic compounds

1. INTRODUCTION

The membrane extraction technique using a sorbent interface (MESI) was developed more than a decade ago.[1] The MESI system can be used for on-site analysis, and exhibits several characteristics to meet the need for continuous monitoring of volatile and semi-volatile organic compounds. These qualities include sensitivity, selectivity, and ruggedness.[1-4] Moreover, MESI is solvent-free and easily coupled to analytical instrumentation.[3] For example, gas chromatography (GC) may be coupled to MESI with either flame ionization detection (FID) or mass spectrometric detection (MS).[1, 2] Alternatively, a portable GC may be connected with MESI which would enable its use outside of a laboratory environment. MESI is especially effective as it pre-concentrates the analytes during the extraction process, to enhance the sensitivity for trace analysis that may be unattainable using GC alone. The components of the MESI system are connected on-line, so that samples can be analysed in real time. Eliminating sample transport and additional preparation steps saves time and reduces the potential for loss of analyte. MESI can be employed in a variety of applications including monitoring compounds present in air, water, plants, and breath.[4-7] The application for MESI in this work involves monitoring a volatile component in human breath and plants.

1.1. Analysis of Volatile Organic Compounds (VOCs) in Breath

1.1.1. History of Breath Analysis

Breath testing dates from the earliest history of medicine and has progressed quite substantially since then. The most primitive form of breath diagnostics began in ancient times, when physicians knew that the odour of breath was characteristic of certain diseases.[8] For example, diabetic patients were diagnosed by the smell of rotting apples as a result of acetonemia.[8] The era of scientific breath testing truly emerged in 1784, with the work of Antoine Laurent Lavoisier, who demonstrated that carbon dioxide was excreted in the breath of guinea pigs. This was later confirmed to be true for humans as well. By the nineteenth century, volatile organic compounds (VOCs) present in millimolar concentrations were detected in breath samples with the development of colourimetric assays.[8] In 1874, Francis Anstie developed a breath test for the detection of ethanol by discovering that breath bubbled through chromic acid turned the solution from red-brown to green in the presence of alcohol. [8] Finally, in 1971, modern breath testing began with the work of Linus Pauling. Pauling used a cold trap consisting of a u-shaped tube immersed in a cryogenic fluid to freeze out the VOCs from breath. The frozen breath VOCs were then heated and injected into a GC for analysis. With his work, Pauling concluded that human breath contains hundreds of VOCs present in picomolar concentrations, providing evidence that human breath is an extremely complex gas.[8]

The foundation of medicine today involves the analysis of urine, blood and other bodily fluids to yield information for the diagnosis of disease, and to monitor

disease progression.[2] Technological developments, however, indicate that breath can also be linked to biological information. For example, a thin barrier, called the pulmonary alveolar membrane, separates the air in the alveoli from the blood in the capillaries.[9] There is a fast gaseous equilibrium that develops between alveolar air and pulmonary blood, based on partitioning into the membrane and passive diffusion across it.[9] This is the reason why breath is a good indicator of what is present in our blood. Testing breath is advantageous because it is a less invasive means of analysis compared to blood. Breath testing is most commonly associated with the analysis of blood alcohol content (BAC) using hand-held devices, yet has recently been capable of much more. During the last decade, breath testing has gained interest as an extremely promising means of early diagnosis and evaluation of metabolic disorders and disease conditions, including lung cancer, heart disease, and occupational exposure or drug monitoring.[10, 11]

Presently, there are seven approved breath tests in clinical use, including the BAC test used by law enforcement officials. [2] In spite of its success, breath testing has not been able to replace blood and urine analysis, due to difficulties in obtaining a standardized method to quantify and characterize trace amounts of important breath volatiles. Researchers in the field of breath analysis therefore need to generate comparable guidelines for the collection and analysis for all molecules found in breath, before the method can be widely used for medical diagnostics. [2]

1.1.2. Biomarkers in Breath

As previously mentioned, exhaled breath contains endogenous compounds, including trace amounts of VOCs, which can be monitored to provide information on the state of a person's health. Volatile organic compounds in the body are commonly known as biogenic volatile organic compounds (BVOCs) and are mainly blood-borne, which allows for monitoring of different processes within the body. To date, approximately 3,000 VOCs have been detected at least once in human breath using various analytical techniques; however, typical breath samples contain around 200 detectable VOCs.[2, 12] Some of the major VOCs present in the breath of healthy individuals include isoprene (12-580 ppb), acetone (1.2-1880 ppb), ethanol (13-1000 ppb), and methanol (160-2000 ppb).[9] These endogenous compounds are a result of normal and abnormal physiological processes and are commonly used for diagnostic purposes. [2, 12] Although endogenous VOCs are the main focus of this research, it is useful to know that a large number of breath VOCs are of exogenous origin.[9] Many of the VOCs present in breath are yet to be characterized, as their source and physiological significance are unknown.

A significant volatile component present in exhaled breath is the light hydrocarbon ethylene, which is the focus of the research outlined in this thesis. Ethylene is a known biomarker of oxidative stress status due to lipid peroxidation.[2, 13] Oxidative stress status is defined as the equilibrium between the formation and removal of free radicals.[13] When the free radical capacity of the cell is overloaded, a complex chain of reactions occur leading to the destruction

of cell membranes, and a release of volatile hydrocarbons. The type of hydrocarbon generated depends on the polyunsaturated fatty acid involved in the lipid peroxidation process.[14] If the polyunsaturated fatty acid targeted in the lipid peroxidation process is linolenic acid, then ethylene and ethane are produced.[14]

The cell damage started by free radical action on biomolecules plays an important role in the pathogenesis of some diseases, such as cancer, Alzheimer's, kidney or liver malfunction, asthma, neurological disorders, as well as aging.[14] In 1974, it was demonstrated that increased concentrations of ethylene were produced in the breath of mice that had been fed with a dose of carbon tetrachloride.[15] The metabolism of carbon tetrachloride involves the generation of free radicals since it is a known hepatotoxin.[15] Today, ethylene has been detected in healthy and unhealthy people using various analytical techniques which will be discussed in Section 1.1.4.[2, 13]

1.1.3. Theory of Breath Sampling

The bulk matrix of breath is a mixture of nitrogen, oxygen, carbon dioxide, water, and inert gases. The remaining fraction of human breath consists of trace components occurring in concentrations in the nmol/L to pmol/L (ppbv to pptv) range.[16] At rest, an adult expires approximately 500 mL with each breath, of which the first 150 mL is dead-space air from the upper airways and nasopharynx, and the subsequent 350 mL is alveolar breath from within the lungs. There are two means of breath collection, including mixed expiratory sampling and alveolar sampling (see Fig. 1). Mixed sampling implies that the

total breath including the dead-space air is collected, while alveolar sampling refers to the collection of pure alveolar gas.[16] Mixed expiratory sampling is the most common method of breath collection, as it is easily performed with a majority of subjects. However, alveolar air sampling is preferred because endogenous volatile substances in alveolar air are two to three times higher in concentration than in mixed expiratory samples, because there is no dilution by dead-space gas.[16] Furthermore, alveolar breath samples have the lowest concentration of contaminants. There is no specific time period for which the breath sample is collected. It may be collected as soon as the volunteer is ready to provide the sample.

Capnography has been a valuable tool utilized for years in hospitals to assist in airway and ventilation management for patients undergoing surgery or for those in an intensive care unit.[17] Its use requires a sensor where breath is monitored and the output is presented as a graphic display, of instantaneous CO_2 concentration versus time, or versus the expired volume during a respiratory cycle.[17] It provides valuable information about cellular metabolism, carbon dioxide transport and pulmonary ventilation.[2] The waveform produced is a capnogram and it indicates the phases of respiration.[8] The capnogram shown in Fig. 1 is an example of a time capnogram.

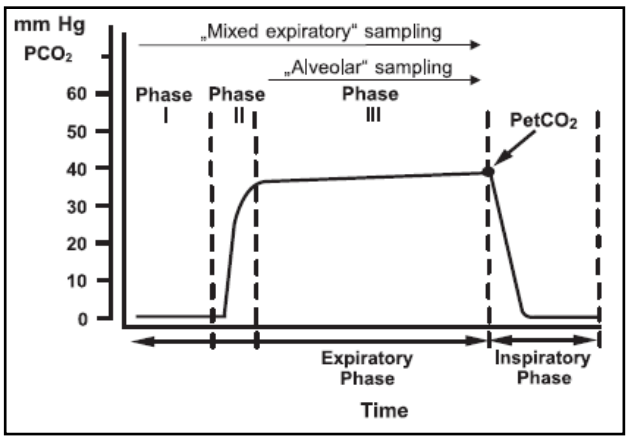

Fig. 1. Schematic of a normal time capnogram showing the typical modes of sampling. $PetCO_2$: pressure of end tidal carbon dioxide (mmHg).[17]

In Fig. 1, phase I is the first expository stage. Gas sampled during this phase represents anatomical dead-space and would typically not contain CO_2 and endogenous VOCs. Phase II represents the appearance of CO_2 depicted by the steep increase of CO_2. Gas sampled during this phase typically contains a mixture of alveolar and dead-space air. Phase III reflects a minimal increase in the CO_2 concentration of the sample as a result of alveolar emptying. This phase is referred to as the alveolar plateau or expiratory plateau. The pressure of end tidal CO_2 ($PetCO_2$) is the terminal portion of exhaled carbon dioxide, which reveals the actual CO_2 concentration at the end of expiration.[7] Volatile organic compounds in breath and air interchange at the alveolar membrane, so only alveolar breath is of any value for analytical purposes.[6]

There are two additional methods for breath sampling. One is referred to as a load method, where a patient consumes a drug or substrate before the sample is taken, and the metabolites are subsequently measured.[18] This is only useful for particular diseases. The other type is a no-load method, where the

patient has not been given a drug or substrate before sampling.[8] Most commonly, breath tests involve the no-load method.[8] Breath sampling is done either one breath at a time or for a certain period of time. Spontaneous breathing may cause the breath-to-breath concentration to vary considerably, and thus, averaging is necessary to ensure that the single breath sample is representative.

Although capturing a sample of breath seems easy, there are difficulties associated with the process. Water condensation is one difficulty in sampling breath. Breath is saturated with water, which may condense in the tubing of the capture system. This may allow breath VOCs to partition from the gas phase to the aqueous phase. Since breath VOCs are present in small quantities, having an abundance of water in the system may deplete the gas phase VOCs, resulting in much lower concentrations then what is really present in the breath.[8]

A difficulty arises with dead-space air dilution as well. Breath is an inhomogeneous sample and the component in the breath that needs to be captured is the portion that reflects the blood solvent concentration. Thus, it is essential that the proper part of the breath be sampled in order to use breath as a biomarker.[6] As mentioned in Section 1.1.3, in the case of mixed sampling, the alveolar breath is diluted with dead-space air, which causes inaccuracy in the findings. This error cannot be ignored, because the dilution factor varies with the tidal volume. There are large variations in the results gathered from different studies as a result of the way a breath sample is captured. Normalization of data can be achieved by using the end tidal partial pressure of CO_2 ($PetCO_2$) in

exhaled breath. This has been demonstrated in a recent study by Ma et al. with the use of a CO_2 monitor.[19]

1.1.4. Current Methods for Breath Analysis

The analytical process for analyzing breath VOCs generally consists of four steps. These steps include capturing the breath, extracting and concentrating the VOCs, analyzing the concentrated VOCs, and quantifying the results. As previously mentioned, either a mixed or expiratory sample of breath can be collected. The next question is how to store the sampled breath. Current techniques involve the use of metal canisters, Tedlar sampling bags, or glass chambers equipped with some sort of orifice from which to extract the collected analytes.[10, 20-23] The use of a container to capture the exhaled breath has a number of disadvantages including the loss of analyte over time, sample contamination from plasticizers or volatile adhesives, sample loss from adsorption on the vessel walls, difficulties associated with transport, and cost.[8] It is important that the collection device be constructed from inactive materials.[8] In addition, breath sampling devices must provide low resistance to expiration for ill patients, and have removable components so that the likelihood of contamination from one patient to the next is avoided. Once the breath sample is collected, the analytes must be concentrated and extracted.

Solid-phase microextraction (SPME) has been successfully used to determine particular VOCs present in breath.[13, 24-18] SPME works well for this application, however, it is limited to the detection of compounds which are

present in relatively high concentrations in breath. Although convenient, SPME is impractical for semi-continuous monitoring without the use of supplementary instrumentation such as auto-samplers, which is not feasible for field analysis.[25] An additional limitation of SPME lies in the analysis of extremely volatile compounds in trace quantities; these compounds can be difficult, if not impossible, to capture.

Recent studies have used the MESI method to analyze breath volatiles semi-continuously, which can selectively concentrate volatile analytes on-line, resulting in improved sensitivity.[25] The term on-line indicates that the MESI system is interfaced with the GC and computer so that data can be collected and analyzed in real-time. MESI involves the use of a hydrophobic membrane which can block water vapour present in breath, but allow the volatile components to pass through and pre-concentrate in the sorbent trap, before being thermally desorbed and introduced into the GC in a narrow band.[26] The principles of this technique are discussed in greater detail in the Section 2. MESI has been used successfully to identify VOCs, including ethanol, acetone, benzene, toluene, *p*-xylene, *α*-pinene, eucalyptol, and *γ*-terpinene.[4, 5, 9, 27] MESI is beneficial because the components of the system are connected on-line so that samples can be analysed as they are taken.

Once the analytes have been extracted, they are typically analyzed using GC. In the case of MESI, the analytes trapped in the sorbent are thermally desorbed and the injection system is bypassed so that the analytes directly enter the column. This is advantageous, as it eliminates a transfer step where precious

analytes may be lost. Most simply, an FID system is coupled with GC; however for conclusive identification of breath volatiles, mass spectrometric (MS) detection must be used.[3]

Quantitation of the analytes is difficult due to background air contamination in the breath sample. Supplying the donor with hydrocarbon free air prior to sampling would be ideal, however not very practical. Another approach is to collect two samples each time, one of the patient's breath and one of the room air, so that the background may be subtracted. This too is undesirable, as it is time consuming and often the VOCs present in room air are of higher concentration than those found within breath samples.[8] This is because compounds present in breath are in trace amounts, and in a laboratory environment some of these compounds may be present in the air (e.g. acetone).

When standard techniques for breath collection and procedures for background correction have been developed, it should then be possible to generate normal concentration ranges for diagnostic breath biomarkers as a function of gender, age, and ethnicity.[2] These ranges will set limits for concentrations of breath biomarkers so that abnormal levels can be detected. The future of clinical breath analysis can only be based on the analysis of molecules whose biochemical pathways for their generation and concentration ranges are well known.[2]

1.2. Analysis of Volatile Organic Compounds (VOCs) in Plants

1.2.1. Biomarkers in Plants

In addition to breath, plants also contain a number of VOCs. These include isoprene, mono- and sesquiterpenes, alcohols, alkanes, alkenes aldehydes, ketones, and esters which may be widely found throughout plant organs.[28, 29] Volatile organic compounds in plants are not only metabolic waste products, but also important plant adaptations. Normal plant growth and development is controlled by compounds produced by the plant itself and are called endogenous plant hormones.[30] They affect the physiological processes of growth and development in plants when present in low concentrations. Individual plant species possess unique combinations of VOCs and the emission pattern is species specific. VOCs also mediate the interaction between plants and other organisms. In addition, they are a defense mechanism against pathogenic insects and herbivores.[30]

There are five classes of naturally occurring plant growth regulators (PGRs), or plant hormones which almost all plants can synthesize. One of the five PGRs is ethylene. The recognition of ethylene as a plant hormone involved in the regulation of many physiological responses, originated in the nineteenth and early twentieth centuries, from observations of premature shedding of tree leaves, early flowering of pineapples treated with smoke, and ripening of oranges exposed to gas from kerosene combustion.[30]

Ethylene is a simple gas with profound growth regulating capability.[30] Unlike other plant hormones, metabolic processes are not required to detoxify

higher ethylene concentrations because ethylene diffuses passively into the ambient atmosphere, depending on the concentration gradient between the inside and outside of the tissue.

Ethylene production occurs in all plant organs, but the magnitude of production varies from organ to organ, and is dependent upon growth and developmental processes.[30] In most cases, the concentration of ethylene produced in the organs is usually very low; however, it increases dramatically during developmental events such as germination, leaf and flower senescence, abscission and fruit ripening.[31] The endogenous level of ethylene in plants is controlled primarily by its rate of production. The measurement of the rate of ethylene released per unit amount of tissue provides information on the relative changes of ethylene in cellular concentrations. In addition to the production of ethylene by various parts of plants growing under normal conditions, any kind of biological, chemical, or physical stress can strongly promote endogenous ethylene synthesis by plants.[30]

Ethylene production within higher plant tissues is contingent upon the availability of its precursor, 1-aminocyclopropane-1-carboxylic acid (ACC). ACC is synthesized from *S*-adenosylmethionine (SAM) by ACC synthase, and is then converted to ethylene by ACC oxidase. Alternatively, ACC can be conjugated with malonate by ACC-N-malonyltransferase to form malonyl-ACC (MACC). [30] MACC is made in the cytoplasm and stored in the vacuole or transported to other tissue by the vascular system. Generally, this conjugation of ACC is thought to slow ethylene production by converting ACC into an inactive product that can be

stored or transported. ACC synthases are cytoplasmic enzymes and are regulated by stress factors such as wounding, auxin treatment, and physiological changes including aging and ripening.[30]

The VOCs released from plants and other live species are typically present in the atmosphere in concentrations between several ppt and ppb.[29] Since biogenic volatile organic compounds (BVOCs) present a significant fraction of the total VOC inventory and possess high reactivity with other atmospheric constituents, they have an influence on tropospheric chemistry.[29] They may react with anthropogenic compounds and form photochemical smog and tropospheric ozone. Thus, BVOCs emitted by plants are important to characterize for the modeling of biogenic emissions in air quality planning.[29] Furthermore, they can provide information about physiological plant processes.[32]

1.2.2. Arabidopsis (Arabidopsis thaliana)

Arabidopsis thaliana is a small flowering plant often used as a model organism in plant biology for convenience, as it is easy to grow.[33] Arabidopsis has no agronomic significance, but it offers important advantages for research in genetics and molecular biology, and consequently was used in this study. The seed pods are known as siliques which can be seen in

Fig. 2, and are where most ethylene is found in both wild and mutant types.[31] Proper comparison of ethylene emission in both types is important for the physical characterization of the mutants.

Fig. 2. Image of the Arabidopsis plant.[34]

1.3. THESIS OBJECTIVE

The primary aim of this project was to demonstrate a working MESI system for the detection of an ethylene standard. The secondary focus was to establish a suitable method for the calibration of ethylene which could be used to quantify amounts in real samples, including human breath and emissions from Arabidopsis plants. Upon investigation, the conditions of the MESI-GC technique were optimized, a new dominant calibration method was established, and was validated using real breath samples. Emissions from Arabidopsis plants were investigated qualitatively.

2. MEMBRANE EXTRACTION WITH A SORBENT INTERFACE

2.1. PRINCIPLES AND THEORY

As a non-exhaustive extraction method, MESI is a unique sample preparation alternative, offering a rapid, solvent-free technique for trace analysis. The MESI system is composed of four sections for multi-component extraction and monitoring as illustrated in Fig. 3: (1) the membrane extraction module; (2) the thermal desorption sorbent interface with cooling; (3) the separation and detection system (GC/FID); and (4) the computer control and data acquisition system.

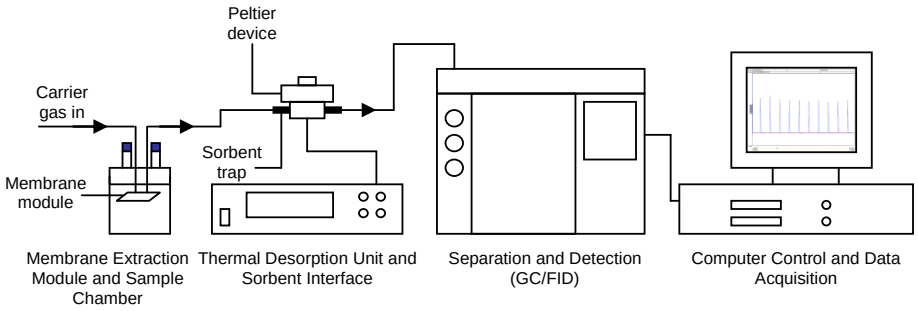

Fig. 3. Schematic of MESI-GC system components.

Membrane extraction consists of two processes: extraction of analytes from the sample matrix by the membrane material, and extraction of analytes from the membrane by the stripping phase.[35] A heating pulse desorbs the analytes collected at the trap and produces a narrow analyte band at the front of the separation column to be analyzed by GC. The transport of analytes through the membrane offers selectivity for the sample preparation process. To increase the capacity of the trap, cooling can be achieved using a semiconductor device such

as a Peltier cooler.[36] The sensitivity of the MESI system is related to the trapping time -- the longer the trapping time, the more analytes are accumulated in the trap and available for desorption. Sensitivity is also related to the affinity that the trapping material has toward the analyte.

Transport through the nonporous membrane occurs by a seven step "solution-diffusion mechanism", and selectivity is achieved either by differences in the membrane-sample material partition coefficient or diffusivity. The basis of membrane extraction procedures is diffusion. A net transport of matter will occur in the presence of a concentration gradient from a region of high concentration to a region of low concentration.[37] This is demonstrated by Fick's law of diffusion which states,

$$J_i = -D_i \frac{dc_i}{dx} \qquad (1)$$

where J_i is the rate of transfer of component *i*, or flux (g/cm^{2}s), and dc_i/dx is the concentration gradient of component *i*. The term D_i is the diffusion coefficient (cm^2/s) which is a measure of the rate of diffusion for the individual molecules. The minus sign indicates that the direction of diffusion is down the concentration gradient (from high concentration to low concentration). Having thin membranes allows faster fluxes across the membrane, which can speed up the diffusion process.[37] In cases where membrane extraction has good flow conditions (agitation) at the sample side of the membrane as well as the stripping side, the rate of mass transport through the membrane is based on the diffusion of

analytes through the membrane.[36] The concentration gradient between the sample side and the stripping side (highest for high flow rates of the stripping phase) facilitates transport across the membrane. Under good convection conditions, the concentration profile for membrane extraction can be seen in Fig. 4.

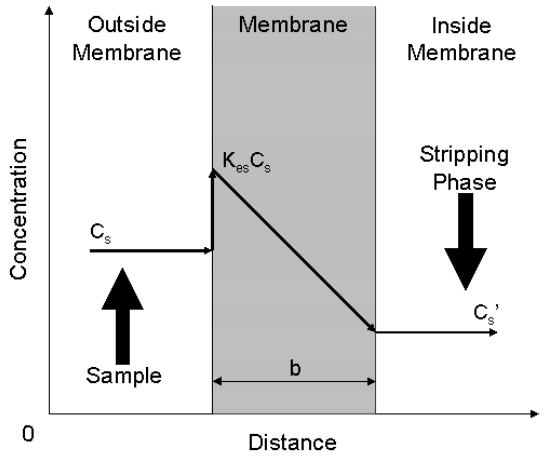

Fig. 4. Membrane extraction concentration gradient under ideal sample flow conditions.[36]

At steady state conditions, the following equation can be used to estimate the rate of mass transfer through the membrane:

$$\frac{n}{t} = \frac{B_2 A D_e K_{es} C_s}{b} \qquad (2)$$

where n is the extracted amount of analytes in the sorbent trap at time t, of A is the surface area of the membrane, D_e is the diffusion coefficient of the membrane material, K_{es} is the membrane material/sample matrix distribution

constant, b is the thickness of the membrane, and B_2 is a geometric factor defined by the shape of the membrane. The concentration of an unknown sample can be calculated by rearranging the above equation (2) into the following form:

$$C_s = \frac{bn}{B_2 A D_e K_{es} t} \quad (3)$$

Since mass transfer involves transport of molecules from the sample matrix into the membrane (contained within the membrane module), a boundary layer model should be included in this discussion. A boundary layer can be used to model the mass transport of molecules in the space surrounding the membrane. The boundary layer is caused by reduced velocity as molecules approach the membrane surface.[37] The extraction process including this boundary layer consists of several steps as illustrated in Fig. 5: (1) mass flux of the analyte from the bulk sample to the boundary layer outside the membrane surface; (2) diffusion of the analyte through the boundary layer to the membrane outer surface; (3) partitioning of the analyte between the sample matrix and the membrane at the membrane outer surface; (4) random movement of the analyte in and through the membrane (diffusion); (5) release and stripping of analyte by the stripping phase at the inner surface of the membrane (partitioning); (6) diffusion of the analyte through the stripping boundary layer which is close to the stripping side of the membrane surface; and (7) mass transfer away from the membrane surface by the stripping phase.[36] The thickness of the boundary layer, b, is determined by the rate of convection in the sample and the diffusion

coefficient of the analyte, and thus, will vary for different analytes.[38] The analyte flux outside the boundary layer is controlled by convection and the analyte flux inside the boundary layer is governed by diffusion.[38] Therefore, as the extraction phase is approached, the analyte flux in the boundary layer will depend more on diffusion than convection.

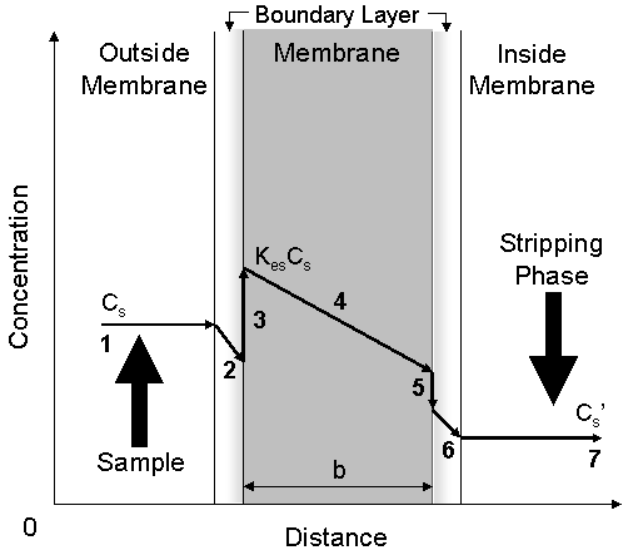

Fig. 5. Membrane extraction concentration gradient profile including boundary layer: (1) convection and diffusion; (2) diffusion; (3) partitioning; (4) diffusion; (5) partitioning; (6) diffusion; (7) diffusion and convection.[36]

2.2. COMPONENTS OF THE MESI SYSTEM

2.2.1. Membrane Module

The membrane module is the assembly that contains the membrane (see Fig. 6). It is composed of a very thin, flat sheet of silicone mounted between two Teflon® spacers. The Teflon® spacers are sandwiched between two steel plates. The upper Teflon® spacer contains two holes that match holes in the upper steel

plate and permit passage of the carrier gas. The lower Teflon® spacer is slightly thinner and has a u-shaped channel cut into it matching the channel cut in the lower steel plate. During operation, the pressure of the carrier gas causes the membrane to balloon into the u-shaped channel in the lower Teflon® spacer.[9] Wire mesh is attached to the lower steel plate to support the membrane and prevent it from ballooning out the bottom of the module during sampling. The module is sealed tight using twelve machine screws that pass through the module to compress two steel plates together as shown in Fig. 6.[9] The membrane module measures 3.8 cm long and 2.6 cm wide, while the effective surface area of the membrane channel is 2.64 cm^2.

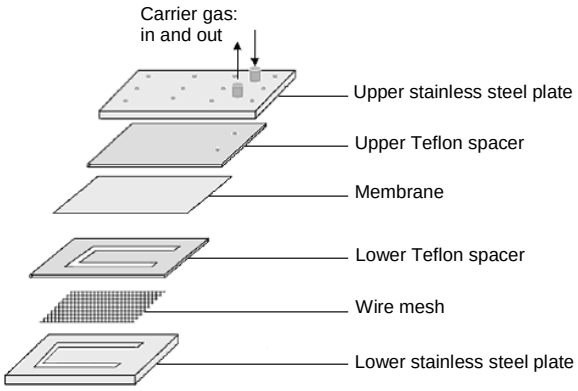

Fig. 6. Schematic diagram of membrane module consisting of a series of plates that support the silicone membrane used in MESI analysis. The 12 points where the machine screws are fixed to the assembly are represented by tiny circles on the upper stainless steel plate.[9]

The silicone sheet moderates permeation and is similar to the non-polar lipid bilayer cell membrane of the alveoli, across which many non-polar and volatile components travel to be expired in air. For the purpose of this project, a dimethyl

silicone thin film membrane (0.005") was used to facilitate high transport rates. A thinner silicone polycarbonate membrane was also tested however its permeability toward the target analyte was not as great, and accordingly not used. Thinner membranes are preferred, but the permeability of the material is also important and often takes priority over thinness. The separation properties and permeation rates of the membrane are determined exclusively by the surface layer, as the substructure functions solely as a mechanical support.[37]

2.2.2. Sorbent Interface

Initially developed for aqueous samples in the 1980's, sorbent materials with a strong affinity towards organic compounds, will retain and concentrate target compounds from a very diluted aqueous or gaseous sample.[35] The porosity of the sorbent material determines the surface area, which ultimately controls the adsorbent strength. Sorbent materials include porous polymers such as Tenax® TA (a polymer of 2,6-diphenyl-p-phenylene oxide), graphitized carbon (Carbotrap), and carbon molecular sieves such as Carboxen™.[36] The sorbent material must be carefully selected to avoid breakthrough and memory effects. Breakthrough occurs when the sorbent material does not retain the analyte either due to its weakness or the amount used. Memory effects are like carry over, where analyte is retained too strongly and becomes difficult to remove. Porous polymers such as Tenax® TA are least affected by high water content present in the samples, but have low breakthrough volumes. Carbon molecular sieves and graphitized carbon have high breakthrough volumes for breath VOCs, however,

during thermal desorption they may have serious memory effects. Memory and breakthrough effects can be reduced by using multi-bed sorbent traps.[16] A multi-bed sorbent trap contains 2 or 3 different sorbent materials in series so that their advantages may be used together to prevent breakthrough and/or memory effects.

The sorbent interface is simply a trap made of stainless steel tubing packed with a sorbent material held in place by two plugs of quartz wool as illustrated in Fig. 7. Analytes stripped by the membrane are transported by the carrier gas to the trap and are held there until a maximum amount has been trapped. The time required to extract the maximum amount of analyte must be optimized. The trapped analytes are then desorbed thermally and are swept by the carrier gas into the column of the gas chromatograph (GC). [9] Details on the preparation of a sorbent trap are discussed in Section 3.3.3.

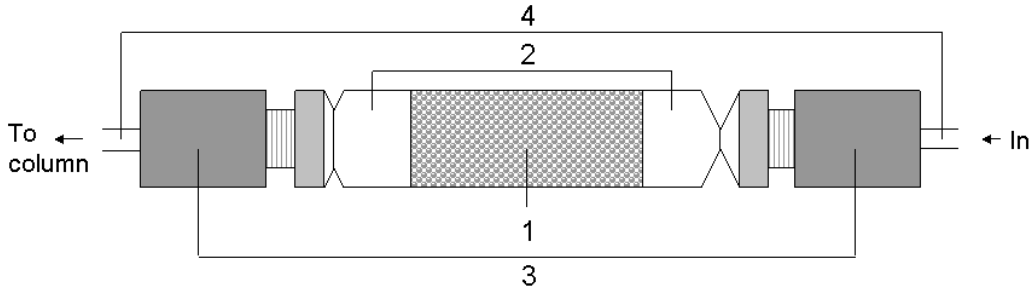

Fig. 7. Schematic of a sorbent trap: (1) sorbent material; (2) quartz wool plugs; (3) internal reducing union with screw; (4) upstream and downstream transfer lines.

2.2.3. Sample Chamber

The samples of breath or plant emissions to be analyzed are collected with the use of a specially designed chamber. For the breath samples a person is instructed to breathe into a sealed device through a disposable mouthpiece. An example of one type of chamber is presented in Fig. 8. This one includes one-way valves mounted at the beginning and end of the cylindrically shaped chamber.

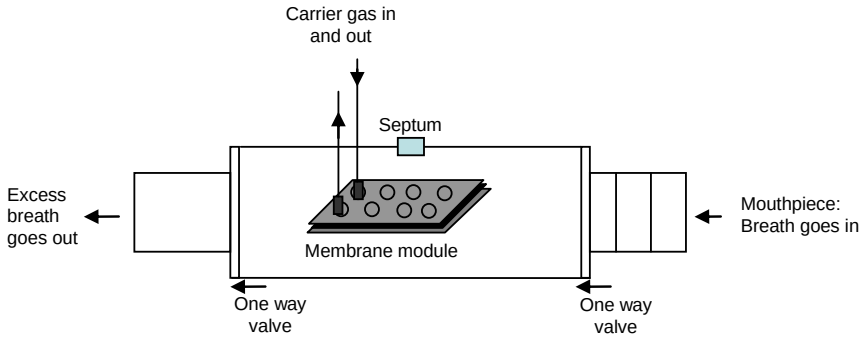

Fig. 8. Sample chamber with one way valves including membrane module.

The one-way valves prevent breath from re-entering the chamber, ensuring that only the last 250 mL of the breath sample will be captured in the chamber. A septum is also available on the device to allow for parallel SPME analysis (if applicable). Alternatively, a chamber constructed of glass can be employed. The glass chamber can be made with a removable top that uses wing nuts for an air-tight seal. This sample chamber is quite useful for the analysis of plant emissions, as it is large enough to fit small stems, and does not require the use of one-way sampling valves. Breath samples can also be collected using this collection device. For this research the glass sample chamber was used for all

experiments because the membrane module was difficult to install and remove from the chamber with one-way valves. A slightly larger cylindrical chamber with one-way valves would have been more reasonable for experiments in this study where components were disassembled and reassembled frequently, however due to time constraints it was not possible to construct one for this study.

2.3. Carbon Dioxide Sensor

The respiratory process occurs in three major steps each of which are involved in the appearance of CO_2 in exhaled gas. First, carbon dioxide is generated by metabolic processes during which the body uses oxygen.[17] Next, oxygen and carbon dioxide are transported between cells and pulmonary capillaries, and diffuse from air or into alveoli. Lastly, ventilation occurs between alveoli and the atmosphere. Monitoring CO_2 is useful in breath analysis and can be measured using a carbon dioxide monitor. The NICO CO_2 monitor was used in this research. The NICO monitor uses a CAPNOSTAT CO_2 sensor to measure CO_2 using infrared (IR) absorption.[39] The carbon dioxide molecules absorb IR light at specific wavelengths; the intensity of absorption is related to CO_2 concentration. When an IR beam is passed through a gas sample containing CO_2, the absorption signal can be obtained from a detector. This signal is then compared to the energy of the IR source, and is calibrated to reflect a known CO_2 concentration. The carbon dioxide sensor allows for quick response to variations in carbon dioxide levels occurring at the end of expiration. The profile of carbon dioxide defines the quality of the breath sample, and the variation of

mouth pressure during the breathing cycle will demonstrate whether the patient is maintaining a tight seal with the mouthpiece. Single breath analysis can be normalized to a physiological parameter such as carbon dioxide concentration. This allows people with different body masses to be compared, as the average CO_2 concentration in alveolar air should be steady for a single subject.[2]

For this work, the CO_2 sensor will be mounted at the output port of the sampling chamber to capture the breath as it leaves the chamber. Thus, it will be used as a natural internal standard for the analysis of breath to improve the reliability of the breath analysis.

3. VALIDATION OF A WORKING MESI SYSTEM

3.1. INTRODUCTION

Prior to optimization of MESI parameters, components of the system were examined and tested for proper functionality. More specifically, the pressure and flow of the system were inspected, along with the membrane module and the sorbent tube. After examination, a working MESI system was verified by using standard ethylene samples, and a real sample that is known to emit a large quantity of ethylene.

3.2. EXPERIMENTAL

A Varian 3800 GC/FID (Varian Inc., Palo Alto, CA) was used for all of the experiments. Other GC systems were used in early experiments but were discontinued as a result of GC electrical and/or mechanical problems including insufficient sensitivity for the analyte of interest. Considerable time was spent trying to optimize the Chrompack and Varian 3400 GCs with the MESI system, until it was decided that the Varian 3800 GC-FID was necessary for producing quality data for this research.

The U-PLOT column (0.32 mm X 30 m) from Restek (Bellefonte, PA, US) was selected for this work as it is insensitive to moisture and capable of retaining volatile, low molecular weight compounds. Helium gas was used as the carrier at a flow rate of approximately 1.6 mL/min. Hydrogen and nitrogen gases were also required for GC operation. These ultra high purity (UHP) gases were maintained at conventional flow rates, and were purchased from Praxair (Kitchener, ON,

Canada). Standard ethylene (99.9% polymer grade) was also obtained from Praxair. A zero air generator purchased from Parker Balston (Haverhill, MA, US) supplied the air for the FID.

The membrane module, Peltier device, sample chamber, and control unit were all designed and manufactured by members at the University of Waterloo Science Shop (Waterloo, ON, Canada). A flat sheet of PDMS membrane (SSP-M823, 0.005") was obtained from Silicone Specialty Products Inc. (Ballston Spa, NY, US). The membrane is reusable and is only changed if there is an issue with leaks in the membrane module. Stainless steel hypo tubing (0.035" ID, 0.042" OD, 19 gauge) for the sorbent tube construction was purchased from Small Parts (Miramar, FL, US). The Carboxen™ 1000 (60/80 MESH) sorbent was supplied by Supelco (Oakville, ON, CA). Flow rates in the system were monitored using an electronic flow meter device from J&W Scientific (Folsom, CA, US). All transfer line connections and the sample chamber were checked for leaks prior to the start of any experiment using an electronic leak detector purchased from Restek (Bellefonte, PA, US). Gas-tight syringes were Hamilton brand also supplied by Supelco (Oakville, ON, CA).

3.3. AREAS INVESTIGATED

3.3.1. Pressure and Flow

The carrier gas helium was regulated by a double stage regulator at 80 psig. Downstream from this regulator, a single stage regulator was installed to reduce the pressure to 5 psig. This configuration was unavoidable since the

helium source also supplied helium for other instruments in the laboratory that required a head pressure of 80 psig. From this line, the helium flow was further controlled by a metering valve. Bypassing the GC injection system is a necessity in MESI, because the analyte enters the column from the outlet of the sorbent tube, located outside of the GC. This modification from conventional GC methods precipitated the need for an alternative means of flow control in the system. Using a flow controller in combination with a low pressure regulator ensured that a flow rate of 1.6 mL/min was maintained. The flow rate was measured using an electronic flow meter. A series of silicosteel transfer lines were connected using Valco unions and attached to the membrane module, which was connected to the trap, followed by the column. The gases required for the GC detection were also checked for appropriate regulator head pressure and flow rate. A schematic diagram of the experimental set-up is shown in Fig. 9.

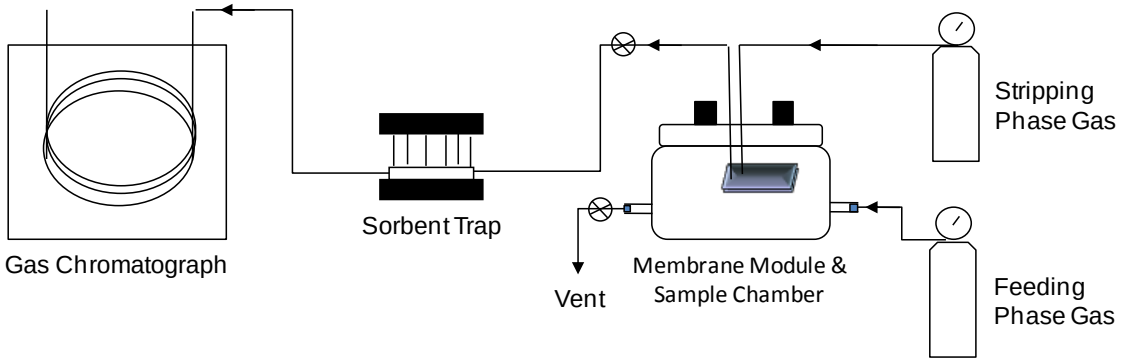

Fig. 9. Schematic of experimental set-up including gases; transfer lines; membrane module and sample chamber; sorbent trap; and GC.

3.3.2. Membrane Module

A piece of membrane was cut from the larger sheet of membrane to fit the module (3.8 cm x 2.6 cm). It was installed as outlined in Section 2.2.1. Once the components were fitted with the screws and tightened, the module was checked for leaks. The module was purged with nitrogen gas while immersed in a beaker of de-ionized water to facilitate the detection of leaks. The problem areas were addressed by tightening the screws. The module was then connected to the lid of the 250 mL glass sample chamber and secured. Both inlets and outlets of the sample chamber were sealed using Teflon® septa to provide a leak free environment for the sample.

3.3.3. Sorbent Tube

A sorbent tube was prepared, installed and tested for trapping capability. Preparation of the tube required a 6.3 cm length of stainless steel tubing cleaned under sonication in methanol for 20 minutes. Once dry, the clean tube was fitted with a Valco union from Supelco (Bellefonte, PA, US) and attached to a suction fitting. The stainless steel tube was crimped first at the far end to immobilize the packing. A 0.5 cm length of quartz wool was placed into the tube, followed by 2 mg of Carboxen 1000™. Another 0.5 cm of glass wool was inserted into the tube, and it was crimped again. The sorbent tube was conditioned in a GC oven at 190°C, while purging with nitrogen gas for several hours. The tube was then installed into the Peltier device which involved securing the sorbent tube into its

holder on the device, and connecting it to 2 alligator clips to allow current flow for heating.

Upon testing the sorbent tube, unidentified peaks were visible in the chromatogram. Moreover, it was often difficult to remove all of the ethylene from the trap once a run had been completed. After building and trying several different tubes, it was eventually determined that poor contact of the alligator clips on the sorbent tube, had been causing damage to the tube resulting in burn spots. This damage had caused heating of the Carboxen material which was the likely the source of the unidentified peaks present in the chromatograms. To solve this problem, the alligator clips were replaced with a flat type of clip which provided a more even contact on the sorbent tube (see Fig. 10).[40]

Fig. 10. Clip types used to connect the Peltier device to the sorbent tube. Alligator style (left); flat style (right).[40]

Exchanging the type of clip eliminated the presence of the unexpected peaks. Although better contact of the tube and clip was achieved, prolonged use of the sorbent tube ultimately required changing the tube. The lifetime of the tube varied with use (from 2 to 8 months), however, once the background chromatograms were unsuitable, a new tube was prepared and installed.

3.3.4. Validation with Sample Injection

At this point, test samples were necessary to confirm that the MESI-GC system was functioning well. Standard ethylene and an unripened Roma tomato were both used to verify a working system. An unripened tomato was used rather than a ripe one, since the ethylene levels would be much lower in the younger fruit and closer to the desired ethylene range. For this, a small quantity standard ethylene of was spiked into the sample chamber using a gas-tight syringe. In another run, ethylene emissions from an unripened Roma tomato were monitored by placing the tomato into the sample chamber. The column temperature was 60°C, the FID temperature was 250°C, and the helium flow rate was 1.6 mL/min. For detection of standard ethylene, the desorption temperature was 180°C held for 10 seconds and occurred every 5 minutes. For the Roma tomato, the conditions were the same except the trapping time occurred every 10 minutes. Since optimization will be discussed in the next chapter, the difference in trapping time is irrelevant here. The difference was primarily because these experiments had been completed at different times. The results can be seen in Fig. 11 demonstrating that the MESI system was functioning well. Standard ethylene and ethylene emitted from an unripened Roma tomato, both were successfully identified by the MESI-GC system. The ethylene peaks in each case eluted at the same time, indicating that the peaks from the Roma tomato do represent ethylene. The retention time was approximately 7.2 minutes. Slight variations in the retention times are expected since there is a slight delay in manually starting the desorptions for each run. It can be noted that the peak

which elutes before ethylene in each run was the unidentified peak present as a result of the damage done to the sorbent tube during heating. It is consistent in both runs and is not present in chromatograms obtained after the clip change was made.

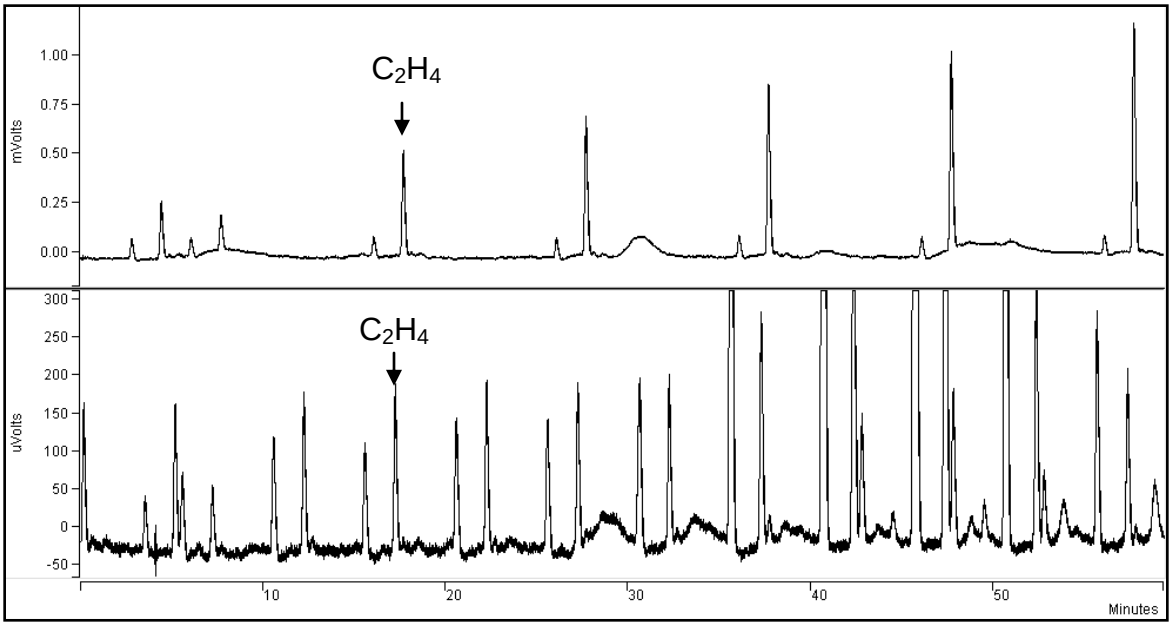

Fig. 11. Chromatograms illustrating a working MESI system. Ethylene emissions from an unripened Roma tomato (upper); and standard ethylene at 0.5 ppm (lower).

3.4. CONCLUSION

It was necessary to ensure the MESI system was functioning properly before carrying out the optimization experiments. This testing process included making sure that all connections were secure without leaks, the head pressures were suitable for generating low flow rates, and the sorbent tube was able to trap

analyte. Moreover, the GC performance was acceptable. With these items checked, the next step of optimization could be completed.

4. OPTIMIZATION OF MESI FOR ETHYLENE ANALYSIS

4.1. INTRODUCTION

Several key areas were investigated in order to optimize the MESI-GC system. A suitable method for operation was established for the GC component of the system. Since the MESI component sets the limits for ethylene extraction, it required the most attention to obtain the maximum amount of analyte. Sorbent capacity and steady state time were determined. The trapping time, trapping temperature, and desorption temperature were also considered in the optimization of MESI.

4.2. INSTRUMENTS, MATERIALS AND METHODS

4.2.1. Gas Chromatograph Component

A Varian 3800 GC-FID and U-PLOT column were used as previously mentioned. Helium gas was used as the carrier at a flow rate of approximately 1.6 mL/min. The column oven temperature was isothermal at 50°C, and the FID was maintained at a temperature of 250°C for all experiments. Flow rates were monitored using an electronic flow meter device. All transfer line connections and the sample chamber were checked for leaks prior to the start of any experiment using an electronic leak detector. The total run time was 60 minutes for each run, yet for some experiments it was not necessary to monitor continuously until the end of the run.

4.2.2. MESI Component

The MESI component of the system included a unit which housed the power supply. This unit was equipped with many options resulting in an extremely practical system. The options included desorption temperature, ramping speed, and holding time. Cold and hot temperature ranges could be achieved from -10°C to 250°C. The ability to meet the upper temperature limit quickly was beneficial for generating analytes in a narrow band and obtaining sharp chromatographic peaks. Nevertheless, a ramp that was too steep (depending on the temperature set) could risk damage to the sorbent tube, so the option to adjust this parameter was advantageous. The frequency and length of the heating pulses could also be varied. For simplicity, the desorption pulses were held for 10 seconds since the length of heating period was meant to provide a fast release of the analyte, and any longer time was unnecessary.

4.2.3. Sample Dilution

The pure ethylene gas required dilution to ensure delivery in small concentrations. Due to the volatile nature of ethylene, this procedure had to be carefully completed for accuracy. The dilution was carried out in a glass gas bulb sampler using Hamilton gas-tight syringes. The glass bulb sampler was supplied by Supelco (Bellefonte, PA, USA). The dilution procedure began with a glass gas bulb purged with nitrogen gas (UHP, 99.999%) for several minutes, after which the stop-cock ports on the bulb were closed. The ports were re-tightened, and

sealed with para-film to ensure leaks were not a concern. A portion of ethylene was taken from an outlet attached to the ethylene cylinder, using a gas-tight syringe. The syringe was capped using a piece of septum and transported to the glass bulb. At this point the ethylene was spiked into the inlet of the glass gas bulb. The glass bulb was left to equilibrate for 2 minutes with shaking. A portion of this diluted ethylene was taken using a clean gas-tight syringe and injected into the inlet port of the MESI sample chamber. Since the injection of ethylene into the sample chamber created an additional dilution, the volume of the chamber was taken into account for all calculations. The schematic diagram shown in Fig. 12 illustrates the dilution procedure. This procedure was used for each experiment with changes in syringe volumes and glass gas bulb volume depending on the desired concentration of ethylene. Great care was taken to reduce the loss of ethylene during each of the dilution steps.

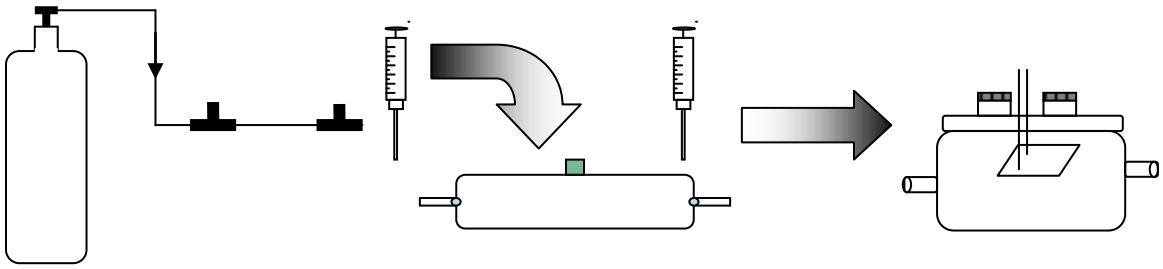

Fig. 12. Schematic of dilution/sampling procedure. (1) portion of ethylene taken from standard cylinder through outlet using gas-tight syringe; (2) ethylene from step 1 spiked into glass gas bulb; (3) after equilibration, a volume is taken from the bulb and injected into the side port of the sample chamber using another gas-tight syringe.

4.3. PARAMETERS FOR OPTIMUM EXTRACTION

4.3.1. Sorbent Tube Capacity

For preparation of well designed experiments for the MESI system, the capacity of the sorbent trap should be determined prior to use. The amount of analyte that can be trapped by the sorbent tube depends on the concentration of analyte trapped, length of trapping time, and of course amount of sorbent (which will stay constant during these experiments). The benefit of knowing the capacity of the sorbent material is primarily to eliminate the chances of breakthrough. Breakthrough occurs when analyte passes prematurely through the trap because the amount of sorbent material is insufficient to trap the total amount. A loss of analyte caused by breakthrough, would lead to an extracted amount that would not be representative of the sample. To test the capacity of the sorbent, a second sorbent tube was connected in series to the original sorbent tube as shown in Fig. 13.

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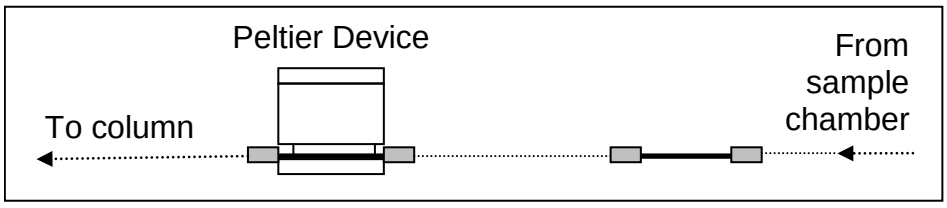

Fig. 13. Schematic illustrating second sorbent tube placed upstream from the original in the Peltier device for breakthrough determination.

Decreasing concentrations of ethylene were injected into the sample chamber from 50 ppm to 0.50 ppm, and were trapped for 10 minutes. The

desorption pulses occurred at 180°C every 5 minutes. The results of this test showed that trapping at 10 minutes for concentrations even as low as 1 ppm caused breakthrough (data not shown). The experiment was repeated using 5 minute trapping, and breakthrough also occurred except for concentrations below 1 ppm. Therefore, in order to avoid breakthrough in the MESI system with 2 mg of Carboxen packed into the trap, trapping times no longer then 5 minutes are essential, and concentrations of sample should be no greater than 1 ppm. This is sufficient since the range of target analyte is between 1 and 100 ppb.

4.3.2. Steady State

Steady state is an important parameter in MESI. Since continuous monitoring in MESI allows for many desorptions, it is necessary to know which peaks accurately represent the sample. Once an injection is made into the MESI sample chamber, it takes time for the analyte to permeate through the membrane, become stripped from the membrane and flow toward the column. Only peaks that have eluted after this time are reasonable to represent the sample, since they are acquired after the system has reached a steady state. The peaks that elute from the first few desorptions represent analyte that has not yet reached steady state in the system and thus should not be used for quantitation.

For the steady state experiment, ethylene was injected into the sample chamber so that the concentration in the chamber was 1 ppm. The desorption

temperature was at 180°C, and occurred every 3 minutes. The trapping time for this experiment was chosen so that the peaks were not eluting too close together, yet were able to provide a more precise value for the steady state time. The results in Fig. 14 illustrate that the ethylene peaks become constant after 15 minutes of desorptions. Therefore, for quantitation purposes, data is best taken after 15 minutes; when the system has reached a steady state.

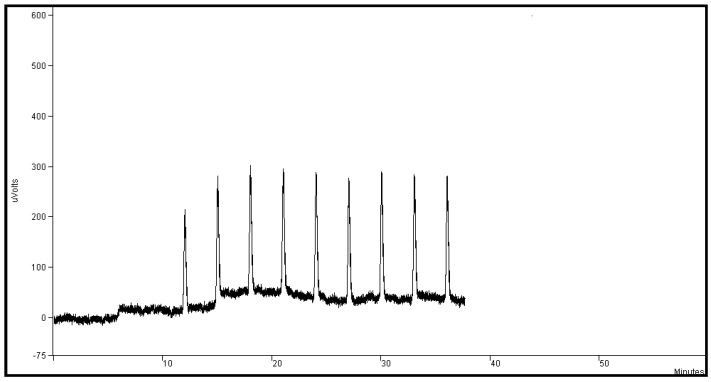

Fig. 14.Chromatogram illustrating time at which steady state is reached; after 15 minutes all peaks are constant in peak height.

4.3.3. Trapping Time

In MESI the analyte accumulates in the sorbent tube for a set amount of time, after which it is thermally desorbed. That time is referred to as the trapping time and can be optimized to increase extraction. It is reasonable that a longer trapping time may increase the extracted amount, yet the challenge is extracting the maximum amount of analyte without causing it to breakthrough.

Ethylene was injected into the sample chamber to give a 0.5 ppm dilution. Desorption pulses were completed at 150°C while the frequency of the pulses were varied from 3 to 30 minutes for each run. Averages of 5 peaks were used to create the trapping time profile shown in Fig. 15. The results show an increase in ethylene signal from 3 minutes to about 10 minutes of analyte accumulation with relative standard deviation (RSD) values between 3 and 7%. At 10 minutes however, the data shows that the extracted amount of ethylene becomes constant (likely a result of breakthrough). This finding suggests that a trapping time around 10 minutes is enough time for a maximum amount of ethylene to accumulate in the trap.

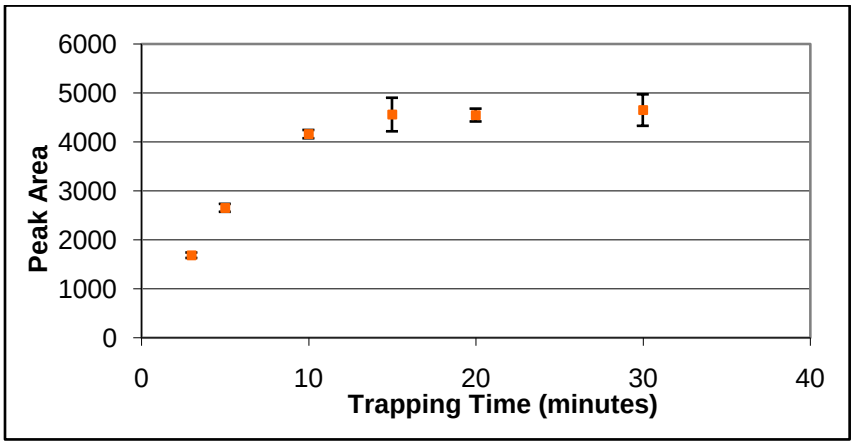

Fig. 15. Trapping time profile indicating time of 10 minutes is sufficient for ethylene.

For 0.5 ppm of ethylene, a trapping time beyond 10 minutes will result in breakthrough, since the sorbent material is unable to retain anymore analyte. The results will likely change as the concentration of analyte changes since trapping time is concentration dependent. If the concentration is decreased, a

longer trapping time will be possible before breakthrough. This is because the capacity of the sorbent material would take longer to reach with less analyte. As demonstrated in Section 4.3.1, a 1 ppm amount of ethylene resulted in a 5 minute trapping time to avoid breakthrough. For the purpose of this thesis, the results from the breakthrough experiment and from the trapping time experiment provide sufficient information for avoiding breakthrough because the target analyte concentration is expected to be in the ppb range.

4.3.4. Desorption and Trapping Temperature

Heat must be applied to the sorbent tube to release the collected analyte as a band which enters the column. This temperature is the desorption temperature which can be varied to increase the efficiency of the extraction. An ethylene concentration of 0.5 ppm was used for this experiment. Desorption pulses were completed every 5 minutes. Desorption temperatures of 100, 150 and 200°C were tested. Fig. 16 shows the results using averages of 5 repetitions with RSD values between 1 and 8%. Thus, the maximum amount of ethylene is extracted at 200°C during room temperature (~25°C) trapping.

In addition to trapping at room temperature, cooling was applied to the trap. To determine the outcome with cooling, the experiment mentioned above was repeated, with the cooling option selected on the MESI control unit. Cooling was maintained at 0°C for the duration of the trapping which was 5 minutes.

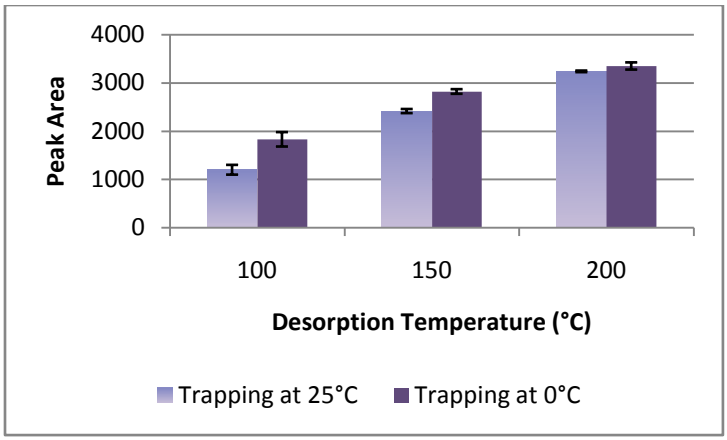

Fig. 16. Optimization results for desorption and trapping temperatures; desorption temperature of 200°C gives best extraction for ethylene regardless of trapping temperature, cooling provides a greater extraction of ethylene.

The results of this experiment showed that using the cooling option increases the extraction of ethylene. Thus, an optimal extraction of ethylene will be obtained through the use of trapping at 0°C and desorbing at 200°C. Although optimal conditions are preferred, a few technical issues have prevented the use of these optimal values. Trapping with cooling especially in humid conditions causes a build-up of condensation around the plates on the Peltier device. This ultimately leads to instabilities in the baseline over time and in the chromatographic peaks. A modification to the Peltier device may reduce moisture build up with cooler temperatures. Since time was limited for this project, the cooling option was not employed. Secondly, heating pulses at 200°C also leads to instabilities due to the damage caused to the sorbent tube from the clips. Moreover, it has been noted that the RSD increases with increasing desorption temperature, thus a final desorption temperature of 180°C was selected to try and improve the reproducibility and precision of the experiments.

4.4. CONCLUSION

In summary, there were a number of parameters to optimize to achieve the most practical MESI-GC system, for the analysis of real samples. The sorbent tube capacity and the time taken to reach steady state were determined. Examination of the effects of trapping time, trapping temperature, and desorption temperature resulted in the best conditions with which to run the MESI system. Two changes were made to circumvent potential problems, and in the end an optimal performing MESI-GC system was established.

5. SYSTEM CALIBRATION

5.1. INTRODUCTION

Calibration is a crucial component in sample analysis. It provides the basis upon which the analytical measurements are made. External calibration is one means of calibration used in MESI to establish linearity for an expected concentration range. However, external calibration is not a definitive means of calibration in MESI, since there are many environmental factors which can affect the extraction rate of the analyte.[27] This is especially true for on-site sampling, as it would be impractical to compensate for all variations in environmental factors, by completing an external calibration for each exposure scenario. The environmental variables which can affect the extraction rate of the analyte include the velocity of the sample, temperature, and UV-radiation.[41] Similarly, internal standardization and standard addition are traditional approaches for calibration that can be used with MESI, yet finding a suitable calibrant can often be difficult, and may complicate the calibration procedure for field work.[27] In order to broaden the span of compounds that can be analysed using the MESI technique, the calibration methods must be appropriate for analysing different classes of compounds in varying environmental conditions. Reliable calibration methods for semi-volatile and volatile analytes in MESI analysis would make the technique more versatile.

Recently, a new method for calibration in MESI was reported. It is the internal calibrant approach and involves a constant concentration of calibrant added to the stripping gas.[6] Adding the calibrant to the stripping side of the

membrane compensates for the environmental variables which can affect analyte extraction rates, while avoiding the complexity of an addition into the sample matrix.[27] When dealing with trace amounts of analyte, calibrant is desired in low concentrations, however, it is a challenge to continuously and consistently supply such low amounts into the stripping phase. The internal calibration method developed by Liu uses a permeation tube to yield a small quantity of analyte and has been successful in furthering calibration for the MESI technique.[6, 27] The permeation tube works well for stable compounds that are liquid at room temperature as it makes preparation of the permeation tube straight forward. For a gaseous analyte like ethylene, the permeation tube is not as simple to prepare. Even if prepared, the lifetime of an ethylene tube may not be practical for this work, due to its volatile nature.

As a supplementary approach, the dominant calibration method is proposed in this research, to address the challenges associated with the calibration of ethylene in MESI. Dominant calibration was initially designed for use in SPME, and was based on the isotropism between absorption and desorption processes occurring with the SPME fiber. [42, 43] The target analyte was actually used as the internal standard by pre-loading it onto the SPME fiber. The details of this process are not relevant to discuss, however, this calibration concept helped to create the dominant calibration method for MESI.

5.2. External Calibration

5.2.1. Experimental

The Varian 3800 GC-FID was also used for this experiment with the U-PLOT column. The column temperature was set to 50°C, while the FID temperature was maintained at 250°C. Trapping was completed at room temperature. Desorptions occurred at 180°C every 5 minutes, and were held for 10 seconds. Ethylene concentrations between 0.05 and 5 ppm were used for the external calibration. The dilutions were prepared as described in Section 4.2.3 using a glass gas bulb sampler and standard ethylene gas. All transfer line connections and the sample chamber were checked for leaks prior to the start of any experiment using an electronic leak detector. Flow rates were monitored using an electronic flow meter device. Starting from the lowest concentration, the diluted ethylene was injected into the side port of the 250 mL sample chamber using a Hamilton gas-tight syringe. Each run was approximately 30 minutes long to ensure three desorptions had occurred within the steady state time.

5.2.2. Results and Discussion

An example of a chromatogram collected from this calibration experiment is seen in Fig. 17. An average of 3 peaks was used for each of the 6 concentration values to create the external calibration curve for ethylene, which can be seen in Fig. 18.

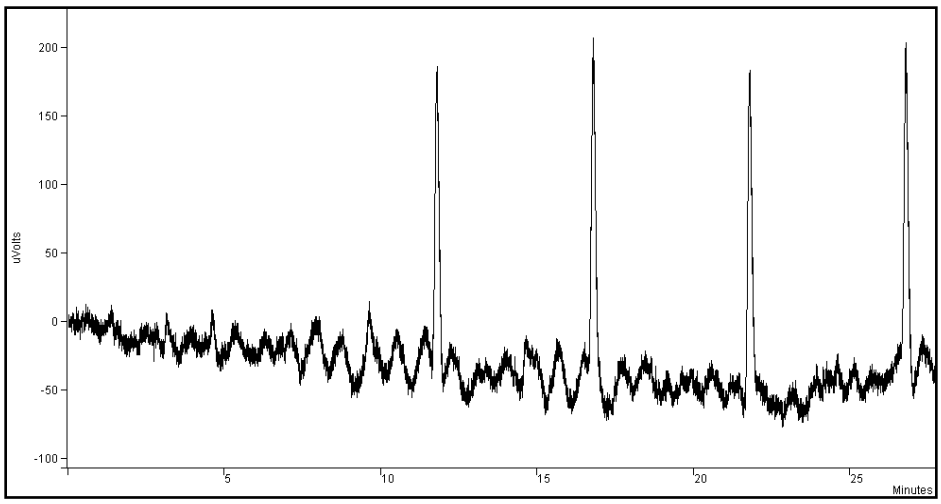

Fig. 17. Example of chromatogram used to construct external calibration, illustrating peaks of 0.5 ppm ethylene from desorptions during steady state period.

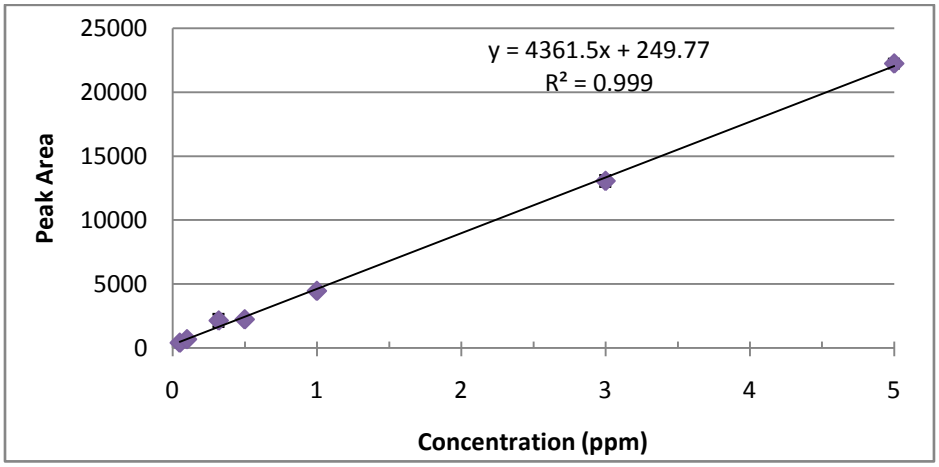

Fig. 18. External calibration curve for ethylene in the concentration range of 0.05 to 5 ppm.

The RSD values lie between 1 and 10%. A correlation value of 0.999 was obtained with a limit of detection (LOD) and limit of quantitation (LOQ) of 40 ppb and 268 ppb, respectively. This calibration method is useful for demonstrating that the instrument is working appropriately and that its response is linear within the range of the expected target analyte. Thus, for a method that can

accommodate fluctuations in environmental conditions, the dominant calibration technique will be used.

5.3. DOMINANT CALIBRATION

5.3.1. Theory

Dominant calibration is based on the mass transfer between analyte and calibrant. An advantage of this approach is that the calibration compound is the same compound as the target analyte. The calibrant ethylene is supplied to the stripping phase in a higher concentration then the expected concentration of ethylene in real samples. The calibrant is used to quantify the analyte by using the isotropic relationship between the mass transfer coefficients of the analyte and calibrant. A solution diffusion mechanism was previously used to illustrate the process of mass transfer into a non-porous polymeric membrane. Just as easily, this mechanism can be used to describe the mass transfer of calibrant present in the stripping phase and analyte in the feeding phase.[27] In the dominant calibration method, calibrant is supplied to the bulk of the carrier gas (BS), diffuses through the boundary layer, and partitions into the membrane from the stripping side. The calibrant then diffuses through the membrane, partitions from the membrane, and finally diffuses through the boundary layer on the feeding side of the membrane, where it escapes into the bulk as the feeding side (BF). A schematic illustrating the permeation of ethylene in both directions is shown in Fig. 19.

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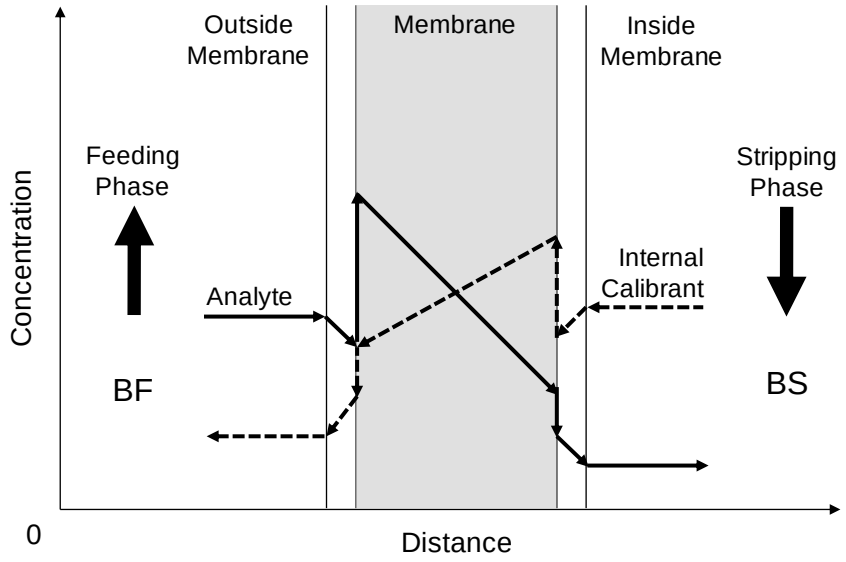

Fig. 19. Concentration gradient profile including internal calibrant and analyte permeation process.[27]

Both calibrant and analyte cannot be analysed simultaneously in this technique. The calibrant is first measured from the bulk of the stripping phase (BS) (that is without the membrane module), and later measured with the membrane module. The difference between the two values gives the 'loss' amount from the stripping phase of the MESI-GC system. Then, the extracted analyte from the feeding side of the membrane is measured to provide the 'gain' amount. Fig. 20 shows the direction of mass transfer for the measured gain and calculated loss.

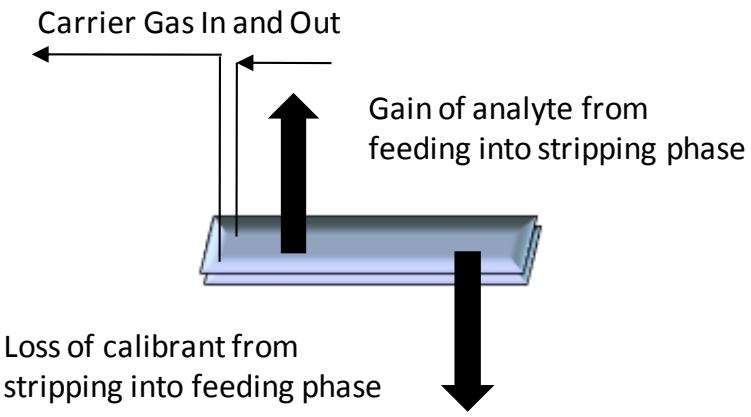

Fig. 20. Illustration of the membrane module indicating the direction for loss and gain measurements which is important to understand for the dominant calibration approach.

The dominant calibration method could only be successful if the mass transfer coefficients of this 'loss' and 'gain' from calibrant in the stripping phase, and calibrant in the feeding phase, were equally affected by changes in environmental conditions. To prove this, the mass transfer values needed to be determined for both calibrant and analyte under varying exposure conditions. The equations used to calculate the mass transfer coefficients of calibrant and analyte can be seen in (4) and (5), respectively. The derivations of these equations are outlined in Liu's work. [27]

$$h_{calibrant} = \frac{QH_{loss}}{A(H_{BS} - H_{loss})} \quad (4)$$

$$h_{analyte} = \frac{QH_{gain}}{A(H_{BF} - H_{gain})} \quad (5)$$

Where Q is the stripping flow rate in mL/min; A is the effective membrane area of the membrane module; H_{BS} and H_{BF} refer to the peak area values of the bulk in the stripping and feeding phase, respectively; H_{gain} is equal to the extracted amount of analyte from the feeding phase, whereas H_{loss} represents the difference in peak area between the stripping phase bulk value without and with the membrane.

To acquire the unknown analyte concentration using the dominant calibration procedure, we must modify the equation derived in previous work from Fick's first law of diffusion.[27] The final equation can be seen in (6),

$$C_a = \frac{n_s}{n_I r} C_0 = \frac{f_s H_s}{f_I H_I r} C_0 = \frac{H_{gain}}{H_{loss}} C_0 \qquad (6)$$

where C_a is the unknown concentration of analyte in the feeding phase; C_0 is the known concentration of calibrant in the stripping phase; n_s is the extracted amount of target analyte in the sorbent trap; n_I is the amount of calibrant lost from the stripping phase; f_s and f_I are GC response factors for the target analyte and calibrant, respectively; H_s is the peak area value for the 'gain' of analyte; H_I is the peak area value for the 'loss' of calibrant into the feeding phase (calculated from the difference between the bulk stripping value with and without the membrane module); lastly, r is the ratio of the mass transfer coefficients or degree of similarity between the target analyte and the internal calibrant during the mass transfer process. [27] Since ethylene is both the calibrant and the analyte, the GC response factors f_s and f_I will be equal, and they will cancel in the equation.

Similarly, the *r* value should equal 1 since the mass transfer trend for the same compound present as calibrant and analyte will be analogous. Equation (3) includes the peak area for extracted analyte, H_S, and the peak area for H_I which can be calculated from the difference between the bulk of the stripping phase (without the membrane module) and the extracted amount from the stripping phase with the membrane module. The parameter H_I can be expressed H_{loss} instead to clarify that it is actually the difference between the peak area of the bulk of the stripping phase (H_{BS}) with and without the membrane module. Likewise, H_S can be replaced by H_{gain} for clarity since it represents the peak area value for the extracted amount of analyte or 'gain' from the feeding phase.

5.3.2. Experimental

All chromatographic work was carried out using the Varian 3800 GC instrument. Praxair (Kitchener, ON, CA) provided all gases used (1 ppm ethylene standard in UHP helium, and the conventional gases required for GC use). The carrier gas for the experiments varied between UHP helium and the 1 ppm ethylene in helium. The U-PLOT column (30 m x 0.32 mm) previously mentioned was also used for these experiments with an isothermal oven temperature of 50°C. The MESI control unit was set to complete desorption pulses every 5 minutes, held for 10 seconds at a temperature of 180°C. The same sorbent tube was employed again with 2 mg of Carboxen™ 1000. The 250 mL glass sample chamber was used. All transfer line connections and the sample chamber were

checked for leaks prior to the start of any experiment using the electronic leak detector. Flow rates were monitored using the electronic flow meter.

To study the mass transfer trends, conditions of flow and temperature were varied. Mass transfer coefficients for the analyte and calibrant were determined under increasing feeding flow. Feeding flow rates were adjusted from 3.0 to 50.0 mL/min, while the stripping phase flow was maintained at 1.6 mL/min for the flow experiments. The experiments completed to determine the effect of membrane temperature on the mass transfer coefficients were completed using temperature ranges of hot or cold. The cold temperature range (approximately between 0-6°C) was achieved by submerging the sample chamber into an ice bath, while the hot range (approximately 50°C) was achieved by wrapping the sample chamber with Omegalux^{TM} heating tape, acquired from Omega (Stamford, CT, US).

5.3.3. Results and Discussion

5.3.3.a Influence of Feeding Flow on Mass Transfer Coefficients

To evaluate the mass transfer coefficient of the analyte ($h_{analyte}$) 1 ppm ethylene standard (in UHP helium) was supplied to the feeding phase at the desired flow rate (i.e., 3.0 to 50.0 mL/min), and UHP helium was the carrier gas in the stripping phase, maintained at a flow rate of 1.6 mL/min. Similarly, the mass transfer coefficient for the calibrant ($h_{calibrant}$) was determined by supplying the stripping phase with the 1 ppm standard ethylene (in helium) at 1.6 mL/min, and the feeding phase with UHP helium at the desired flow rate (i.e., 3.0 to 50

mL/min).The bulk values were also determined. The peak area value for the bulk of the stripping phase (H_{BS}) was determined using the 1 ppm standard ethylene in the striping phase without the membrane module, whereas the bulk of the feeding phase (H_{BF}) was determined by supplying the 1 ppm ethylene standard into the feeding phase (sample chamber) and connecting it directly to the sorbent trap. An example of a chromatogram for the bulk of the stripping side of the membrane is demonstrated in Fig. 21.

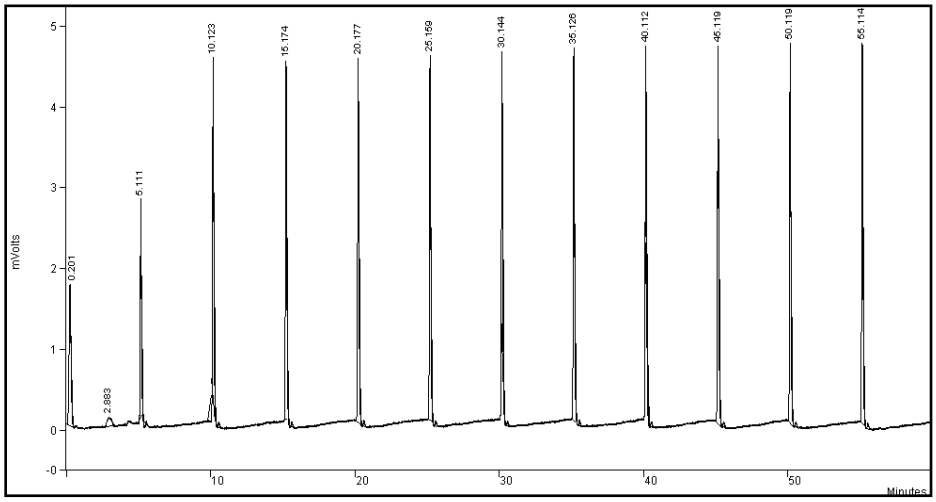

Fig. 21.Chromatogram illustrating 1 ppm of standard ethylene measured from the bulk of the stripping phase without the membrane module.

It was important to maintain the flow rates of the ethylene so that the amount trapped from either side of the membrane could be comparable. If the flow rates were not equal from run to run then there would be more or less moles of gas trapped in the sorbent trap. In this application the concentration of the ethylene depends on the pressure and flow rate of the gas, so consistency was essential.

The results from the effect of feeding flow on the mass transfer coefficients were calculated by taking an average of 5 repetitions. The RSD values were between 1 and 6%. The feeding flow equally influenced the mass transfer of ethylene into the membrane as it does out of the membrane. This is denoted by the mass transfer coefficients being identical to each other with increasing feeding flow. The values for calibrant increase from 0.29 to 0.43, while the values for analyte increase from 0.28 to 0.41 as seen in Table I . This is further demonstrated by the consistency in mass transfer coefficient ratios spanning from 0.96 to 1.01. A graphical presentation of the data is displayed in Fig. 22.

TABLE I. MASS TRANSFER COEFFICIENT DATA FOR ANALYTE AND CALIBRANT WITH CHANGES IN FEEDING FLOW

Feeding Flow Rate (mL/min)	Mass Transfer Coefficients (cm/s)		Ratio of Mass Transfer Coefficients
	Calibrant	Analyte	
3	0.29	0.28	0.99
6	0.33	0.33	1.00
9	0.38	0.39	1.01
20	0.40	0.41	1.01
50	0.43	0.41	0.96

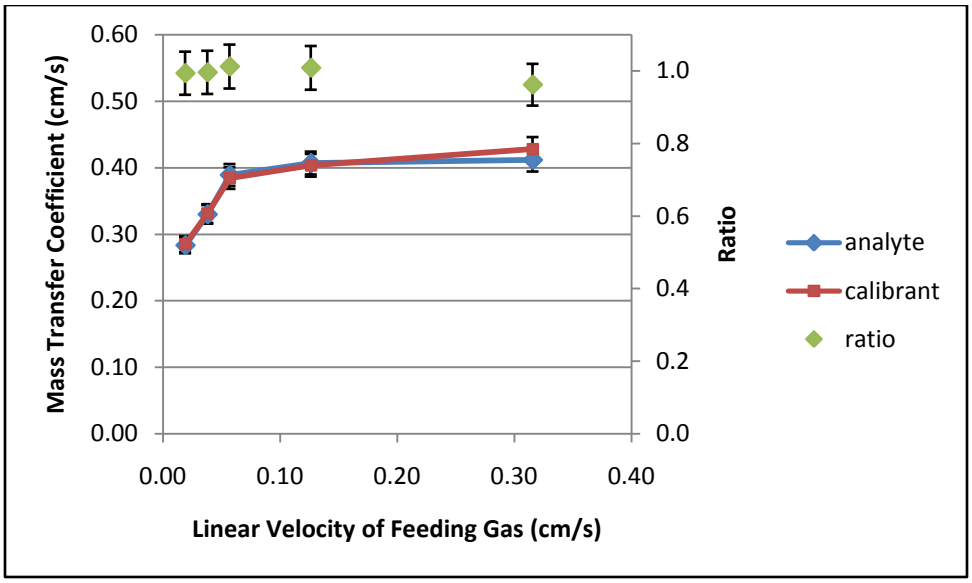

Fig. 22. The effect of feeding flow on mass transfer coefficients for analyte and calibrant along with their respective ratios. Feeding flow values between 3.0 and 50.0 mL/min were tested.

5.3.3.b Influence of Temperature on Mass Transfer Coefficients

The membrane module temperature was altered from cold to room temperature to hot and the mass transfer coefficients of the calibrant and analyte were determined in each case. The flow rates on both sides of the membrane were adjusted to 1.6 mL/min. An average of 5 peak area values were used to calculate the mass transfer coefficients, with RSD also between 1 and 6%. The results are presented in Table II and Fig. 23. The mass transfer coefficient trends for analyte and calibrant were very similar, even though they were not exactly equal as in the feeding flow experiment. The ratio values were around 0.6 rather than being equal to 1 (Table II).

TABLE II. MASS TRANSFER COEFFICIENT DATA FOR ANALYTE AND CALIBRANT WITH CHANGES IN TEMPERATURE

Temperature Range	Mass Transfer Coefficients (cm/s)		Ratio of Mass Transfer Coefficients
	Calibrant	Analyte	
Cold	0.42	0.24	0.57
Room	0.32	0.20	0.63
Hot	0.36	0.23	0.64

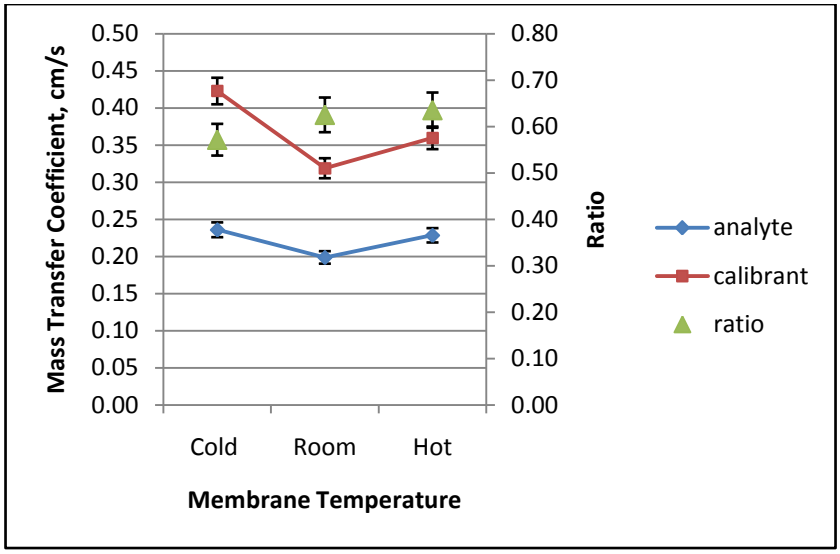

Fig. 23. The effect of membrane module temperature on mass transfer coefficients of calibrant and analyte at cold, room, and hot temperatures.

The explanation for why the *r* value does not equal 1 is due to the flow rate of the ethylene from one side of the membrane to the other. The metering valves used to the control the flow are not extremely stable at low flow rates of 1.6 mL/min. In order to achieve equal concentrations of analyte on both side of the membrane, the flow must be constant at 1.6 mL/min each time. This was difficult to deliver since the flows had to be adjusted each time the experiment changed from one side of the membrane to the other. Thus, although the ratio of mass transfer coefficients should be one, it is a challenge to practically accomplish using this experimental apparatus. This suggests that equation (6) will have to

include the *r* value to compensate for the inequality in the flow rate on both sides of the membrane. This problem could likely be resolved by using a more appropriate method for flow control at lower flow rates.

Therefore, changes in feeding flow and temperature influence the mass transfer of ethylene equally from both sides of the membrane, suggesting that a dominant calibration strategy is suitable for the quantification of ethylene in real breath samples.

5.4. VERIFICATION USING CO_2

Another useful calibration technique involves the use of CO_2 in expired breath. When a volunteer provided a breath sample, the end tidal concentration was measured as a means of normalizing varying breath samples from different volunteers. Our goal with this research is to relate the concentration of ethylene in breath to the concentration in our blood. That is how breath provides information. Since the CO_2 value in our breath is also representative of what is present in our blood, it can be used as an internal standard. The CO_2 amount expelled with every exhalation varies depending on the size of the breath/person and accordingly the amount of analyte (ethylene) will also vary. The results from this supplementary calibration method can be viewed in Tables III and IV in Section 6.2.2. The CO_2 was examined using the NICO Capnostat CO_2 sensor and monitor, provided by Respironics Novametrics (Wallingford, CT, US).

5.5. CONCLUSION

To conclude, there are various calibration methods available for use in analytical analysis. Some of these traditional methods like external calibration can be used with MESI-GC system, but are not very practical for field analysis. The dominant calibration technique has been investigated to guarantee that it is not affected by changes in environmental conditions. This would make the MESI-GC technique much more versatile. Completion of this work will use real samples to verify its success.

6. MESI APPLICATIONS

6.1. ARABIDOPSIS THALIANA

6.1.1. Experimental

This experiment was completed using the Varian 3800 GC-FID. The carrier flow was helium at 1.6 mL/min. The MESI conditions were as follows: desorption temperature of 200°C; trapping time varied between 5 and 10 minutes; column temperature of 60°C; FID temperature of 250°C, and cooling at 0°C. Most of these experiments were actually carried out prior to optimization, which is why optimum conditions were not used. Due to time limitations during the project, the experiments were not repeated using the optimized conditions.

Arabidopsis plants were grown in chambers with artificial light in a cycle of 8 hours of dark followed by 16 hours of light. They were grown from seed in soil medium in various sized cell packs, obtained from the Moffatt Laboratory in the Biology Department at the University of Waterloo (Waterloo, ON, CA). The variety of Arabidopsis available for analysis included wild-type, mutant, and ethylene over producers (Eto_3).

6.1.2. Results and Discussion

Different Arabidopsis lines were tried with the hope of detecting ethylene emissions. Wild-type, mutant, and known ethylene over expressers were obtained. Wounding the plant was certainly a way of generating ethylene, so that was tried first. This involved removing a few stems of the wild-type plant from

their roots, cutting them and placing them into the 250 mL glass sample chamber. The GC run was started immediately after closing the sample chamber. A whole wild-type Arabidopsis plant at 5 weeks old (containing many siliques) was also tried by placing the cell pack containing 1 plant into a 500 mL glass sample chamber. No peaks were visible from any of the wild-type whole plant trials, thus over-expressing Arabidopsis were the next to try. The over-producing plants were tested in the same way and small peaks were visible in the chromatogram. There are more peaks in the baseline of this trial likely due to soil components. The chromatograms of wound ethylene, whole plant ethylene emissions, and standard ethylene at 0.5 ppm can be seen in Fig. 24.

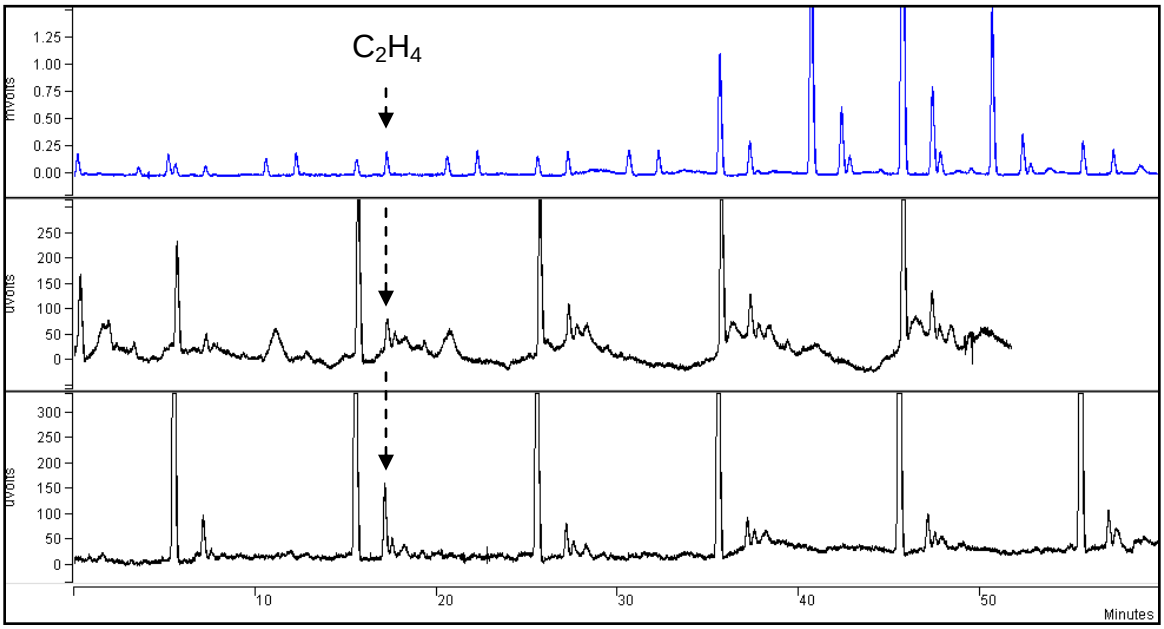

Fig. 24. Chromatograms illustrating 0.5 ppm standard ethylene (top); small peaks detected from whole Eto_3 plant emissions (middle); and wild-type wound emissions (bottom). Dashed line indicates the ethylene peaks of interest which occurs every 10 minutes.

Since the quantity of ethylene is so small and barely distinguishable from the baseline, quantitation of ethylene in Arabidopsis was not completed. Although the MESI-GC system shows promise for wound emissions and emissions from the over-producing variety of Arabidopsis, ethylene was not detected in wild-type or mutant whole samples. As a result the MESI-GC system is not quite sensitive enough for this application in spite of the fact that there was much interest in characterizing ethylene amounts during different stages of Arabidopsis growth.

6.2. HUMAN BREATH

6.2.1. Experimental

The carrier gas for the experiments also varied between UHP helium and the 1 ppm ethylene in helium. The U-PLOT column (30 m x 0.32 mm) was used again with the Varian 3800 GC-FID for these experiments with an isothermal oven temperature of 50°C. The MESI control unit was set to complete desorption pulses every 5 minutes, held for 10 seconds at a temperature of 180°C. The sorbent tube contained 2 mg of Carboxen™ 1000 and was prepared as previously outlined. The 250 mL glass sample chamber was used. All transfer line connections and the sample chamber were checked for leaks prior to the start of any experiment using an electronic leak detector. Flow rates were monitored using an electronic flow meter.

Six volunteers were selected for the donation of breath samples. Three were smoking individuals, while the others were non-smoking. The smoking volunteers provided breath samples immediately after smoking a cigarette. The

time the samples were taken was not monitored, nor were the types of cigarettes smoked. The experiments were kept simple as the point was to provide evidence that the MESI-GC system was able to detect low levels of ethylene in breath samples. This research never intended to be conclusive about the amounts present in the breath of smokers or non-smokers. For this many more variables would have to be monitored and more volunteers would need to be tested. When ready, the subject was asked to inhale through the nose and exhale fully into the 250 mL glass sample chamber. The CO_2 amounts were measured shortly thereafter and the GC run was immediately started.

6.2.2. Results and Discussion

The data collected in this experiment has been tabulated in Table III and IV. The tables include the sex of the volunteer, the amount of end tidal ethylene measured in mmHg, the average peak area measured, and the calculated amount of ethylene. Equation (6) has been used to calculate the ethylene in the breath samples. The result from non-smoking volunteers is presented first. Between 7 and 16 ppb of ethylene was determined with RSD values of 33 and 48%. Higher RSDs existed for lower concentrations of ethylene since those values were much closer to the LOD. The data captured from smoking volunteers is considerably higher between 35 and 221 ppb, with RSD values of 2 and 31%. The reason why ethylene may be larger in the breath of smokers is due to the ethylene present in the fumes of cigarette smoke.[44]

TABLE III. PEAK AREA VALUES FOR NON-SMOKING VOLUNTEERS WITH END TIDAL CO_2 MEASUREMENT

Volunteer	Sex	$EtCO_2$ (mmHg)	Average Peak Area	Amount of Ethylene Detected (ppb)
1	F	38	70	16
2	F	36	64	15
3	M	36	31	7

TABLE IV. PEAK AREA VALUES FOR SMOKING VOLUNTEERS WITH END TIDAL CO_2 MEASUREMENT

Volunteer	Sex	$EtCO_2$ (mmHg)	Average Peak Area	Amount of Ethylene Detected (ppb)
1	M	49	944	221
2	M	48	149	35
3	F	38	227	53

Chromatographic examples from the breath of non-smoking and smoking volunteers can be seen in Fig. 25 and 26, respectively. Ethylene peaks eluted every 5 minutes. A blank run (Fig. 25-upper) was carried out before each sample run to ensure ethylene was cleared from the sorbent tube. The retention time of ethylene detected from breath samples was in agreement with the retention time of the 1 ppm standard ethylene (see Fig. 26-lower).

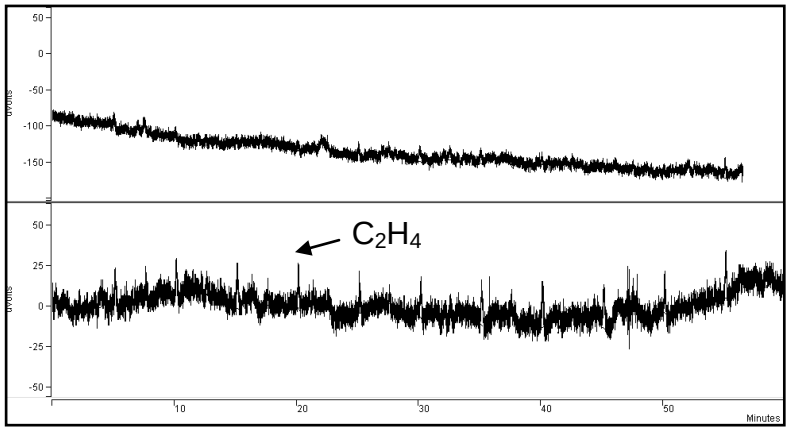

Fig. 25. Chromatogram illustrating an example of the background signal before analysis S/N: 2 (upper) and ethylene detection from a non-smoking volunteer S/N: 5 (lower).

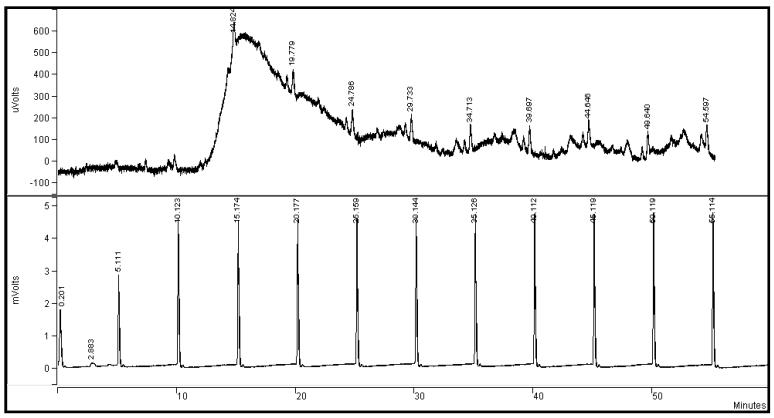

Fig. 26. Chromatogram illustrating an example of ethylene detection from a smoking volunteer immediately after smoking (upper), and 1ppm ethylene standard (lower).

There has not been an extensive amount of research confirming the amounts of ethylene in the breath of non-smoking and smoking volunteers. Yet, the results from the breath experiments in this study correlated well with values cited in literature. Literature values for ethylene detected in the breath of healthy volunteers are reported below 5 ppb, while smoking breath samples contained anywhere from 60 to over 1000 ppb of ethylene.[13, 45] The literature data was obtained using different methods including sampling directly using laser detection or collecting prior to analysis in aluminized bags.[13, 14, 44]

Therefore, the data obtained from the volunteer samples shows that the MESI-GC system is capable of detecting low (ppb range) concentrations of ethylene in breath. Moreover, the dominant calibration technique is a valuable tool for the quantitation of ethylene in human breath, which is a positive contribution toward expanding the limits of MESI analysis, so that ultimately it may be used as a primary source for medical information.

SUMMARY

In short, this research has made a positive impact on the use of the MESI-GC technique. The system was optimized for the analysis of the important biomarker ethylene. The MESI component was examined for breakthrough, steady state time, and for general use using standard ethylene. Finally the dominant calibrant technique was proven possible, by the isotropic relationship between the mass transfer of the calibrant and analyte with altering conditions. Broadening the range of compounds that can be analysed and calibrated using the system makes it a much more versatile technique. The MESI-GC system was successful for the detection and quantification of ethylene in human breath as well as for qualitative results with the Arabidopsis plant. This research is a positive contribution toward expanding the limits of MESI analysis, so that ultimately it may be used as a primary source of medical information or a means of monitoring ethylene emissions from plants.

SAFETY CONSIDERATIONS

It is common practice to be cautious in the laboratory since it is crucial for the safety of everyone working in the lab environment. Eye protection, nitrile gloves and a lab coat are highly recommended when handling hazardous chemicals. Also, when handling sorbent materials such as Tenax® TA, it may be necessary to wear a respirator, as inhalation may cause irritation of the mucous membranes and the upper respiratory tract. These hazardous chemicals should be used in a well ventilated fume hood to avoid direct exposure. Finally, this research project relies heavily on the use of gas cylinders. Gas cylinders are extremely dangerous and proper precautions should be taken during handling. This includes storing the cylinders upright and only moving them with the cap securely in place by rolling the cylinder on its base. Also, compressed gas cylinders should be equipped with the proper regulator when in use and should be routinely checked for leaks. For this research, hydrogen, nitrogen, helium and ethylene cylinders are required which are extremely flammable compounds. Personnel should be trained in the safe handling of compressed gases as well as WHMIS to be aware of the risks associated with working in a laboratory.[2]

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