DEVELOPMENT OF MEMBRANE EXTRACTION WITH A SORBENT INTERFACE FOR THE ANALYSIS OF ENVIRONMENTAL AND CLINICAL SAMPLES

by

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ABSTRACT

There is an increasing need for simple and rapid sample introduction techniques. This need has driven the development of solvent free sample preparation techniques, like solid phase microextraction (SPME) and membrane extraction with a sorbent interface (MESI). Membrane extraction with a sorbent interface is a single step sample preparation technique, which was developed to enable fast routine analysis, and semicontinuous monitoring of volatile organic compounds in various matrices.

Hollow fiber membranes present the advantage of being self-supported and very easy to couple to the carrier gas line. However, due to their thick wall, they have a slow response time and a long lasting memory. Flat sheet membranes are thinner, but they need to be placed in special holders in order to be connected to the carrier gas line.

A membrane module was constructed to enable the connection of flat sheet membranes in MESI systems. The response time and memory effect of the system were examined using a standard mixture of benzene, toluene, ethylbenzene and xylene. The permeation of analytes through silicone membranes and silicone polycarbonate membranes of different thickness was compared. The analytes permeate faster through thinner membranes. However, due to the higher permeability of the silicon membrane compared to the silicone polycarbonate membrane, the amount of analytes extracted using a silicone membrane of 55 μm thickness is higher than the amount extracted using a silicone polycarbonate membrane of 25 μm thickness.

A new sorbent trap has been designed to allow the use of longer trapping for improved sensitivity. A piece of stainless steel tubing was packed with an appropriate sorbent, and for analytes desorption a voltage pulse was applied to the walls of the sorbent trap. The use of the packed design favors the retention of the analytes by the sorbent, minimizing the channeling effect, making it more convenient for use in field analysis. Even with the use of a short trapping

time of one minute, the sensitivity was improved by more than 100 times, versus the sensitivity of a normal injection. The efficiency of the trap was further improved by placing it on a Peltier cooler. A piece of fused silica capillary, having an inner diameter of 100 μm was placed in front of the trap to minimize the back flush of the analytes during the desorption pulse.

The newly designed MESI system was coupled to different detectors and was applied for the analysis of various environmental samples. Complex chromatograms were obtained by analyzing tap water, eucalyptus leaves, and cigarette smoke using the MESI system and a flame ionization detector. The limit of detection obtained for the analysis of benzene by MESI-GC-FID was determined to be 10 ppt. By using a quadrupole mass spectrometer as detector, the peaks from tap water, fume hood air, and parking lot samples were identified. A field portable system was obtained by coupling MESI to a Micro GC equipped with a thermal conductivity detector. Even though TCDs are not very sensitive detectors, a detection limit of 60 ppt was achieved for the analysis of toluene, by preconcentrating the sample with MESI.

MESI was also applied for the analysis of various compounds in human breath. Complex chromatograms were obtained when a flame ionization detector was used as detector in the analysis of breath samples. The permeation of ethanol through skin was monitored using an ion mobility detector. Chloroform was detected in the breath of a swimmer and the variation of acetone in breath over a 4.5 hour period was monitored, using the same detector.

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DEDICATION

I dedicate this thesis to the most important people in my life: Sorin, my parents, Mona and Adela.

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CHAPTER 1

INTRODUCTION

1.1. Volatile Organic Compounds Considerations

1.1.1. Pollution potential of volatile organic compounds

Volatile organic compounds evaporate easily and can be present in air, water and soil. Fossil fuel deposits, including oil sands, are natural sources of volatile organic compounds (VOC) as are volcanoes, vegetation and bacteria. Trees also emit different volatile organic compounds. For example, deciduous trees emit a great amount of isoprene during a hot day; coniferous trees emit the volatile organic pinene day and night. Man-made VOC emissions come from transportation, solvent use, industrial processes and gasoline evaporation.

The industrial revolution has been the central cause for the increase in pollutants in the atmosphere over the last three centuries. Before 1950, the majority of this pollution was created from the burning of coal for energy generation, space heating, cooking and transportation. Under the right conditions, the smoke and sulfur dioxide produced from burning of coal can combine with fog to create industrial smog. In high concentrations, industrial smog can be extremely toxic to humans and other living organisms.

Today, the use of other fossil fuels, nuclear power, and hydroelectricity instead of coal has greatly reduced the occurrence of industrial smog. However, the burning of fossil fuels like gasoline can create another atmospheric pollution problem known as photochemical

smog. Photochemical smog is a condition that develops when primary pollutants interact under the influence of sunlight to produce a mixture of hundreds of different and hazardous chemicals known as secondary pollutants.

The incomplete combustion of residential fuel wood is one source of primary pollutants. Primary pollutants consist of materials (dust, gases, liquids and other solids) that enter the atmosphere through natural and human-made events. The main primary pollutants influencing our atmosphere are carbon monoxide, sulfur oxides, nitrogen oxides, volatile organic compounds and particulate matter.[1]

The most common volatile organic compound released into the atmosphere is methane. Methane poses no direct danger to human health; however it does contribute to global warming through the greenhouse effect. Other VOCs released into the atmosphere include benzene, formaldehyde and chlorofluorocarbons. Of these chemicals, benzene and formaldehyde are the most dangerous to human health because they are carcinogenic.[2]

Air pollution can also occur indoors. The quality of indoor air has become a major concern to the entire population. The "sick building syndrome" has been associated with the poor quality of indoor air in public buildings. Buildings-related health problems occur due to contamination of indoor air by emission of volatile organic compounds from a variety of sources including construction materials, fabrics, furnishings, maintenance supplies, adhesives, paints, caulks, paper and cleaning products.[3,4] Many of these VOCs and their by-products are very toxic.

The Clean Air Act of 1990 requires the use of emission reducing oxygenated fuels in areas failing to meet the air quality standards. Methyl tert-butyl ether and ethanol are most commonly selected by petroleum refiners and distributors as additives for producing cleaner burning gasoline. However, ethyl tert-butyl ether, tert-amyl ether, diisopropyl ether, tert-butyl

alcohol, and methanol are also used.[5] Due to increased use of methyl tert-butyl ether (MTBE) since the 1980s and its environmental mobility and persistence, reported detection of MTBE in groundwater and surface water is on a dramatic uprise.[6] Recently the USEPA Office of Ground Water and Drinking Water established an advisory panel to examine the behavior of oxygenated fuels.[7] The information currently available on gasoline oxygenates in ground and surface water has focused on the presence of ethers, primarily MTBE.

VOCs have been shown to affect a wide number of biological and environmental systems. They are known to influence various atmospheric processes, some are carcinogenic and/or mutagenic, while others are persistent and show bioaccumulation effects[8]. Many VOCs also affect aquatic organisms. Trace levels of monocyclic aromatic hydrocarbons and halocarbons have been found in Antarctic waters and surface snow. [9,10,11] In the list of 36 priority toxic pollutants established at the Third International Conference on the Protection of the North Sea, nine VOCs, all chlorinated C1- and C2-hydrocarbons, were mentioned.[12]

Another pollution issue results as a consequence of water chlorination. There is increased evidence for an association between rectal, colon and bladder cancer in humans and consumption of chlorinated drinking water[13,14].

Chlorination is used extensively by municipal water treatment plants to disinfect water. However, the gaseous chlorine used by the plants is much too dangerous for home use. When chlorine is fed into water, it first reacts with any iron, manganese, or hydrogen sulfide that may be in the water. If any residual (un-reacted) chlorine remains it will next react with any organic material (including bacteria) present. In order to ensure that the water remains protected throughout the distribution system, an excess of chlorine is available to fully react with the organics present.

Chlorine is a very effective disinfectant killing many pathogenic bacteria (including those that cause typhoid, cholera and dysentery). However, cyst-forming protozoa, which cause amoebic dysentery and giardiasis, are extremely resistant to chlorination.

Because of its efficiency, chlorine has been used for drinking water disinfection for nearly 100 years. What concerns health officials are the chlorination by-products, chlorinated hydrocarbons, known as trihalomethanes (THMs). THMs are formed in drinking water when chlorine reacts with naturally occurring substances such as decomposing plant and animal materials.

One of the THM found in chlorinated drinking water is chloroform. Chloroform is strongly absorbed by the pulmonary, oral and cutaneous routes. It has adverse cardiac, hepatic, renal, dermatological, neurological and ophthalmological effects. In animals, chloroform is carcinogenic and embriotoxic.[15]

Air and water quality issues cannot be addressed in isolation. Their complex inter-relationship make achieving the goal of a future clean environment a challenge for individuals, industry and government alike.

1.1.2. Significance of volatile organic compounds for toxicology and clinical chemistry

Mixtures of volatile organic compounds can be credited with pleasant fragrances of flowers or perfumes. Nonetheless, they are also responsible for the less pleasant odors emitted by adhesives, caulks, paints and dies. As previously mentioned, exposure to some of these compounds can be harmful to humans. Protection of human health from pollutants begins with the accurate assessment of the actuai exposure. Such information is used to plan further health-related research and to serve as a baseline to confirm the efficacy of remedial actions.

Toxicology is a science that deals with poisons and with the problems involved (clinical, industrial, or legal). The toxicological data for a chemical are evaluated using parameters such as the lethal dose, 50%, (LD_{50}) and the lethal concentration, 50% (LC_{50}).[16] LD_{50} is the amount of analyte per kilogram of body weight that produces 50% mortality in the test animal population. An analyte having a LD_{50} of 50 to 500 mg/kg is considered to be a highly toxic compound, while one having a LD_{50} of 5 to 15 g/kg is considered to be slightly toxic. LC_{50} refers to the concentration of an analyte that produces 50% mortality in the test population.

The development of an industrial hygiene program as a complement to the environmental assessment of chemical contaminants has sparked enormous interest in the search for biological indices of occupational exposure.

For industrial workers, the respiratory system represents a facile port to the body for the ingress of volatile organic compounds that may be present in the inspired air, often leading to adverse health effects. Numerous studies[17,18] have focused on the uptake and elimination of organic solvents in subjects exposed to controlled levels of the solvent in air, in order to elucidate the processes of ingestion of volatile organics via the respiratory system. Generally, hydrophilic compounds are readily absorbed into the blood stream and are not accumulated in any specific body tissue but rather are distributed throughout the body.[19] Such compounds have a tendency to remain in the bloodstream and following the exposure period are rapidly excreted, unchanged via the respiratory system. On the other hand, lipophilic compounds have a tendency to accumulate in the adipose tissue.[20] Such compounds are slowly excreted via the respiratory system.

Workers from paint industry are exposed daily to compounds like toluene, xylene and styrene. Toluene is the most widely used organic solvent, constituting up to 80% of paints.

62% of inks, 56% of thinners and 51% of adhesives.[21,22] Gasoline contains 5-7% toluene by weight, and about 92% of the United States production, 6.03 billion pounds in 1992,[23] is used for gasoline formulation. The highest current levels of toluene exposure are seen in the printing, painting, automotive, shoemaking, and speaker-manufacturing industries. Occupational overexposure is associated with mucous membrane irritation, decrements in central nervous system function, and endocrine disruption.[24] In women, occupational exposure to toluene has been reported to produce spontaneous abortion and menstrual dysfunction.[25] Another major component of glues used in shoe manufacturing, n-hexane, has been shown to act as a peripheral neurotoxic.[26]

JP-8 jet fuel is considered the most common chemical exposure in the military. It is the fuel for all land-based internal combustion engines including trucks, jeep, tanks, and generators; and cooking and heating for forward-deployed forces. Additionally, JP-8 is essentially identical to Jet A-1, the commercial aircraft aviation fuel. JP-8, like other petroleum distillate fuels, is a complex mixture of aromatic and aliphatic hydrocarbons. [27] Several studies have indicated the toxic effect of JP-8 on the immune system, postural balance, pulmonary function and embryo growth.[28,29] Therefore, occupational exposure to JP-8 in Air Force has now become a real concern in terms of long-term health consequences.

Occupational exposure to inhalation anesthetics is an undesired consequence of the work in the operating room. Anesthesia is currently practiced using nitrous oxide associated with one or more potent anesthetics (halothane, enflurane, isoflurane). Some epidemiological evidence suggests that exposure to waste anesthetic gases may be associated with reproductive toxicity. Accordingly, National Institute of Occupational Safety and Health has established recommended exposure limits for nitrous oxide (25 parts per million) and volatile

anesthetics (2 parts per million). However, data suggest that exposure of healthcare personnel may exceed recommended levels in poorly ventilated postanesthesia care units.[30]

Many people participate in regular physical fitness activities to maintain or improve physical well being. While any level of physical activity can cause injuries or other ailments, participation in athletics is generally considered to be a long-term physical benefit for most people. However, there are some risks associated with long-time participation in certain sports. For example, debilitation due to practicing football or boxing has been encountered. A more unusual risk is represented by the exposure to chloroform while swimming. Because swimming pools are treated with chlorine-based disinfection compounds, a few studies have shown significantly increased levels of chloroform in blood and breath after swimming.[31,32] As mentioned before, chloroform is a known animal carcinogen, and it has been linked to bladder and rectal cancer in humans.

A high number of endogenous volatile organic compounds are present in the human body. The increased or decreased concentration of some of them has been associated to certain diseases. Thus, isolation and quantification of VOCs is extremely important in clinical chemistry.

Premature infants have an increased susceptibility to brain damage partially due to the immature development of their free radical defense. They have low antioxidant vitamin status, low levels of reduced glutathione and elevated prooxidant content.[33] In addition, premature infants encounter situations in which oxidative stress is unavoidable. Parturition, in itself, may be a mild form of oxidative stress since the fetus with a low arterial pO_2 (hypoxic relative to maternal arterial blood) abruptly enters a relatively hyperoxic environment. Furthermore, because of the immaturity of their lungs, premature infants have to be kept in

hyperoxic incubators for a period of time. Due to the higher pO_2, lung and eye damage, mediated through free radical mechanisms, have been reported.[34]

Oxidative stress processes in the central nervous system have been reported in children suffering from bacterial meningitis. Oxidative stress is also related to coronary arterial diseases. It contributes to endothelial dysfunction in human obesity, and is a major problem for hemodialysis patients.

Lipid peroxidation can be assessed by measuring ethane and pentane in breath, which are produced from the peroxidation of omega-3 or omega-6 polyunsaturated fatty acids.

Another example of the use of VOCs in clinical chemistry is acetone, which is successfully used for monitoring diabetic patients and the diet of obese subjects. The presence of higher concentrations of dimethyl- and trimethylamine in the body has been related to some renal diseases. Increased concentrations of dimethyl sulfide and mercaptans have been found in the blood and breath of cirrhotic patients.

The usefulness of volatile organic compounds in toxicology, and as biomarkers in clinical chemistry, as well as for the assessment of the environmental pollution depends on the analytical technique used for their measurement. Sometimes very low concentrations of VOCs have to be measured, and conventional analytical techniques are incapable of reaching such low limits of detection. There is also a great need for on-line methods capable of delivering the result in real time. There is also a great demand for the development of field analytical techniques, in environmental monitoring especially. They eliminate the inconvenience of having to transport the sample to the laboratory and the chance of damaging or partially losing the sample during transportation.

Therefore, an analytical technique suitable for these kinds of applications would have to be able to reach very low limits of detection, deliver the results in real time, and be field portable.

1.2. Sampling and Sample Preparation

An analytical procedure has several steps: sampling, sample preparation, separation, quantitation, statistical evaluation, decision and action. The analytical steps follow one after another, and the next step can not begin until the preceding one has been completed. Each of the steps is very important for obtaining correct results. If one of the steps is improperly done, the overall performance of the procedure will be poor. However, mistakes that occur in the sampling step are extremely important since they can not be corrected and the entire analysis is compromised. If a mistake is made in one of the following steps, the analytical chemist has the choice of repeating the analytical procedure, if the sample amount permits a repeat.

In the sampling step, a decision of how to obtain a representative sample in the right amount has to be made. Thus, the precision of the analytical measurement is predetermined by this step. Most traditional sampling methods involve the use of special containers or sorbents for sample loading.

If the sample is to be sent to a laboratory for analysis, precautions must be taken regarding its delivery. It is this step that usually contributes significantly to sample loss and sometimes sample damage. This is extremely important if volatile organic compounds or unstable compounds have to be analyzed.

On the other hand, a very important issue is the time spent between the sampling step and the moment the analytical result is being obtained. Very often fast decisions have to be

made, and these are the cases in which on-line and on-site analytical techniques are most welcome.

In the sample preparation step, the analytical chemist tries to isolate the analytes of interest from the sample matrix, or tries to eliminate the compounds in the matrix that could interfere with the analysis of the target analytes. This makes sample preparation the most time consuming and critical step in an analytical procedure.

Traditional sample preparation methods are labor intensive and have multi-step procedures that lead to loss of compounds, and most often, require the use of toxic and expensive solvents. Over 75% of the analysis time is spent on sampling and sample preparation steps.

Very often, the concentration of analytes in the matrix is below the limit of detection of the instrument. In these cases, sample preparation step has to be combined with a sample preconcentration step. The traditional sample introduction techniques are laborious, time consuming and sometimes of low efficiency.

These are the reasons why there was a great need for a change in the methodology of sampling and sample preparation, and solvent-free alternatives were needed.

A relatively new and revolutionary sample introduction technique called solid phase microextraction (SPME) has been introduced to the analytical chemistry scene by Dr. Janusz Pawliszyn and his coworkers. SPME has gained the interest of scientists around the world for its simplicity and ability to preconcentrate the samples, allowing them to reach very good limits of detection.

SPME is a single step sample preparation technique[35] that uses a polymer coated on a fused silica rod (SPME fiber) to extract and trap the analytes from their matrix. The technique

provides significant advantages over traditional methods, being very simple, accurate, reproducible, portable, cost effective, and reusable.

In SPME, the sample has to be introduced into the analytical instrument manually or by using an autosampler. As an alternative to SPME, Dr. Pawliszyn and his coworkers have developed an on-line, semicontinuous monitoring method, called membrane extraction with a sorbent interface.[36,37]

1.3. Membrane Extraction with a Sorbent Interface

Membrane extraction with a sorbent interface (MESI) is a very simple and efficient analytical technique that was developed to allow routine analysis and long time monitoring of volatile organic compounds. In the MESI process, the sampling and sample preparation steps are integrated within the analytical instrument. MESI is capable of measuring VOCs in different matrices, without the requirement of sample pretreatment. Like SPME, MESI is a solvent free sample preparation technique that is also capable of preconcentrating the sample.

MESI uses a membrane module, a sorbent interface, a gas chromatograph and a computer for data acquisition. A schematic diagram of the MESI system is presented in Figure 1- 1. The membrane module represents the sampling part of the system. The volatile organic compounds from the sample permeate the nonporous membrane, which can be in direct contact with the sample or exposed to its headspace. The membrane represents a barrier between the sample and the carrier gas that flows through the MESI components. It is usually nonpolar, thus keeping water from entering the system. It also acts as a selective element, since permeation rates of different molecules vary with membrane material. During sampling, the analytes are transported through the membrane by diffusion.

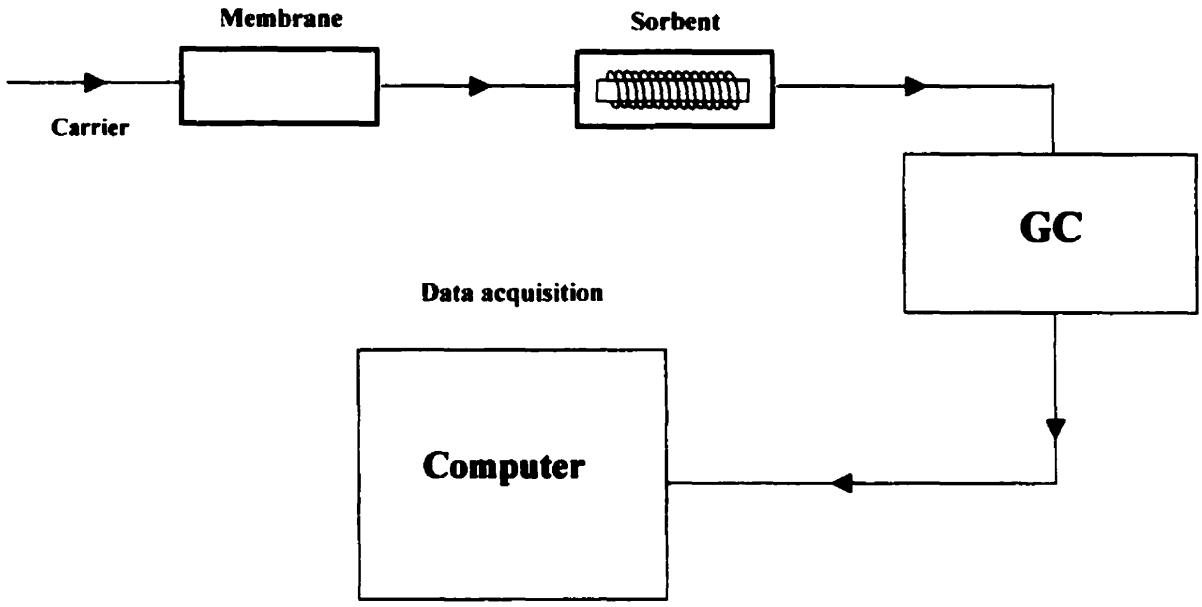


Figure 1- 1. Schematic diagram of the MESI system

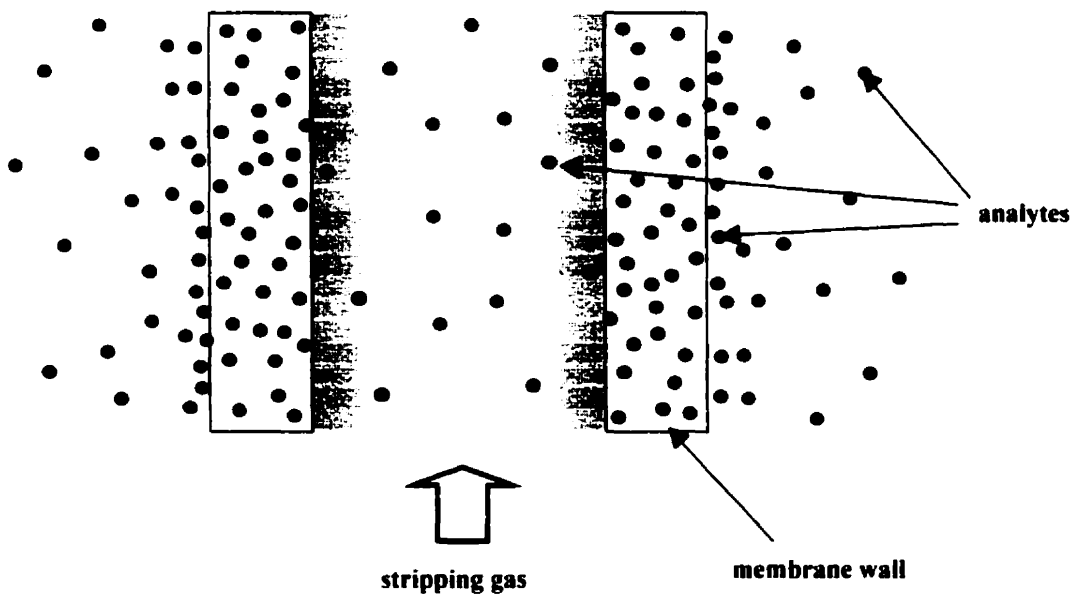


Figure 1- 2. Schematic diagram of analyte permeation through the membrane

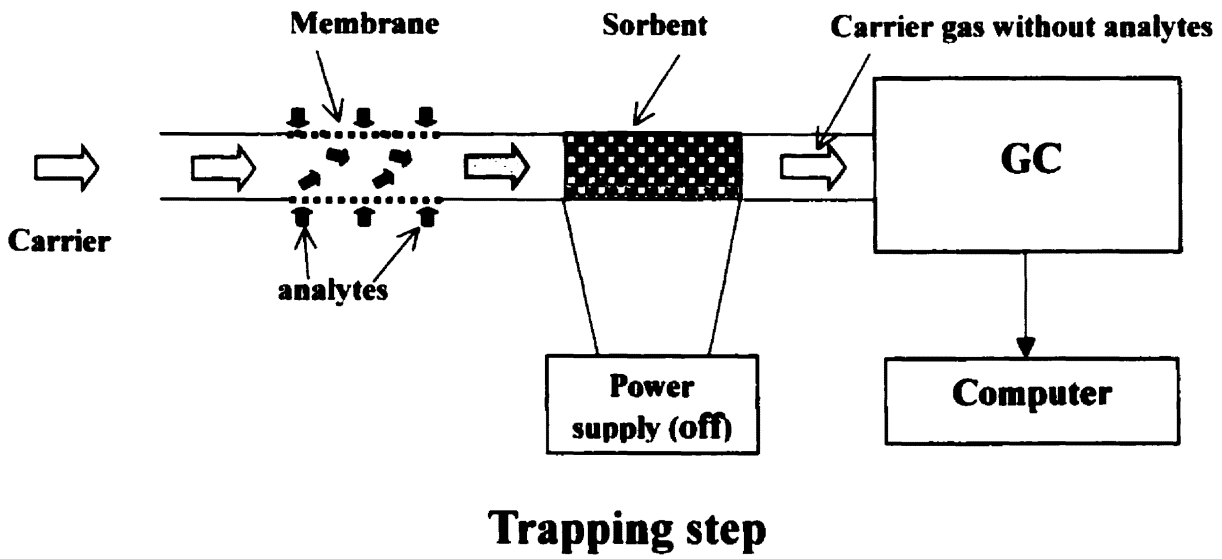
Once they reach the other side of the membrane, the inner side, they are stripped by the carrier gas and conducted to the sorbent interface.

Figure 1- 2 illustrates the permeation of analytes through the membrane. A concentration gradient is established across the membrane wall and the analytes diffuse toward the stripping gas. The membrane is nonporous and the most commonly used material is silicone.

The sorbent interface has a very important role in MESI. It is the element that provides sample preconcentration, and it also acts as an injector for the gas chromatograph. The sorbent interface consists of a sorbent material and a heating element. The analytes present in the carrier gas are passed over the sorbent which retains them. Thus, the carrier gas is free of analytes after passing through the sorbent interface. If desired, a cooler can be used to decrease the temperature of the sorbent, making trapping more efficient. After a desired preconcentration time, a heating pulse is applied on the sorbent interface, and the analytes are released. The sorbent interface is heated by applying a voltage pulse from a power supply.

Figure 1- 3 schematically presents the two steps of a MESI process, trapping and desorption. In the trapping mode, the analytes permeate through the membrane and are retained on the sorbent. During this step, the sorbent is kept at room temperature, or if a cooler is used, the temperature can be considerably lowered (reaching negative values). The power supply is off, and the carrier gas is free of analytes after passing over the sorbent.

During the desorption step, the analytes are still allowed to enter the system, if the membrane is exposed to the sample. They are again carried to the sorbent interface, where a considerable amount of analytes has already accumulated. The power supply is then turned on by a timer, thereby heating the sorbent. The analytes present in the sorbent at the time the voltage pulse is applied are being released and enter the carrier gas stream in a very narrow sample band.



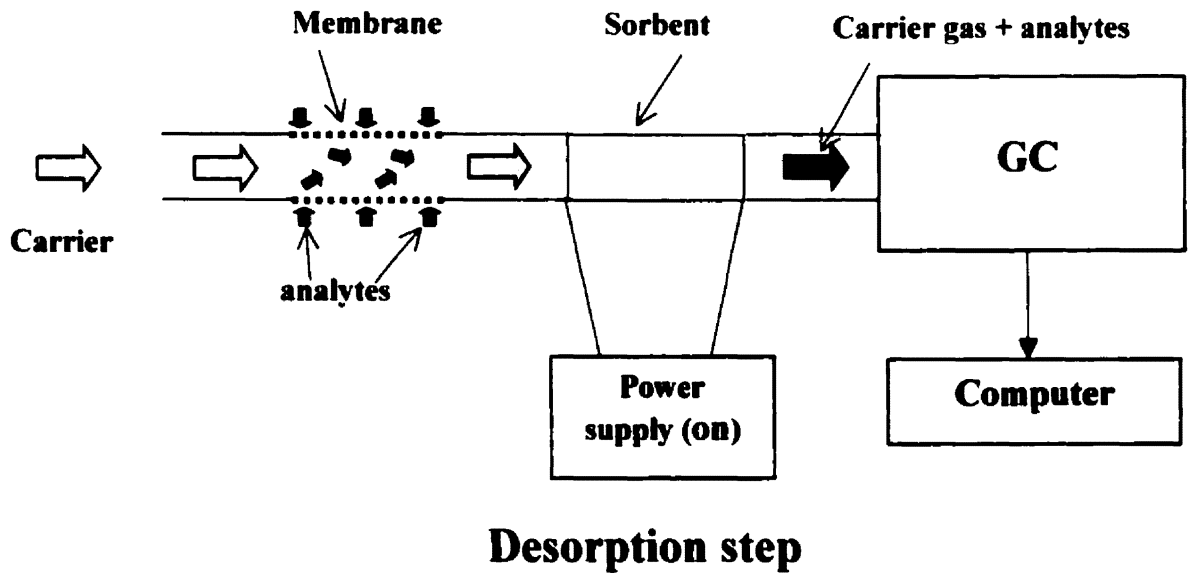


Figure 1- 3. Schematic diagram of the trapping and desorption processes in a MESI system

The analytes are then carried directly into the gas chromatographic column where they are separated and then detected by passing through an appropriate detector. Thus, in a MESI system, the injector from the GC is bypassed.

MESI presents numerous advantages:

➤ It is a solvent free analytical technique.

➤ During the analysis, the membrane is simply exposed to the sample that is to be analyzed. No additional instrumentation is used, and no sample pretreatment is required.

➤ The membrane introduces selectivity in the system, which is extremely important when complex samples have to be analyzed.

➤ Sampling and preconcentration occur in one step.

➤ The sensitivity is very much improved by preconcentrating the sample prior to introduction into the gas chromatograph.

➤ On-line continuous monitoring of industrial processes can be accomplished by exposing the membrane to the sample for a long period of time and desorbing the analytes at desired time intervals.

➤ The membrane and the sorbent interface are reusable.

➤ The system is cost and time effective, and easily automated.

Because of the above advantage, choosing MESI technique to analyze volatile organic compounds seems to be a very good choice.

1.4. Thesis Objectives

The overall objective of the herein-described research is to demonstrate that MESI is a viable tool for the analysis of volatile organic compounds in environmental and clinical chemistry applications.

Previous MESI research has focused mainly on the theoretical aspects of the method, and less in the area of applications. It is the goal of this thesis to prove that MESI can be successfully applied for the analysis of VOCs in different matrices.

The two main components of the system, the membrane module and sorbent interface, will be optimized.

Chapter 2 describes the optimization of the membrane module. Given the theoretical advantages of using flat sheet membranes versus hollow fiber membranes, this chapter describes the design of the membrane holder that has been constructed to facilitate faster transport of analytes through the membrane. Response time and memory effect of the system are two important issues in systems using membranes. They have to be evaluated for a better understanding of the system's potential.

Chapter 3 deals with the design of the sorbent interface. One of the major advantages of using MESI is its preconcentration capability. The extent of the preconcentration depends on the permeation rate through the membrane and the capability of the sorbent material to trap the analytes for as long as possible. On the other hand, the analytes have to be efficiently desorbed from the trap and introduced into the chromatographic column. This depends on the design of the heating element and the power supply used. Optimization of the sorbent interface has to be done in order to achieve low limits of detection.

In analytical chemistry, choosing the right detector is as important as choosing the right technique itself. Different detectors are more or less sensitive for different analytes. Detectors are also demanding elements that sometimes require certain conditions, that are not always easily achieved. Different detectors are evaluated in Chapter 4 and examples of applications for environmental monitoring, obtained with those specific detectors, are presented.

Breath analysis is a noninvasive technique. Its use in clinical chemistry has several advantages versus blood analysis. Chapter 5 focused entirely on the development of MESI for the analysis of human breath. Sampling breath is very challenging, since it is a non-homogeneous sample. A special breath-collecting device has to be designed in order to be able to measure VOCs in the exhaled air, by MESI. This author's personal interest in clinical chemistry resulted in the devotion of a large amount of time into the development of the breath analysis technique. However, quantitative measurement of analytes in the breath was not the purpose of this research. Quantitation can be achieved in a further step, once the analytical device has been successfully developed.

Chapter 6 provides an overall summary of the research described herein, and the scientific advancements obtained from this research.

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CHAPTER 2

MEMBRANE EXTRACTION MODULE

2.1 Introduction

Several methods are currently used for the analysis of volatile organic compounds in environmental samples. For aqueous and solid sample, the procedure implies the collection of the sample into vials, followed by transfer to a laboratory where they are analyzed usually by static or dynamic headspace gas chromatography.[1,2] These methods are simple since no extensive sample preparation is required. However, there are also some disadvantages. The sensitivity of static headspace is limited and the method is thus restricted to samples with relatively high concentrations of VOCs. Dynamic headspace, i.e. purge and trap, provides higher sensitivity, but more complicated instrumentation is required, and the inconvenience of having to transport the sample to the laboratory remains a major concern.

As regulatory requirements for pollution monitoring become more stringent, continuous monitoring methods capable of tracking emission sources such as industrial stacks and vents on a continuous basis are becoming more important. Continuous monitoring is also useful for keeping an emission inventory and for process control. The advantages come from the fact that continuous monitoring methods can detect, in a very short time, an upset in a chemical process, so that corrective actions can be taken immediately. Not only does this reduce environmental problems, but it can also save money for the industry in terms of resource conservation and recovery.

In general, spectroscopic techniques are ideal for process monitoring because of their analysis speed. For example, infrared methods are used in real-time monitoring of compounds such as ammonia, hydrochloric acid, ozone and carbon dioxide, as well as some organic compounds.[3] However, water vapor, which commonly exists in emission streams can seriously interfere with IR analysis. Another problem is the difficulty of identifying individual organic compounds in complex mixtures, due to the overlapping of absorbance bands from the different compounds.

Gas chromatography (GC) is an excellent technique for separating individual compounds from a complex mixture. It has been used in process monitoring since 1956[4] even though GC separation is much slower than spectroscopic measurements. However, recent developments in GC have significantly reduced the separation time, making GC a viable technique for on-line continuous monitoring.[5] A critical component of on-line GC monitoring systems is the sample introduction device, which has to make automatic injections at certain time intervals.

Membrane techniques are used for a number of applications such as microporous filtration, ultrafiltration, reverse osmosis, dialysis, electrodialysis and gas separation.[6] In recent years, the membrane introduction mass spectrometry (MIMS) technique has gained interest from scientists around the world. In MIMS, the analytes are transported from the bulk solution to the surface of the membrane, then diffuse across the membrane and evaporated from the other side either directly into the mass spectrometer vacuum system or into a stream of inert gas which transports the analytes to the ion source. The technique is very rapid and offers increased sensitivity. However, the use of a GC for the separation of compounds from very complicated matrices makes the identification of individual spectra much easier. Moreover, simpler and less expensive detectors than mass spectrometers can be used.

2.2 Sampling volatile organic compounds by MESI

The membrane module represents the sampling part of an MESI system. It consists of the membrane itself and its support (if necessary), and additional tubing that connects it to the rest of the elements of the system.

The membranes used in MESI are nonporous. If porous membranes would be used, part of the carrier gas that flows along the inner side of the membrane would escape from the system, resulting in fluctuations of the flow rate of the carrier gas. The most used membrane material is silicone rubber. Silicone membranes discriminate against water. Thus, it is advantageous to have such a membrane connected to a gas chromatograph, since water is undesirable in a gas chromatographic column. Other advantages are: chemical inertness, thermal stability, and good solubility for numerous solutes.[7] The high energy of the Si – O bond gives the membrane a high thermal and oxidative stability compared to that of other organic polymers.

Hollow fiber membranes have been mostly used in MESI experiments so far. They present the advantage of being self supported and very easy to couple to the carrier gas line.

In order to connect a piece of silicone hollow fiber membrane to the carrier gas line, the outer diameter of the connection tubing (fused silica, stainless steel, Teflon) has to be equal to or slightly smaller than the inner diameter of the membrane. If the ends of the membrane are immersed into an appropriate solvent (usually toluene) for about 30 seconds, the membrane swells. The connection tubing can then be easily slipped into the membrane. Once the solvent has evaporated, the membrane remains tightly sealed to the ends of the tubing. Figure 2- 1 illustrates a schematic diagram of the hollow fiber membrane probe.

The several different ways in which the extraction can be performed are schematically presented in Figure 2- 2. For liquid samples, the extraction can be done in the

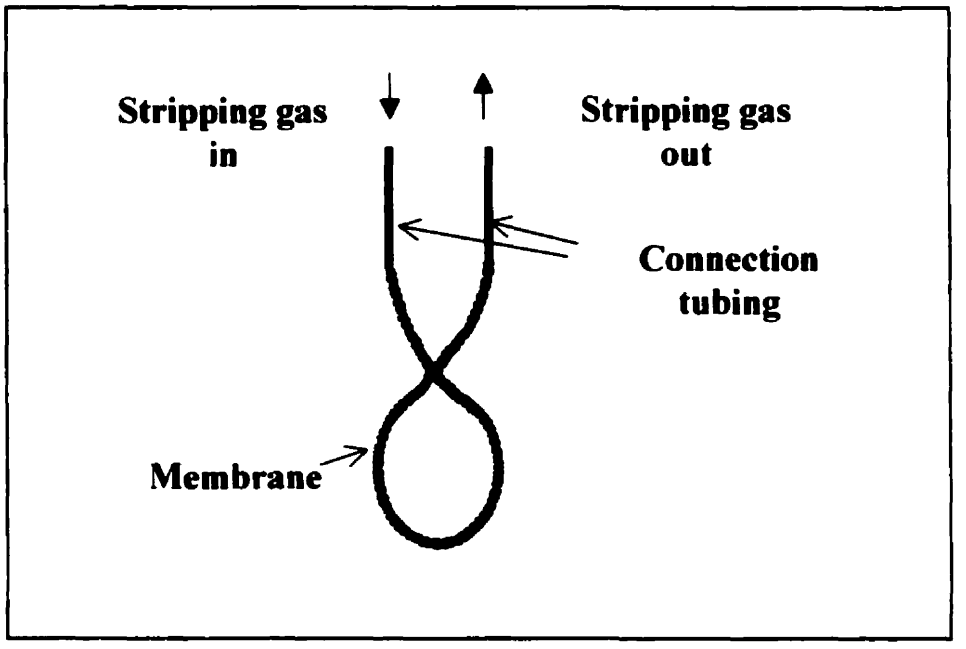

Figure 2- 1. Schematic diagram of the membrane module

direct-static mode, direct-stirred mode or by headspace extraction. In the direct-static mode the membrane is submerged in the liquid and the sample is not stirred. In the direct-stirred mode the membrane is placed in the liquid and the liquid is stirred. The headspace extraction is accomplished by placing the membrane above the liquid, in its headspace, and the sample is usually stirred.

For gaseous samples, either a static or dynamic extraction can be performed. In the dynamic mode the membrane can be placed into a sampling chamber through which a gas stream flows. This sampling procedure is particularly useful in the monitoring of industrial

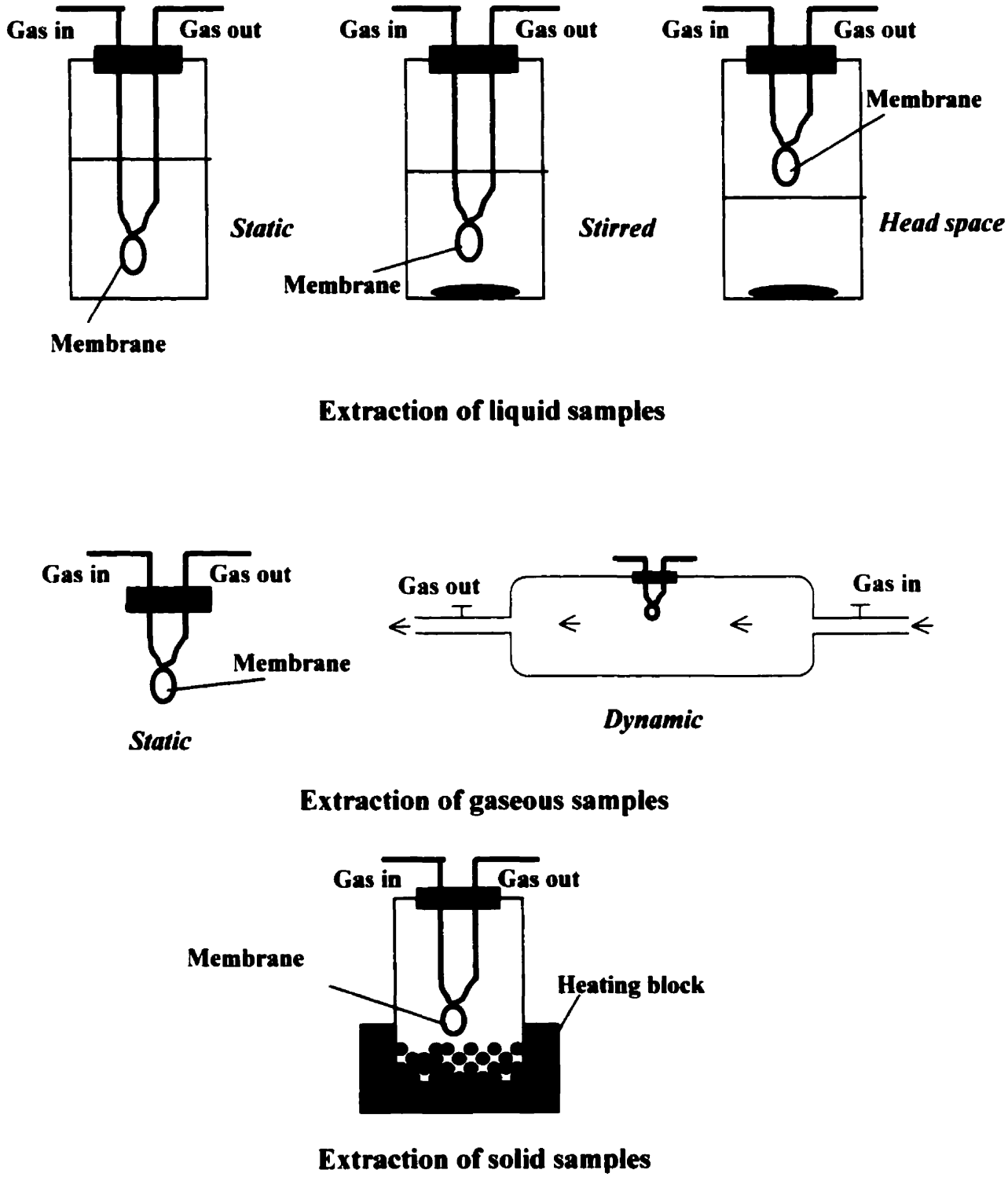

Figure 2- 2. Membrane extraction from liquid, gaseous and solid samples

processes. The static mode is used mainly in environmental monitoring and implies the exposure of the membrane to the gaseous sample, without forcing the sample to flow across the membrane.

Solid samples can also be analyzed for volatile organic compounds by means of membrane extraction. The samples can be analyzed either at room temperature, or they can be heated in order to facilitate the release of VOCs.

In a membrane extraction process, sampling becomes extremely simple since it only involves the exposure of the membrane to the sample. Another big advantage is that the samples do not have to be placed in sampling containers, which enables on-site monitoring.

So far hollow fiber membranes have been used in MESI applications. However, because they are self-supported, they are thicker than the flat sheet membranes. A big concern related to the thickness of the membrane is the memory effect. Some analytes remain trapped in the walls of the membrane, after the sampling process has ended (the membrane is no longer exposed to the sample). These analytes escape the membrane later on, giving an analytical response. This process is called "memory effect" and it is proportional to the thickness of the membrane. The thicker the membrane, the more analytes get trapped in the walls and the longer it will take to eliminate them. Another implication of the wall thickness is the response time of the membrane. The thinner the membrane wall, the faster the analytes reach the detector. This is particularly important for continuous monitoring processes because it dictates the rapidity with which the membrane can respond to variations in the concentration of analytes in the sample that is being monitored.

In order to eliminate some of these inconveniences, flat sheet membranes can be used. The fact that they are not self supported, and thus do not have to be that robust, gives the manufacturers flexibility in terms of membrane materials. This is why the variety of the

commercially available flat sheet membranes is much larger than the variety of hollow fiber membranes.

2.3 Design of the membrane module for flat sheet membranes

Flat sheet membranes need to be placed in a supporting module in order to be connected to the carrier gas line. Such a module should be constructed from an inert material that does not react with, or adsorb, analytes.

The membrane extraction process involves the following steps: 1) diffusion of

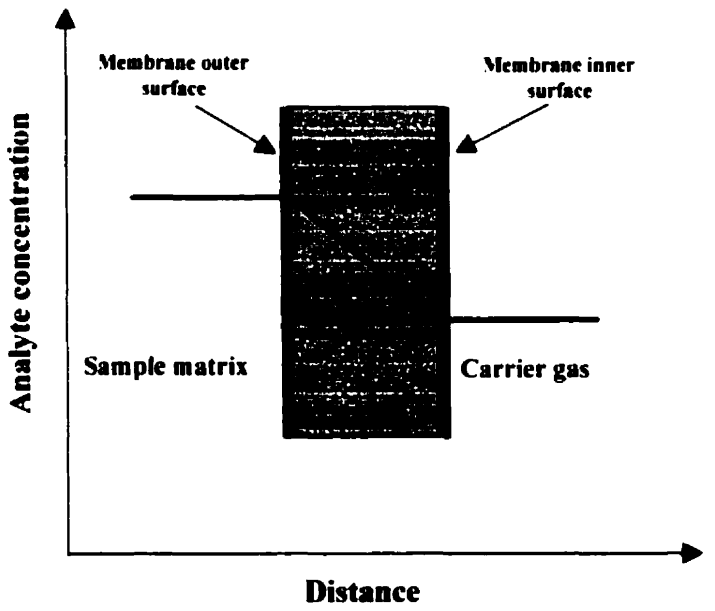

Figure 2- 3. Schematic representation of the membrane extraction process

analytes from the bulk solution to the membrane surface; 2) once they have reached the membrane, the analytes firstly dissolve in the polymeric material and then diffuse through it under a concentration gradient; 3) after reaching the inner wall of the membrane, they get stripped by the carrier gas. The process is schematically illustrated in Figure 2- 3.

Good agitation can be applied on both sides of the membrane. On the sample side, stirring can be applied for liquid samples, or dynamic extraction can be performed if gaseous samples are analyzed. A fast turbulent stripping gas flow can be applied on the inner side of the membrane. Assuming that the agitation is perfect, the flux of analytes across the membrane can be estimated using Fick's first law of diffusion:

$$F = -D\frac{\partial C}{\partial x} \qquad \textbf{Equation 1}$$

where F is the flux, D is the diffusion coefficient of the analyte in the membrane, and $\partial C/\partial x$ is the concentration gradient of the analyte across the membrane wall. The flux can be expressed as the number of moles of analyte "m" passing through the membrane wall per unit time "t", per unit area "A":

$$F = \frac{m}{At} \qquad \textbf{Equation 2}$$

By assuming that:

- the concentration of the analytes in the sample is equal to the concentration of the analytes on the outer side of the membrane ($C = C_O$)
- the flow stripping process is very fast (the concentration on the inner side of the membrane is $Ci = 0$)
- the concentration profile inside the membrane wall is linear.

the concentration gradient can be expressed by:

$$\frac{\partial C}{\partial x} = \frac{Co - Ci}{b} = \frac{KC}{b} \qquad \textbf{Equation 3}$$

where Co and Ci are the analyte concentrations on the outer and inner wall of the membrane. K is the distribution constant of the analyte between the membrane and the sample. C is the analyte concentration in the sample. and b is the membrane thickness. By combining the three equations. the analyte mass transfer through the membrane wall results:

$$\frac{m}{t} = D\frac{AKC}{b} \qquad \textbf{Equation 4}$$

It can be seen that the extraction rate at the membrane interface is directly proportional to the surface area of the membrane and inversely proportional to the membrane thickness.[8]

The assumption that the concentration of the analytes on the inner side of the membrane is Ci = 0 has also been made.

MESI uses membranes for sample introduction. However. when the membrane extraction module is being designed. the other parts of the system have to be taken into consideration. The Ci = 0 condition can be achieved by applying a very high gas stripping flow rate on the inner side of the membrane. However. the carrier gas flow rate is limited by two factors: the breakthrough of analytes in the sorbent trap and the chromatographic separation. Since the gas flow through the membrane module is the same as the gas flow through the sorbent trap and through the chromatographic column. a proper value has to be found in order to achieve the best sensitivity and analyte separation. A too high flow rate would cause a fast break through of the analytes through the sorbent trap. In such a case only

short trapping times could be used. Short trapping times imply lower preconcentration. A fast flow rate could also affect the separation of the compounds in a complex mixture. Thus, depending on the nature of the analytes, the matrix in which they are present and their concentration, the optimum flow rate has to be found.

Another solution for the achievement of the Ci = 0 condition is the variation of the inside volume of the membrane module. If the inside volume is very small the carrier gas can replace it very rapidly, and it can be considered that the analytes will not accumulate on this side of the membrane. In such a case, the concentration of analytes on the inner side of the membrane can be considered to be close to zero.

Taking into consideration all these factors, a membrane module has been designed.

Figure 2- 4 presents a schematic description of the membrane module.

The body of the module is made out of stainless steel, and it has a sandwich-like design. In between the two stainless steel pieces, a Teflon washer is inserted. Teflon is relatively inert, and, because it is soft, it also helps to seal the membrane without the need of an o-ring. Carrier gas was supplied through a small diameter Teflon tubing, fitting tightly into the Teflon washer. There was no need to use glue or other sealants.

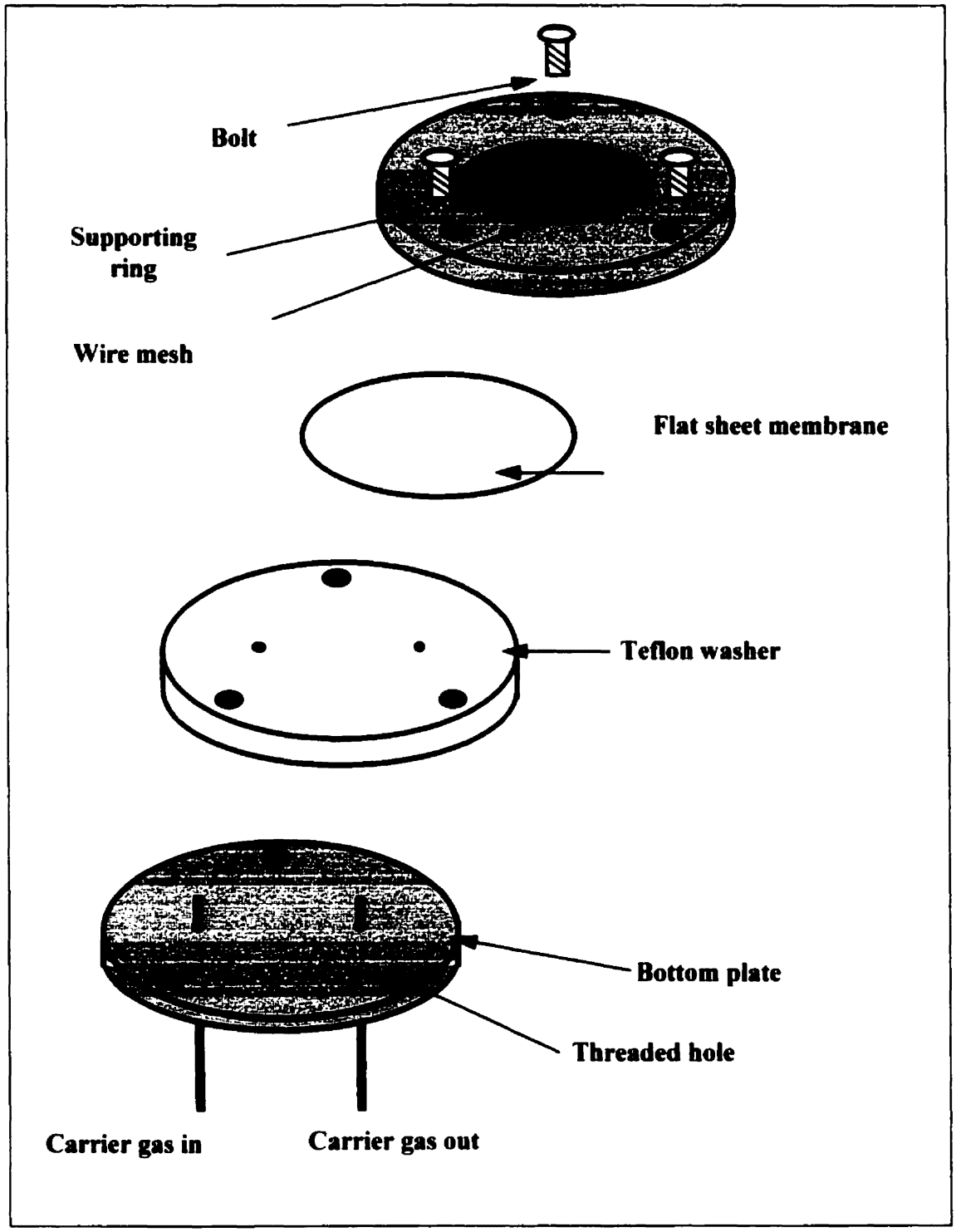

Figure 2- 4. Flat sheet membrane module

Due to the very small dead volume of the module, high pressure is applied on the membrane when the carrier gas flows along its inner side. As a consequence, if the membrane is not supported, it can expand (the silicone membrane is elastic) and eventually break. To prevent this, a very fine stainless steel wire mesh has been spot welded to the supporting ring of the module. The wire mesh and the supporting ring form an even surface.

The supporting ring was screwed to the bottom plate of the module using three bolts. This way, the membrane was pressed against the Teflon washer and could be sealed into the module without using an o-ring.

The round design was chosen at the beginning because it was the easiest one to machine. It was used to obtain preliminary results and understand how the membrane can be sealed inside the module, and how the carrier gas lines can be attached to the module. Once the module was leak free and it proved to work for the extraction of VOCs, improvements were made.

The main concern about the initial design was the flow path of the carrier gas inside the module. The flow profile of the carrier gas inside the round module is illustrated in Figure 2-5. A thin layer of carrier gas next to the walls of the membrane module does not move at all. The speed of the gas increases with distance from the walls of the module.[9] Because of that, the extraction efficiency is different in different parts of the membrane module, the extraction becoming more efficient along the median line of the module. The increasingly thicker arrows suggest the more efficient stripping flow towards the center of the membrane module.

The uneven flow profile affects the performance of the membrane module not only during the extraction step, but also after it. When the membrane is removed from the sampling site, it is desirable that the membrane frees from any traces of analytes in as short a time as possible. However, the part of the membrane that is inefficiently flushed by the carrier

gas releases the analytes at a much slower rate than the rest of the membrane. This can significantly affect the memory effect introduced by the membrane module, making it more pronounced.

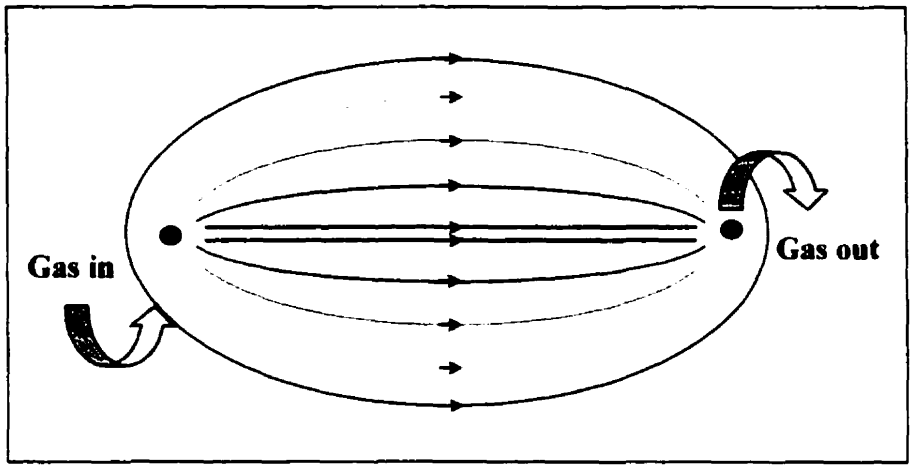

Figure 2- 5. Flow profile in the round membrane module

One way of minimizing the memory effect of the membrane, is by making the opening of the membrane module longer and narrower. The flow profile in such a module can be seen in Figure 2- 6.

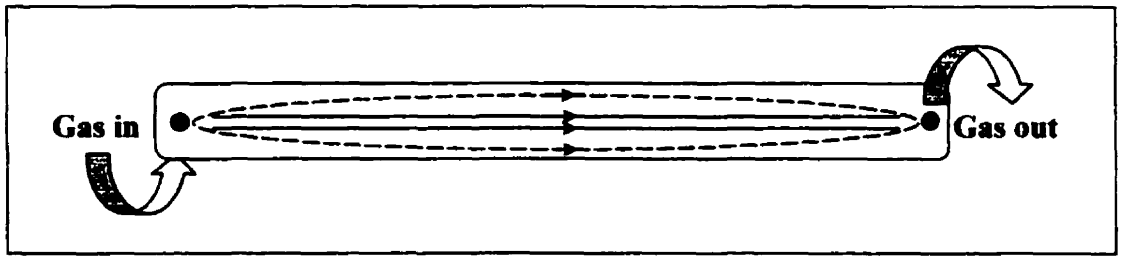

Figure 2- 6. Flow profile in a narrow membrane module

Such a design would provide a more uniform stripping flow. However, the module would have to be significantly longer in order to provide the same extraction area for the membrane. This might be inconvenient sometimes because the membrane could not be placed

in short sampling containers. In the round design, the exposed area of the membrane had a diameter of 2.5 cm, making an area of 4.9 cm^2 available for analyte extraction. The new design was 0.5 cm wide. For this width, the length of the module should have been 9.8 cm to provide the same area. However, a more convenient length has been chosen (3 cm), to enable extraction from different containers.

2.4 Characterization of the performance of the membrane module

As mentioned before, the response time and the memory of the membrane are very important parameters of the system.

The faster the analytes can pass through the membrane wall, the faster they can reach the sorbent interface. As a consequence, a higher amount of analytes get to be preconcentrated in a certain time interval, increasing the sensitivity of the method.

Another consequence is the rapidity with which the system can respond to variations in the concentration of the analytes in the monitored sample. If the system has a slow response time, the variations can be averaged out and an accurate concentration profile can not be obtained.

The main factors influencing the response time and memory of the membrane module are the membrane thickness and the carrier gas flow. As mentioned before, a compromise has to be made when choosing the flow rate. When a slow flow rate is chosen, a smaller amount of analytes reach the sorbent interface in a certain period of time, and the sensitivity is decreased. When a high flow rate is chosen, a higher amount of analytes reach the membrane in the same period of time, but the trapping time can not be too long, because the analytes would break through the trap, increasing the level of the baseline. Another very important

factor should not be forgotten: the MESI system uses a gas chromatograph for the separation of analytes. The flow rate of the stripping gas has to be optimal for the separation of the compounds in the studied mixture. Thus, usually the chosen flow rate is the highest one with which a good separation can be obtained. This way a high amount of analytes permeate the membrane and the analysis time is as short as possible.

The other very important parameters are the thickness of the membrane and the membrane material. The analytes cross the membrane wall via diffusion, which is a slow process since the silicone membranes are liquids. The shorter the distance the analytes have to travel to reach the carrier gas, the larger is the amount getting trapped at the sorbent interface in a defined period of time. Thus, the sensitivity is directly related to the thickness of the membrane.

When the analytes reach the surface of the membrane, they firstly have to dissolve into the polymeric material, and then they start diffusing through it. Nonpolar compounds will easily dissolve into nonpolar membranes, and polar compounds into polar membranes. This is why the membranes act as selective barriers, being able to discriminate against some of the analytes in the matrix.

Water is present in different amounts in most of the real samples. Thus, in the case of MESI, mostly nonpolar membranes are being used, because a high amount of water should not enter the chromatographic column (oxygen can destroy the stationary phase of the column). However, if the analysis of polar compounds is required, a more polar membrane can be used at the expense of the column.

2.4.1 Response time and memory effect

In order to evaluate the performance of the flat sheet membrane module, the response time and memory effect have been investigated.

Experimental setup

A flat-sheet Silicone Polycarbonate Membrane (SSP-M213), of 25 μm thickness, purchased from Membrane Components (Ballston Spa, NY, USA) was used. This membrane material has been chosen because it is less permeable to oxygen than Poly(dimethylsiloxane). XAD-2 resin (Polystyrene divinyl benzene) purchased from Supelco (Oakville, ON, Canada) was used as packing material for the sorbent trap. For the separation of the compounds, a CP-9002 gas chromatograph (Chrompack, Middelburg, The Netherlands) was used. The GC was equipped with a flame ionization detector (FID) and a 10 m, 0.25 mm i.d., 0.15 μm film thickness CP Sil 5 CB column (Chrompack). BTEX (benzene, toluene, ethylbenzene, and xylenes) compounds of 99+% purity were purchased from Aldrich Chemical Company, Inc. For the connection of the membrane module to the rest of the system deactivated stainless-steel tubing (MXT guard column) and Valco connectors obtained from Chromatographic Specialties Inc. were used. Ultra high purity nitrogen, helium, hydrogen and air required for the FID and the chromatographic column were purchased from Praxair (Kitchener, ON). A three stage Peltier (Melcor Corp., Trenton, NJ) cooler was used to decrease the temperature of the sorbent trap. A custom-made power supply containing a computer-grade 16000 μF/50 V capacitor was used to supply the required voltage for compounds desorption. The trapping time was controlled using a H3CR-F external timer (OMRON Corporation, Kyoto, Japan).

Isothermal separation at 65°C was performed. The flow rate was set at 1.3 mL/min, and the carrier gas was helium. A desorption voltage of 47 V was applied at the sorbent interface.

Four individual standard stock solutions were prepared. 91 μL benzene (B), ethylbenzene (E) and xylene (X), and 93 μL toluene (T) were added to methanol to obtain a final volume of 100 mL for each stock solution.

The sampling jar had a volume of 120 mL. By placing 40 mL water into the jar and spiking it with 5 μL of the stock solution, a solution of 100 parts per billion (ppb) was obtained for each individual compound.

The membrane was placed into the vial containing the deionized water. A 5 cm magnetic stir bar was used to agitate the sample at a rate of 500 rotations per minute. After the desired stirring rate was achieved, the water was spiked with the stock solution. Desorption pulses were applied at the sorbent interface every minute (a trapping time of one minute was used).

For the determination of the response time of the membrane module, the chromatographic run started at the time the first desorption pulse was applied.

For the determination of the memory effect of the membrane, the same procedure was followed as for the response time experiment. After a steady-state was reached (a peak maximum was obtained), the membrane was taken out of the jar and exposed to clean air. Desorption pulses were applied every minute and the chromatogram was recorded.

Results and discussions

The response time of the membrane to toluene is illustrated in Figure 2- 7. It can be seen that steady-state was reached after the third desorption. The profile looks similar for all four compounds, and a comparison diagram is illustrated in Figure 2- 8.

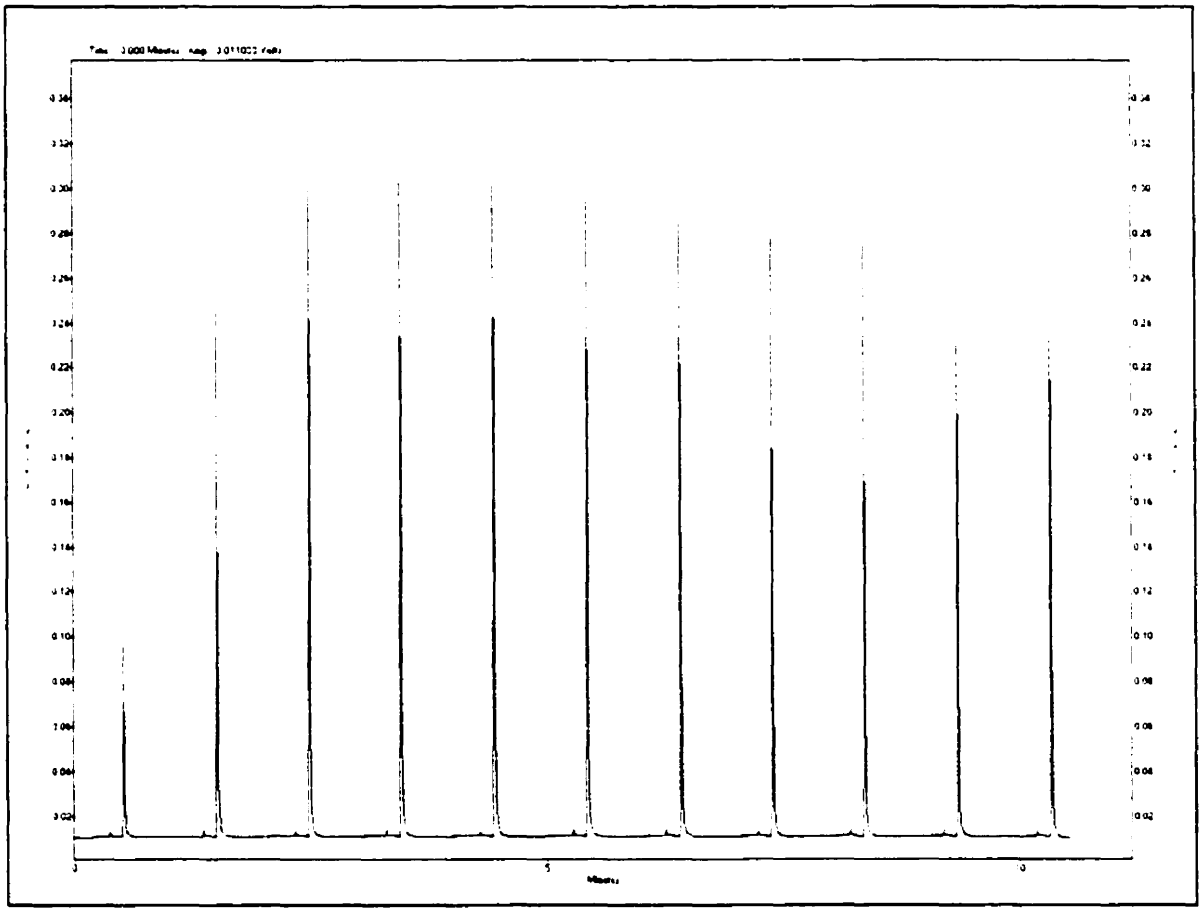

Figure 2- 7. Chromatogram obtained for the extraction of toluene

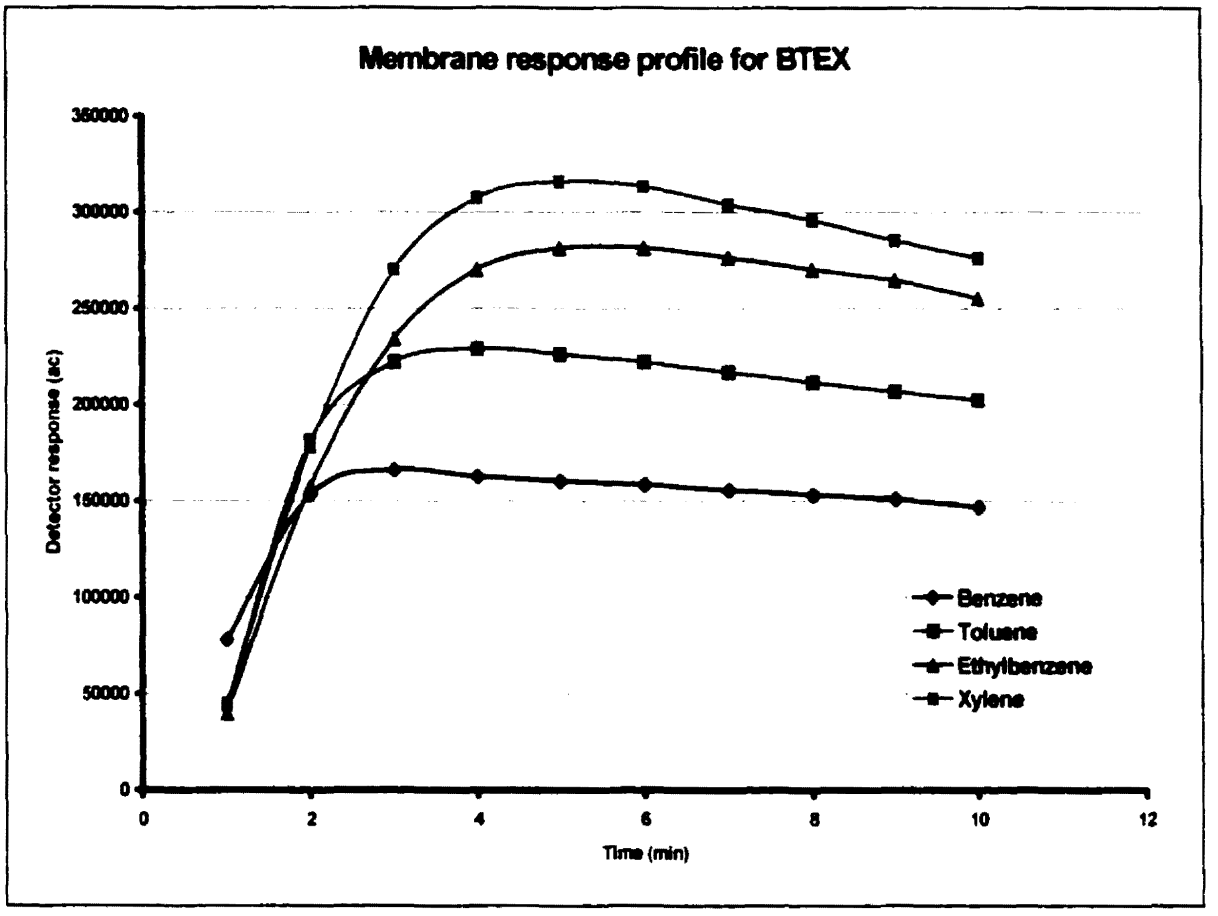

Figure 2- 8. Membrane response profile for BTEX

The peak produced by the first desorption was smaller for all four compounds due to the fact that the equilibrium was not achieved in the jar, at the time desorption occurred. After the analytes were introduced into the jar, they had to mix with the water and then partition in the headspace where they were extracted from. The analytes started to be extracted by the membrane before they could reach equilibrium with the headspace.

The compound that reached steady-state the fastest was benzene. In its case, a maximum extracted amount was obtained at the second desorption. Toluene reached steady-state after about three minutes, and ethylbenzene and xylene after about four minutes.

The memory effect introduced by the membrane for toluene extraction is illustrated in Figure 2- 9.

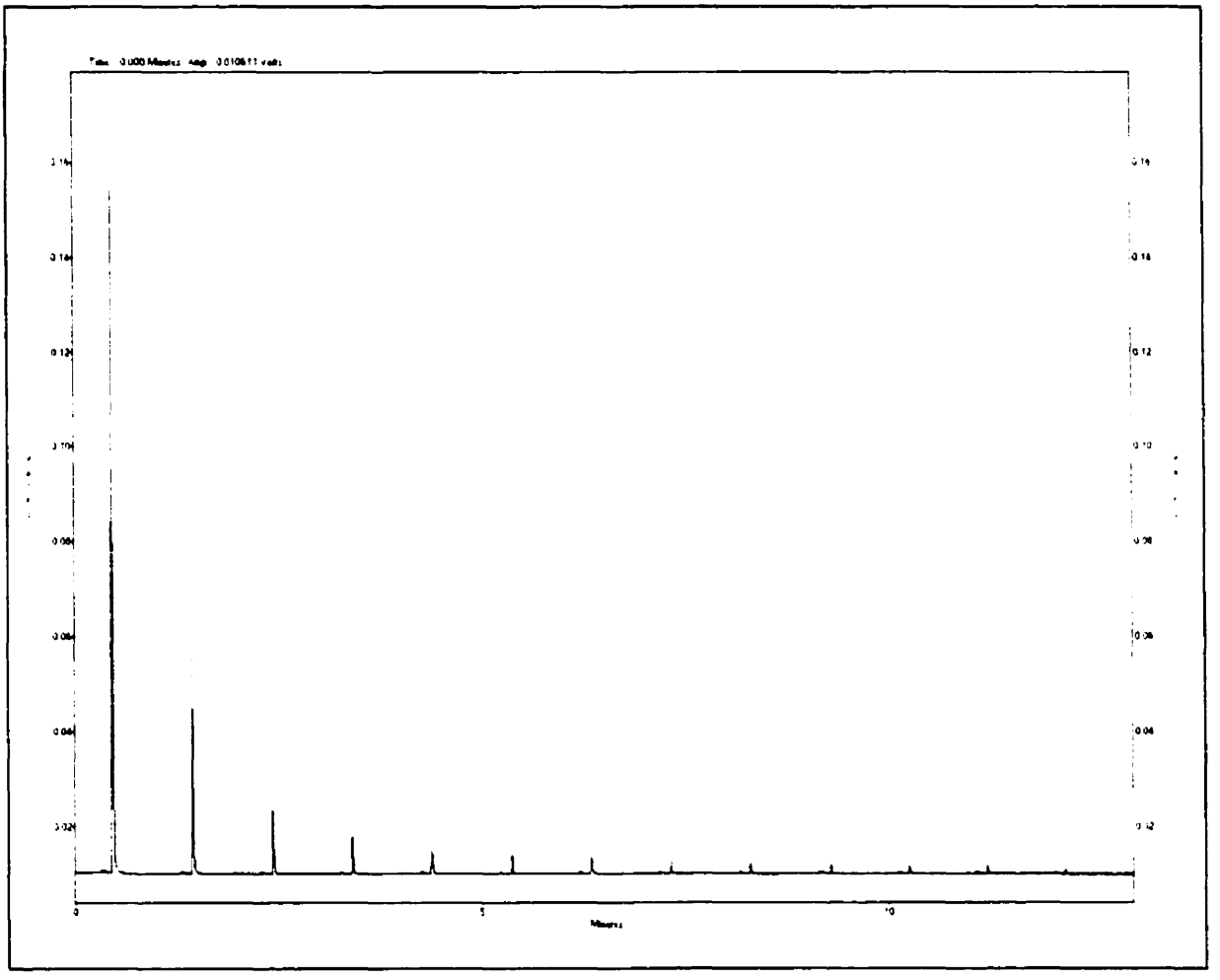

Figure 2- 9. Memory effect introduced by the membrane after extraction of toluene

A comparison of the memory introduced by the membrane for the extraction of all four compounds is presented in Figure 2- 10.

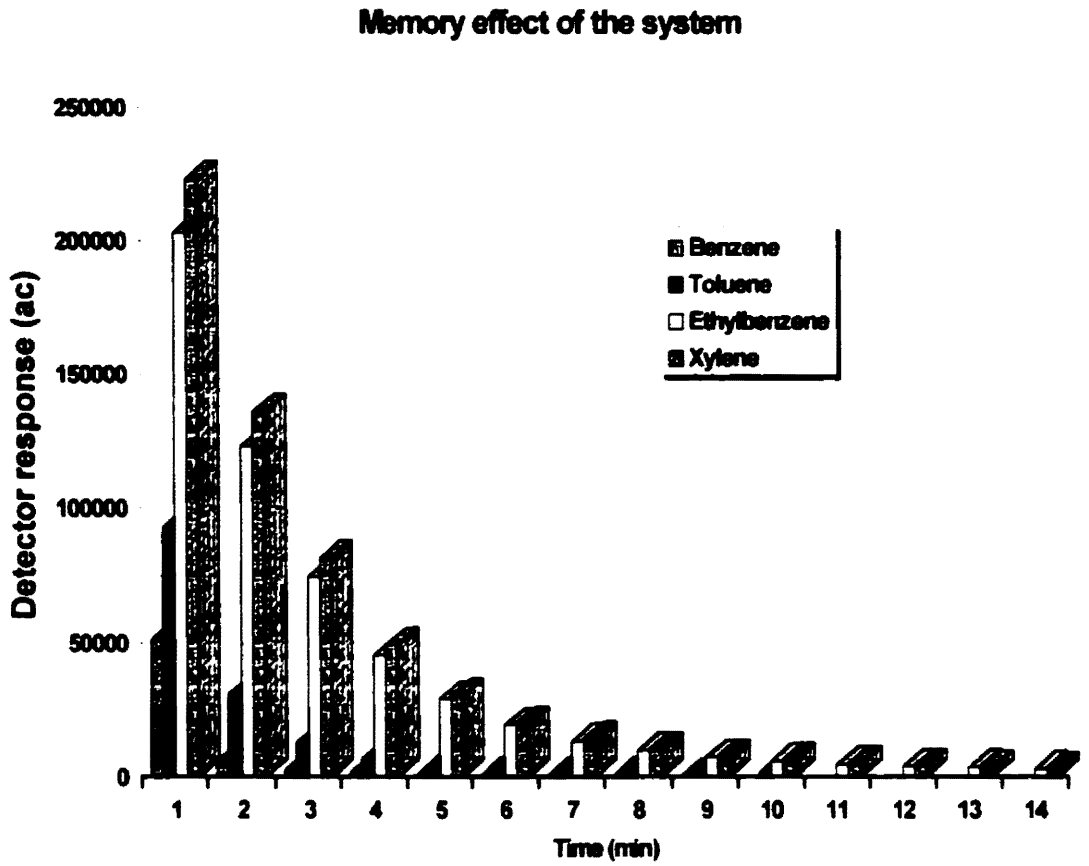

Figure 2- 10. Memory effect introduced by the membrane after BTEX extraction

When benzene was analyzed, the signal of the detector dropped to a value close to zero about 3 minutes after the membrane was taken out of the sampling jar. For toluene it took about 7 minutes, while for ethylbenzene and xylene approximately 14 minutes had to pass before the detector signal became negligible.

In MESI systems the membrane is the component responsible for most of the memory effect. However, the tubing that connects the membrane module to the sorbent trap can introduce some memory, even though deactivated stainless steel is used. Another source of

memory could be the trap carryover. In order to determine the source, each segment of the system had to be examined. To evaluate the memory introduced by the membrane module alone, the memory introduced by the rest of the system had to be evaluated.

Xylene was chosen for the experiment since it was the one that persisted in the system for the longest time after the membrane was removed from the sampling jar. The experimental setup was identical to the one described previously. The only difference was that after reaching steady-state, the membrane was disconnected from the system (by-passed). The Teflon tubing that conducts the carrier gas in and out of the membrane module is connected to two pieces of stainless steel tubing via two Valco connectors. The membrane was bypassed by opening these connections and reestablishing the gas flow through another connector body. The memory was measured again, by pulsing the sorbent trap every minute.

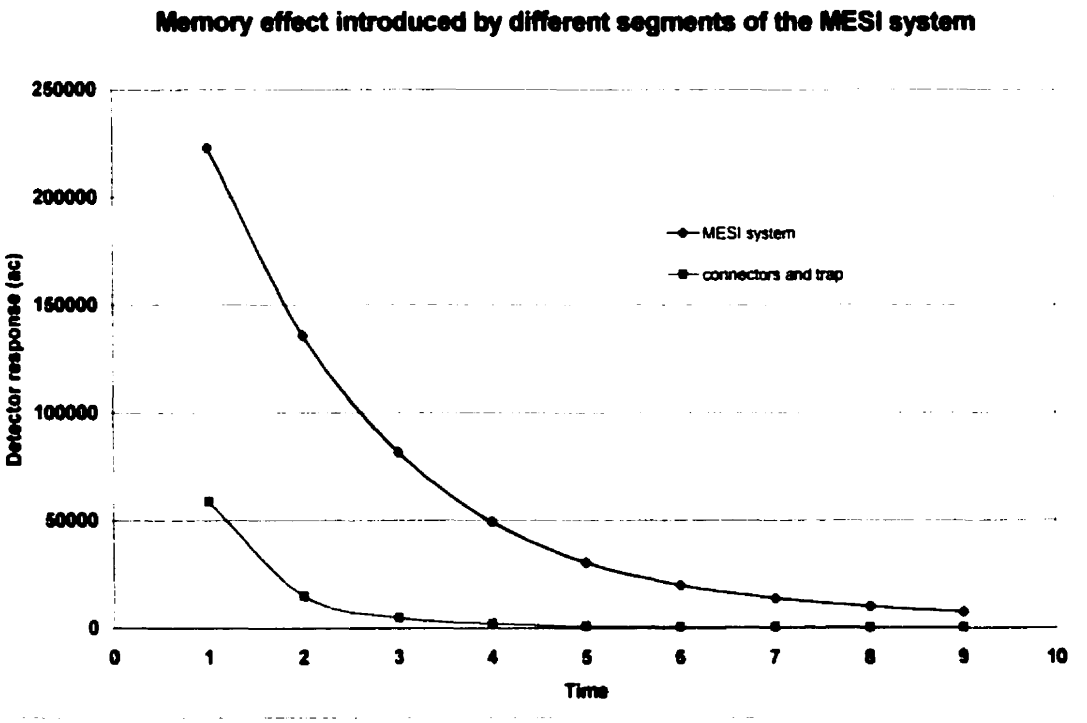

Figure 2- 11. Memory effect introduced by different segments of the MESI system

Figure 2- 11 illustrated the memory effect introduced by different segments of the MESI system. The upper trace represents the memory introduced by the membrane module, connectors, and the sorbent trap together. The lower trace was obtained for the memory introduced by the connectors and the sorbent trap. The memory of the membrane module can be obtained by subtracting the lower trace from the upper trace. For the analysis of xylene, the memory introduced by the membrane module is illustrated in Figure 2- 12.

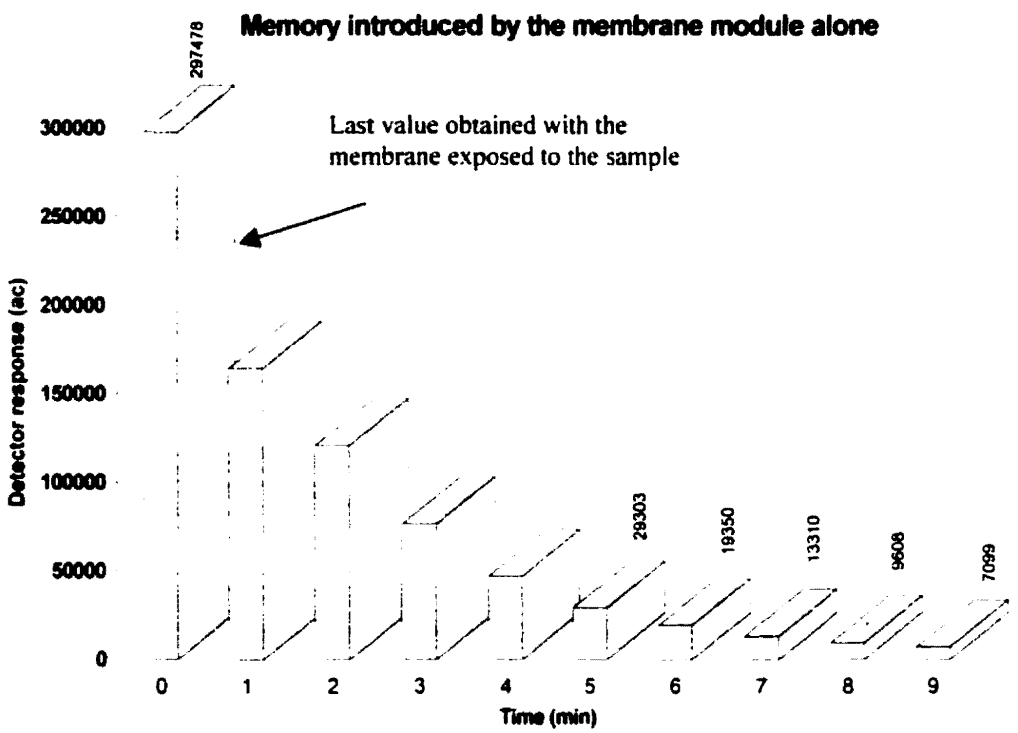

Figure 2- 12. Memory effect introduced by the membrane module

After five desorptions the analyte peak has a value equal to 9.8 % of the last value obtained with the membrane exposed to the sample, and after 9 minutes the peak area is still detectable (2.4 %).

It follows that the memory effect of the membrane is still significant for some compounds. For such analytes it would be difficult to monitor sharp variations of their concentration.

According to theory, the thin flat sheet membranes should have a faster response and a shorter memory than the hollow fiber membranes. However, the fact that the flat sheet membranes are not self-supported represents a big disadvantage. For MESI applications, the size of the active surface of the membrane is comparable to the one of the unexposed membrane. This unexposed part sits inside the membrane module and serves to seal the membrane inside the module. However, even though the carrier gas does not vent this part, it can trap analytes in it. The analytes can reach this unexposed part of the membrane by diffusion through the membrane wall. Because the carrier gas does not reach this part of the membrane, the analytes can escape from it only by diffusing back to the active part of the membrane, through the membrane wall. This process is very slow and it can significantly contribute to the memory of the membrane.

It follows that the design used for the membrane module should be improved in order to see a significant decrease in the memory. Also, depending on the application, hollow fiber membranes can be considered because it is much easier to use longer hollow fiber membranes and increase the extraction area to obtain better sensitivity, than to obtain it by building bigger supports for flat sheet membranes.

2.4.2 Evaluation of permeation through membranes of different material and different thickness

According to theory, the amount of analytes extracted by the membrane per unit time is directly proportional to the membrane surface and inversely proportional to the membrane thickness. Another very important factor for the permeation rate is the membrane material.

Two membrane materials were examined. One of the membrane materials was poly (dimethylsiloxane) (PDMS) and the other was silicone polycarbonate. Of the commercially available flat sheet membranes, the two materials were chosen because they were very permeable for our target analytes, and less permeable for water. The silicone polycarbonate membrane was easier to work with because it did not stretch like the silicone membrane. In the case of the silicone membrane, the thinner the membrane was, the harder it was to place it in the membrane module because it rolled as soon as it was taken off the manufacturer's support. It also stretched very much and that made it very hard to determine the real thickness.

According to the manufacturer, the silicone polycarbonate membrane is less permeable to oxygen and water. It is also easier to assemble into the membrane module. There were no data available from the manufacturer to indicate the permeation rates of organic compounds through the two membrane materials. Membranes of different thickness were purchased and evaluated.

Experimental setup

For the extraction, Silicone Membrane (SSP-M100) and Silicone Polycarbonate Membrane (SSP-M213) purchased from Membrane Components (Ballston Spa, NY, USA) were used. XAD-2 resin (Polystyrene divinyl benzene) purchased from Supelco (Oakville, ON, Canada) was used as packing material for the sorbent trap. For the separation of the compounds, a CP-9002 gas chromatograph (Chrompack, Middelburg, The Netherlands) was used. The GC was equipped with a flame ionization detector (FID) and a 10 m, 0.25 mm i.d., 0.15 μm film thickness CP Sil 5 CB column (Chrompack). Toluene and xylene of 99+% purity were purchased from Aldrich Chemical Company, Inc. For the connection of the membrane module to the rest of the system, deactivated stainless-steel tubing (MXT guard column) and Valco connectors obtained from Chromatographic Specialties Inc. were used. Ultra high purity nitrogen, helium, hydrogen and air required for the FID and the chromatographic column were purchased from Praxair (Kitchener, ON). A three stage Peltier cooler (Melcor Corp., Trenton, NJ) was used to decrease the temperature of the sorbent trap.

A standard gas generator was used to deliver the standard gas mixture that served as sample for the experiments. A schematic diagram of the standard gas generator is presented in Figure 2- 13.

A syringe pump purchased from Sage Syringe Instruments was used to deliver the analyte mixture. The 100 μL gas tight syringe used with the syringe pump was purchased form Supelco. A very fine metering valve (Swagelock, ON) was used to control the flow of the diluting gas. Copper tubing and copper and stainless steel connectors (Swagelock, ON) were used to connect the parts of the generator. They were thoroughly cleaned with solvent, allowed to dry and then flamed.

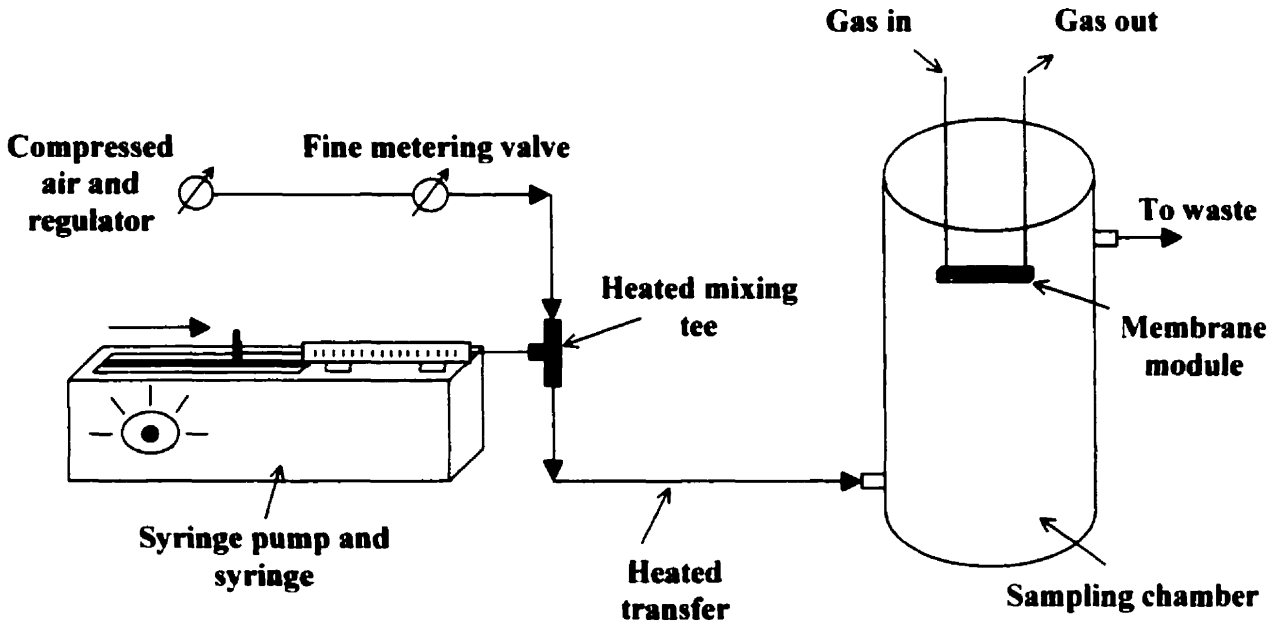

Figure 2- 13. Schematic representation of the standard gas generating device

High purity compressed air (Praxair, Kitchener, ON) was used as diluting gas. The tee where the analyte mixture combined with the air was heated using heating tape. The same heating tape was used on the transfer line that conducted the analyte gas mixture to the sampling chamber.

The delivery rate of the syringe pump was calibrated using a mixture of benzene, toluene, trichloroethylene and tetrachloroethylene in methanol. The mixture contained 11.9862 g methanol, 24.1 mg benzene, 23.5 mg toluene, 25.5 mg trichloroethylene and 25.7 mg tetrachloroethylene. The mixture was placed in the 100 μL gas tight syringe, and the amount delivered by the syringe pump (the speed adjusted for the lowest delivery rate) in 12,

24, 48 and 72 hours was collected into 2 mL vials and weighed. The delivery rate proved to be constant in time, and the calibration is presented in Figure 2- 14.

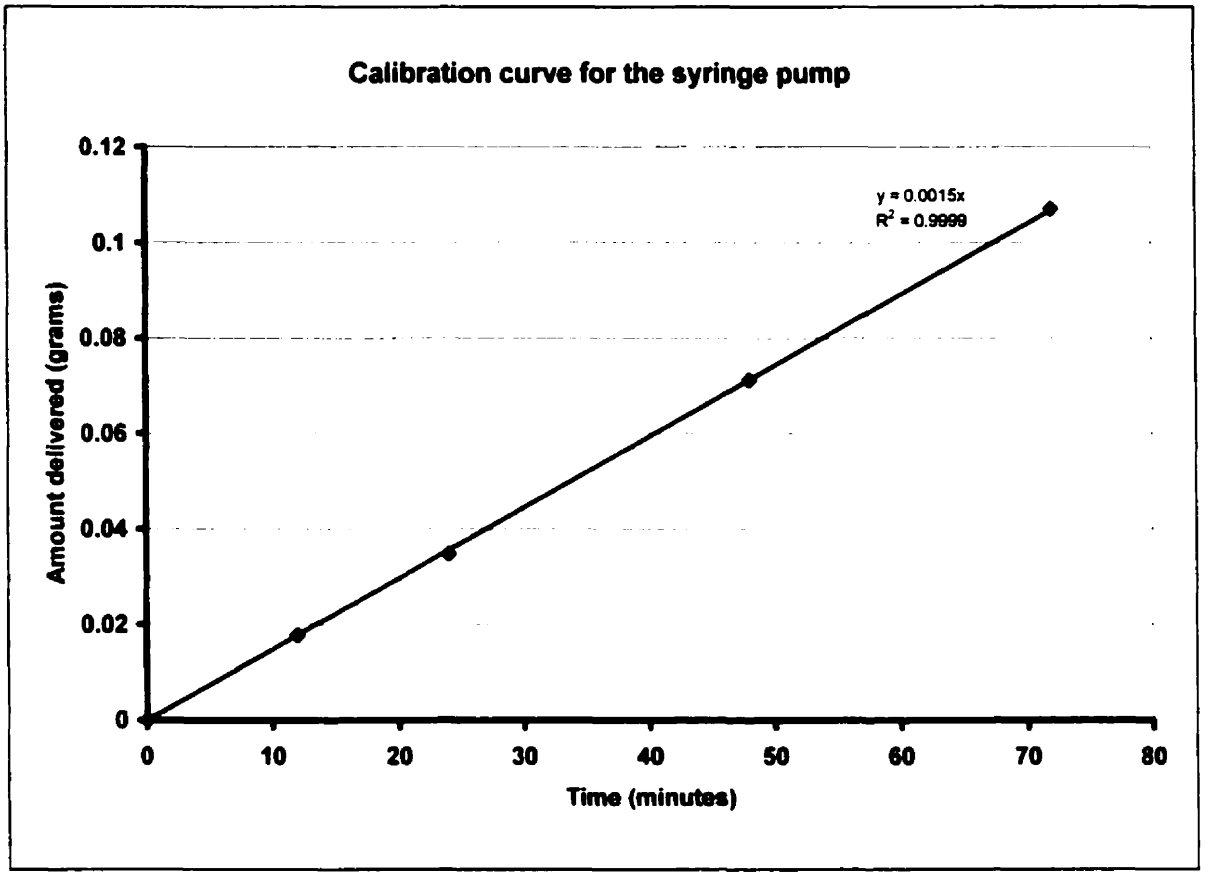

Figure 2- 14. Calibration curve for the syringe pump

The amount of mixture delivered in one hour was 1.478 mg.

For the experiments in which the evaluation of the permeation through the membrane was performed, a mixture of toluene and xylene was used. The mixture contained 3.8820 g of toluene and 3.8706 g of xylene. The air flow rate was 500 mL / min.

Isothermal separation of the two compounds at 70 °C was performed. The carrier gas used in the gas chromatograph was helium, and it was set for a flow rate of 1.4 mL / min.

The sorbent trap was desorbed using 50 volt pulses.

Results and discussions

For the evaluation of the permeation through membranes of the same material and different thickness, silicone polycarbonate membranes with thickness of 32 μm and 25 μm, and silicone membranes of 50 μm and 55 μm were used.

It was the initial intention to use 16, 32, and 50 μm silicone polycarbonate membranes and 25, 50, and 100 μm silicone membranes for the experiment. The membranes were purchased from the manufacturer and were used to obtain preliminary results. However, the results indicated that the membranes actually were of different thickness than the ones reported by the manufacturer. Approximate thickness values were obtained by measuring the membranes in the Science Shop of the University.

The thinnest available membrane was made out of a silicone polycarbonate and it had a thickness of approximately 25 μm. For the silicone membrane, the thickness varied very little.

The membranes were sealed into the membrane module. The membrane module was placed into the sampling chamber of the standard gas generator. A 0.5 cm wide and 3 cm long membrane area was exposed to the gas mixture. Trapping times of 1, 2, 4 and 8 minutes were used. The membrane was never taken out of the sampling chamber, so it was always at steady-state.

The results obtained for toluene extraction with the two silicone polycarbonate membranes are illustrated in Figure 2- 15. The ones obtained with the two silicone membranes are presented in Figure 2- 16.

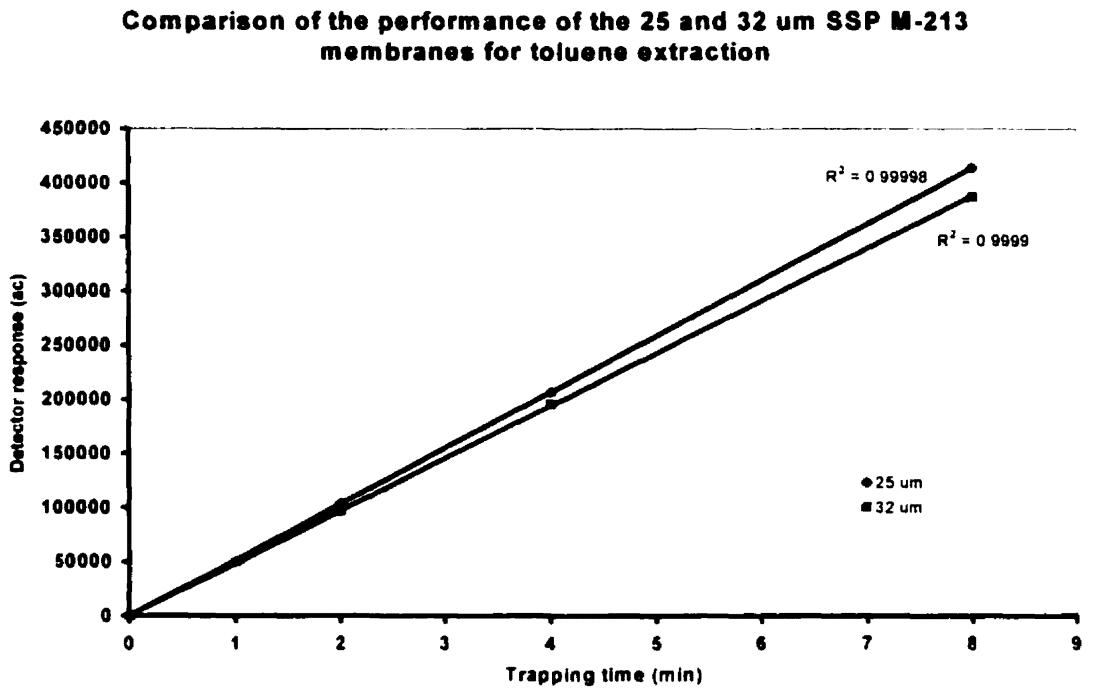

Figure 2- 15. Toluene extraction by 25 and 32 μm silicone polycarbonate membrane

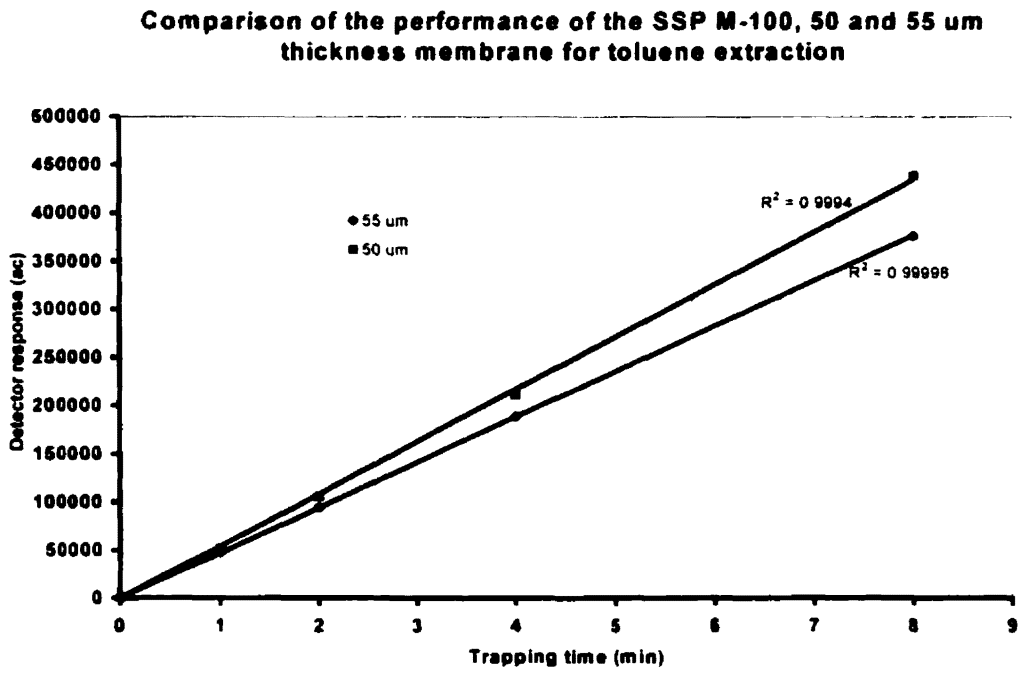

Figure 2- 16. Toluene extraction by 50 and 55 μm silicone membrane

As predicted by theory, for both membrane materials the thinner membrane allows for more analyte extraction per unit time. A difference in the amount extracted can be noticed even for a small difference in thickness (5 μm for the silicone membrane).

In practice, if the permeation rate through a membrane material is known, the thickness of a membrane of the same material can be determined very precisely by measuring the permeation of a compound through it. The commercially available membranes are measured for the determination of the thickness because permeation measurements with conventional methods are very time consuming (days or even weeks are spent for such determination). The measurements are not accurate, so the values offered by the manufacturers are not reliable.

Because silicone and silicone polycarbonate membranes of the same thickness were

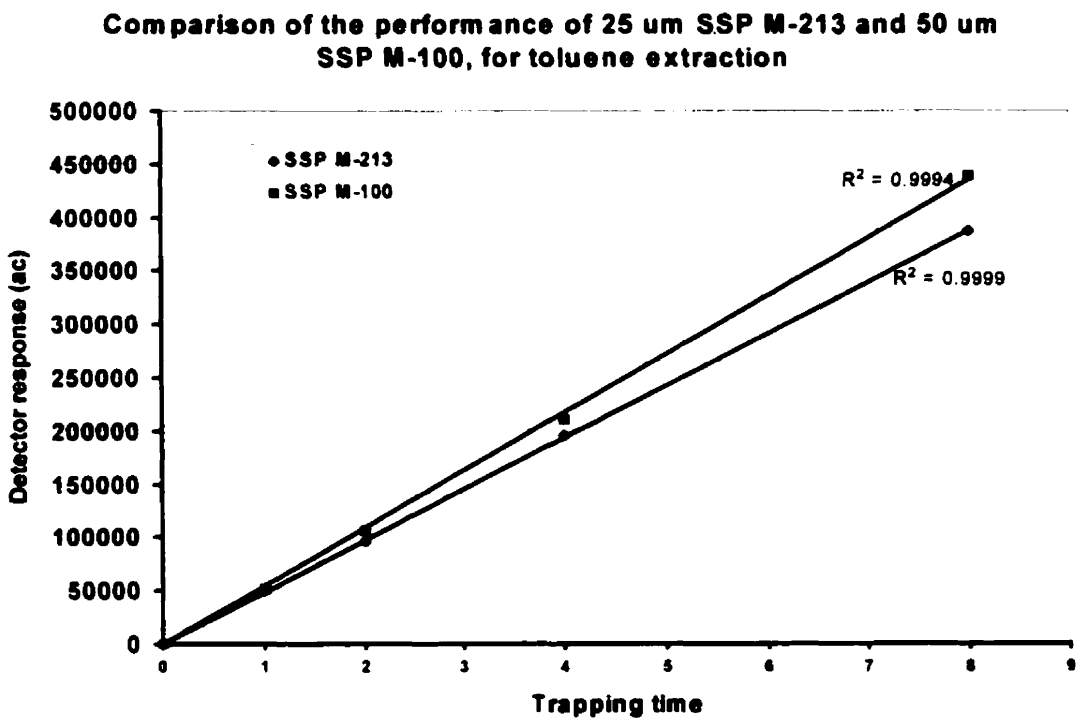

Figure 2- 17. Toluene permeation through membranes of different materials

not available. 25 μm silicone polycarbonate and 50 μm silicone membranes were used for the comparison of the performance of the two membrane materials. The results are presented in Figure 2- 17.

As expected, the silicone membrane proved to be more permeable than the silicone polycarbonate one. Even though the thickness of the SSP-M213 membrane was half the thickness of the SSP-M100 one, the permeation through the silicone membrane was higher.

The silicone membrane is more permeable for oxygen and also for water. Besides the risk of damaging the chromatographic column in a short period of time, the high permeation of water through the membrane can compromise the analysis if certain detectors are used. For example, the flame of the flame ionization detectors could be extinguished by high amounts of water; thermal conductivity detectors respond to water, and thus the peak of water could interfere with the peaks of the target analytes. In such cases the silicone polycarbonate membrane would be more useful.

The results obtained for the extraction of xylene are summarized in Table 2- 1.

SSP-M213 **Silicone polycarbonate membrane**			**SSP-M100** **Silicone membrane**		
	25 μm ***(Peak area counts)***	***32 μm*** ***(Peak area counts)***		***50 μm*** ***(Peak area counts)***	***55 μm*** ***(Peak area counts)***
1 min	50449 ± 190	467701 ± 252	***1 min***	52922 ± 356	48945 ± 176
2 min	103080 ± 516	95260 ± 420	***2 min***	109321 ± 1058	97879 ± 332
4 min	206201 ± 2266	189580 ± 1948	***4 min***	202346 ± 2728	189674 ± 1363
8 min	404128 ± 2814	375906 ± 4135	***8 min***	425544 ± 4486	384072 ± 9366

Table 2- 1. Comparison of xylene extraction using different membranes

2.5 Summary

Hollow fiber membranes are self supported and easily connected to the carrier gas line. However, they have thick walls making the response time and memory effect more pronounced, as demonstrated by longer decay times. The flat sheet membranes have thin walls, and because they do not need to be self-supported, the variety of materials they can be made out of is much larger. However, in order to be connected to the carrier gas line, they have to be placed into special holders. A part of the membrane is used to seal the membrane into the holder. This part is not exposed to the sample and the carrier gas does not flush it either. The analytes that diffuse to this area get trapped there for a longer time. This contributes to an increased memory effect. In cases in which the active area of the membrane is considerably larger than the unexposed area of the membrane this effect becomes less important. In such a case, the amount of analytes extracted by the membrane is much higher than the amount released from the unexposed membrane area. Thus, when changes in the concentration of the analytes in the monitored sample occur, they can still be seen, because the signal due to memory effect is less pronounced.

As a conclusion, considering the present design of the membrane module, the advantages of using flat sheet membranes are not exploited to their full potential. Hollow fiber membranes (average thickness of 300 μm) can be successfully used in applications in which the memory is not a big issue.

It is also very important to know that the membrane material that most efficiently keeps the water and oxygen away from both the chromatographic column and the detector is not as permeable for organic compounds as the pure silicone membrane. At the same time, the less permeable membrane can be manufactured in thinner sheets and it is much easier to

work with. The thinnest commercially available unsupported nonporous flat sheet membrane is the 16 μm silicone polycarbonate membrane. The thinnest silicone membrane available has a thickness of 25 μm. For this particular thickness, the silicone membrane would still be more permeable than the thinner silicone polycarbonate one.

Another point worth repeating is that the thickness reported by the manufacturers does not always correspond to the real values. As an application, MESI could be used to precisely determine them.

2.6 References

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CHAPTER 3

THE SORBENT INTERFACE

3.1 Introduction

The detection limit can be a significant limitation for an analytical method. Usually the detectors are not powerful enough to respond to the low concentrations that chemists are trying to analyze. This is why preconcentration is frequently used in analytical chemistry. Depending on the sample that is to be analyzed, different preconcentration methods can be used.

Liquid-liquid extraction (LLE) is a sample preparation technique that also preconcentrates the sample. In LLE an organic solvent is added to the sample; the sample is then agitated in a separatory funnel that separates the aqueous phase from the organic phase. The organic phase is then removed and concentrated by evaporation. Thus, LLE is a multi-step procedure that often results in loss of analytes during the process, frequently making the sample preparation the major source of errors in the analysis.[1]

Solid phase extraction (SPE) was developed in the 1980s, and has emerged as a powerful tool for chemical isolation and purification. From trace levels to industrial scales, SPE plays an important role in a broad range of applications. In SPE, the aqueous sample is passed through a column packed with a suitable stationary phase that selectively sorbs the analytes of interest. The target compounds are then flushed from the stationary phase with a

suitable solvent. The technique is limited to semivolatile or nonvolatile compounds.[2]

For the extraction of volatile compounds from liquid samples, the purge and trap technique can be used. The VOCs are extracted by purging the liquid sample with a gas. The analytes are then trapped and preconcentrated by using either a cryogenic or a sorbent trap.

More recently, solid phase microextraction (SPME) is used for sampling and preconcentrating VOCs in aqueous as well as gaseous samples. For the preconcentration of the analytes present in gases, the samples are pumped through special tubes packed with a suitable sorbent. The analytes of interest are trapped by the sorbent and they can later be desorbed by heating or by using small amount of solvents. However, most of the solvents used are expensive and toxic, and sample dilution occurs when using them.

Cryogenic trapping is also used sometimes. In cryogenic trapping the analytes are condensed onto cold surfaces, and later desorbed by heating. However, water condenses in these traps as well as the target analytes and that is why the use of water traps in front of the cryogenic traps is required. Analyte loss can be encountered when the sample is passed through the water traps. It is also very inconvenient to cool the traps to the required temperature, especially for field analysis.

3.2 The sorbent interface in MESI

In a MESI system the sorbent interface is the element that provides sample preconcentration and it also acts as injector for the gas chromatograph. It consists of a sorbent material that is placed into a piece of capillary tubing, a heating element and sometimes a cooling element.

The sorbent traps used so far in the MESI experiments were either pieces of gas chromatographic columns, or SPME fibers placed into fused silica capillaries (Figure 3- 1).

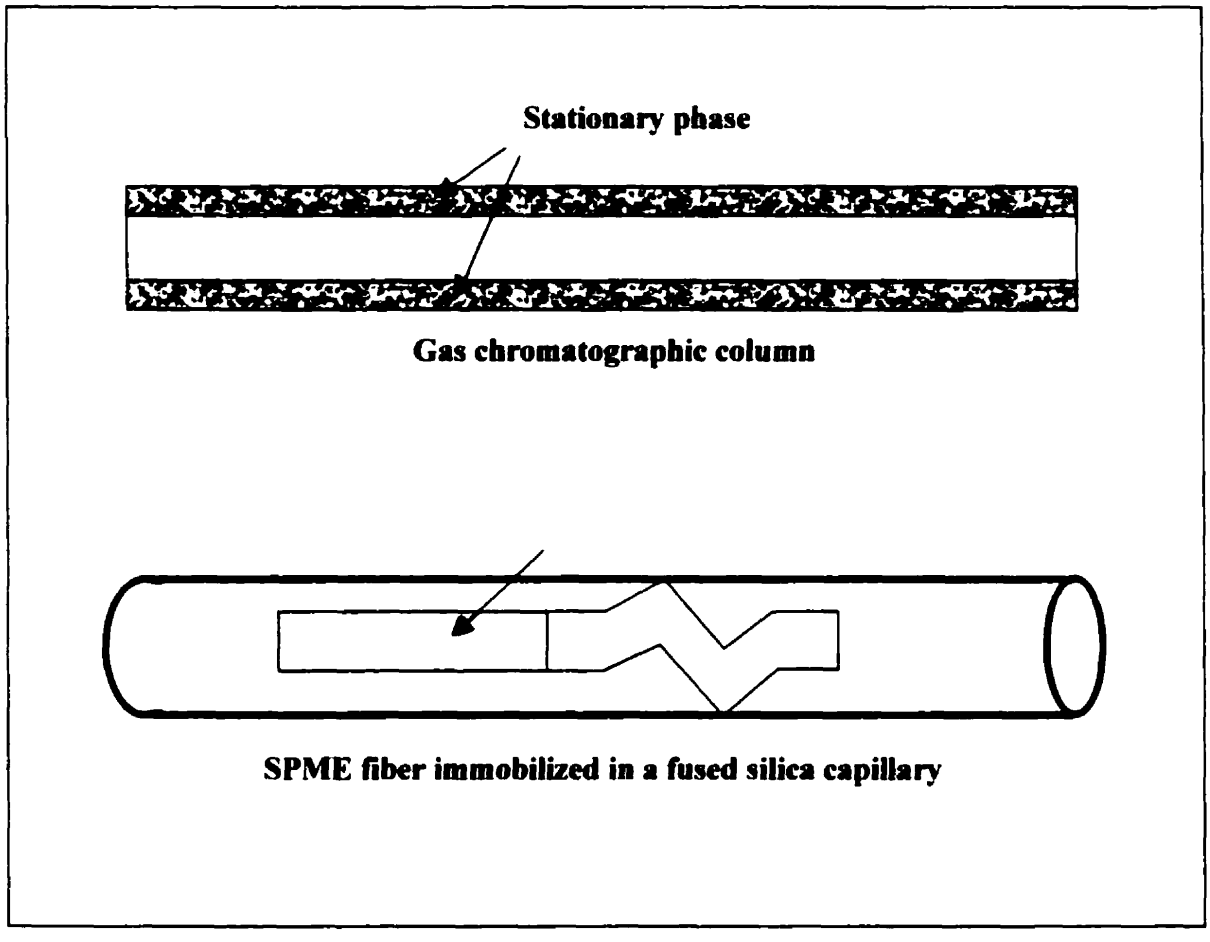


Figure 3- 1. The sorbent trap in MESI

A Ni - Cr wire wrapped tightly around the sorbent trap, covering the entire region where the sorbent was located, served as heating element (Figure 3- 2).

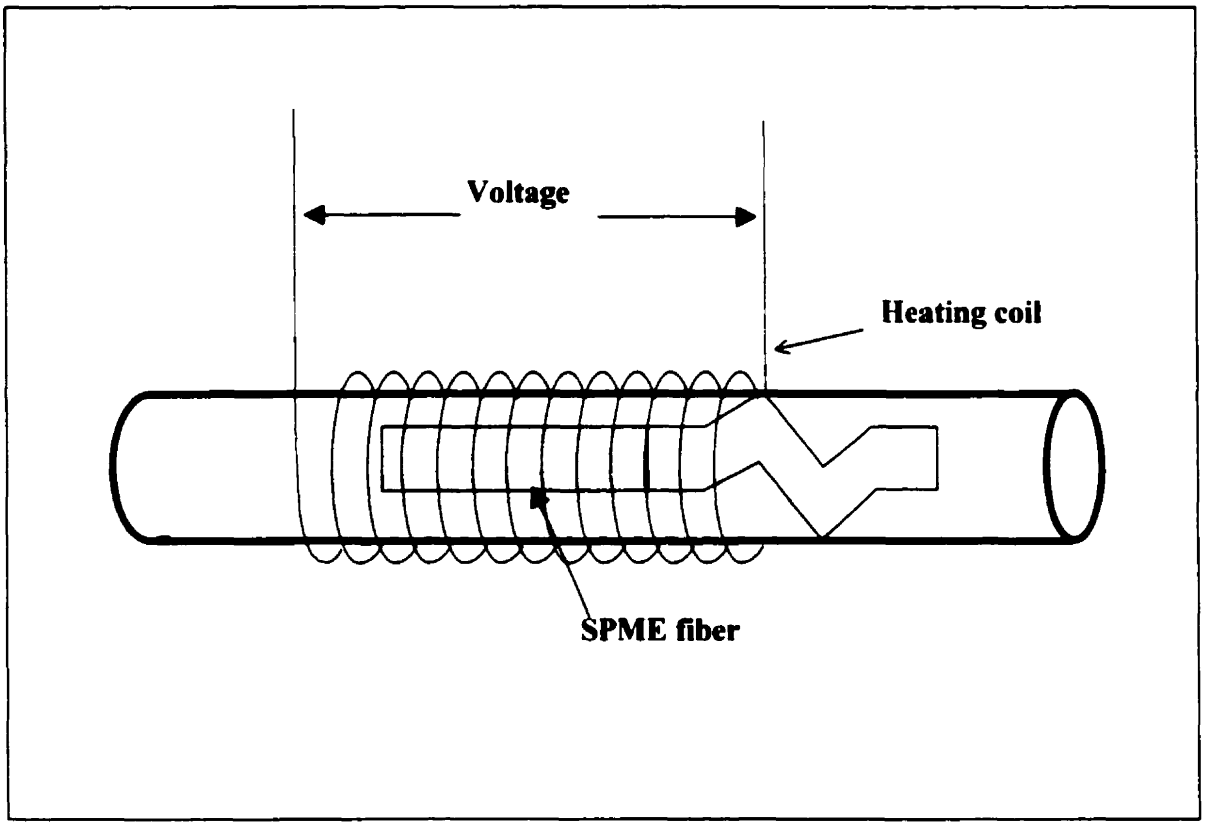


Figure 3- 2. Schematic diagram of a heated sorbent trap

The heating coil was connected to a power supply. The desorption of analytes was achieved by applying periodical voltage pulses to the heating coil.

In order to increase the efficiency of the sorbent and lower the limits of detection, the trap can be cooled. Peltier coolers use semiconductors to achieve negative temperatures. They are small and can be connected to 12 V power supplies for operation. By using multi-stage Peltier coolers, temperatures as low as -70° C can be achieved with good thermal insulation. The trap can be placed on a Peltier cooler, or it can be sandwiched in between two coolers (Figure 3- 3).

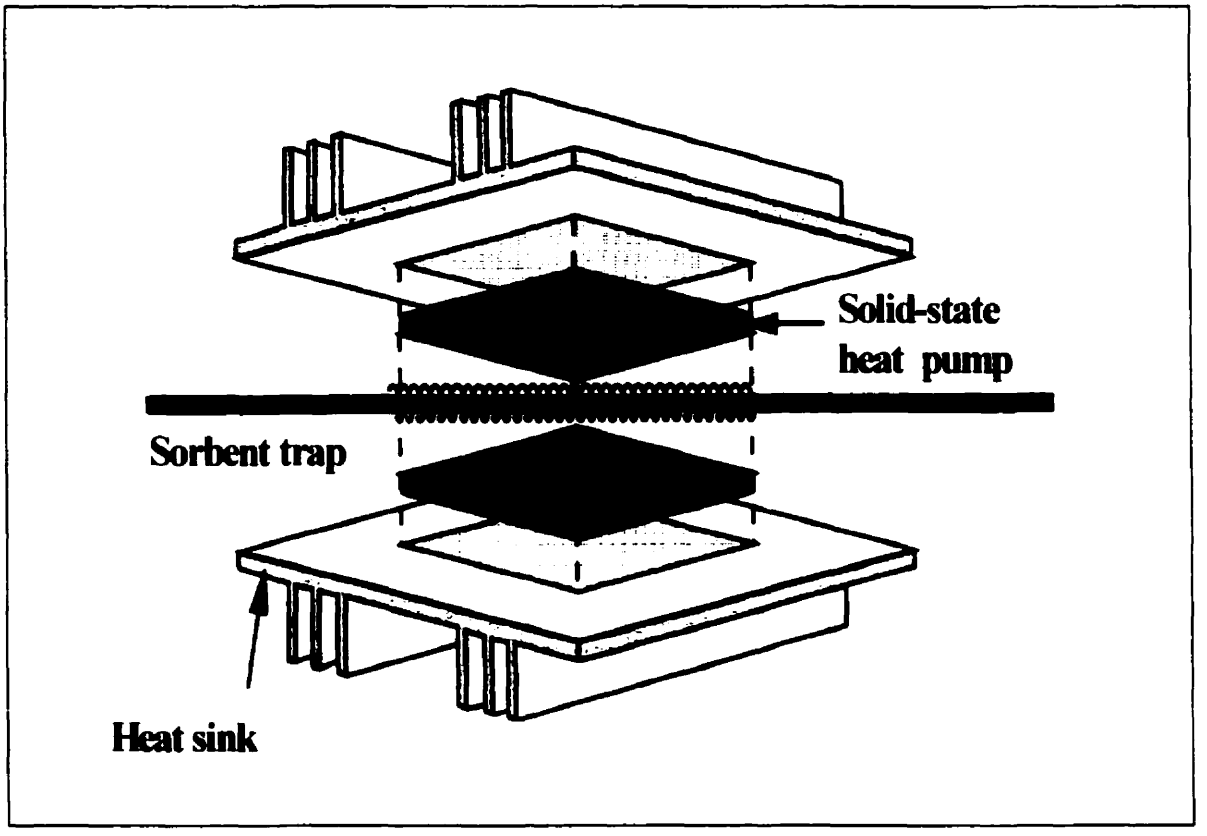


Figure 3- 3. Schematic diagram of a cooled sorbent trap

The analytes that penetrate the membrane are transported by the carrier gas to the sorbent interface. The sorbent material retains the analytes, allowing only the clean gas to pass through. When the voltage pulse is applied, the sorbent material becomes hot and it releases the analytes in a narrow injection band. A sharp increase in the temperature of the sorbent produces a narrow injection band. A schematic representation of the trapping and injection process is illustrated in Figure 3- 4.

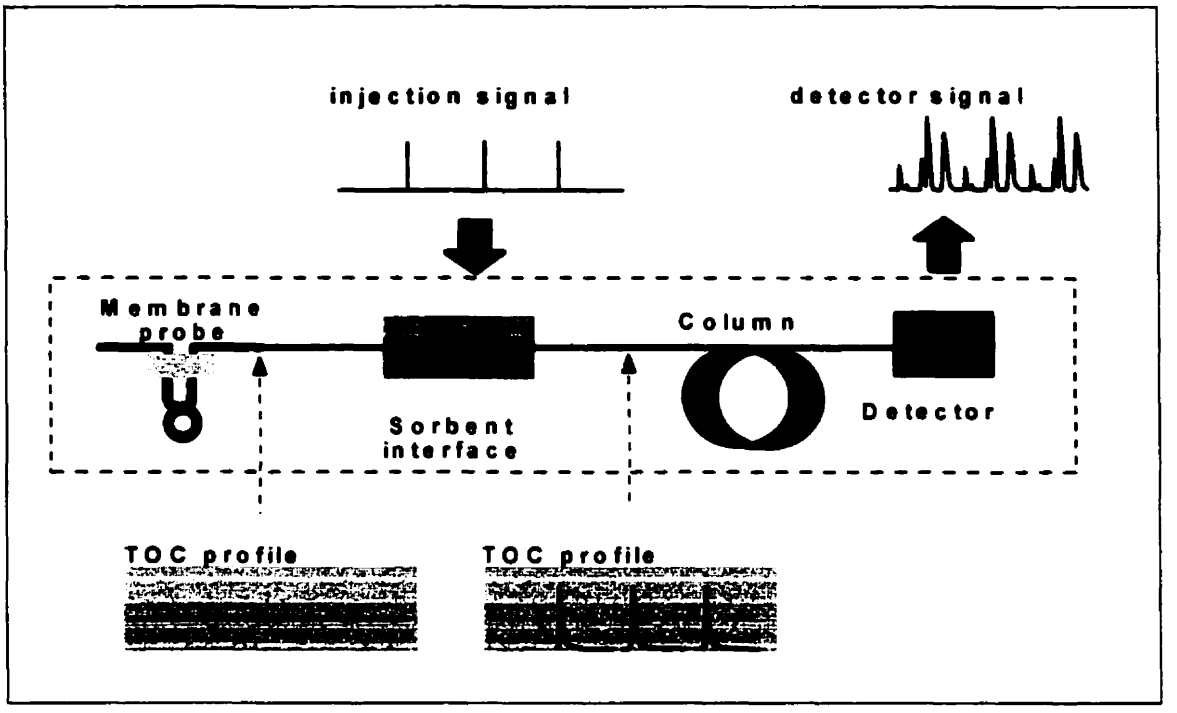


Figure 3- 4. Schematic representation of the trapping and injection process

3.3 Development of the sorbent interface

Pieces of capillary column can be used as sorbent traps only if the trapping times are very short. There is not enough sorbent material in the capillary column to allow long time trapping of the analytes. The compounds present in the carrier gas partition in and out of the stationary phase, and travel along the trap in the same way the analytes travel through a gas chromatographic column. In order to be able to retain the analytes long enough to obtain significant preconcentration, the piece of the column would have to be very long. However, if the sorbent trap is too long, the injection band becomes broad and the separation of the compounds is affected.

At the same time, in a capillary column there is discrimination between the

molecules of the compounds that travel through the middle of the column and the ones that are closer to the stationary phase. The ones in the middle have to travel longer distance to reach the stationary phase in order to be retained. Thus, these molecules would break through the trap faster than the other molecules, causing an elevation of the baseline.

By placing a SPME fiber into a fused silica capillary the amount of sorbent available to trap the analytes is increased and the injection band is narrowed. The efficiency of the trap is thus increased. Trapping times as long as three minutes can be used.

The use of a heating coil wrapped around the sorbent trap could be very inconvenient if the MESI system were to be moved, for field analysis for example. The coil is delicate and can break or move from the optimum position, affecting the desorption of analytes.

A new sorbent interface was constructed. The goals were to improve the trapping efficiency and obtain a convenient design for portable MESI systems.

In order to increase the trapping efficiency particles of sorbent were used. By using small diameter particles and packing them in a capillary tubing, the amount of the sorbent available for trapping was increased (Figure 3- 5).

The carrier gas transporting the analytes is forced to travel between the sorbent particles, which are close to each other. In this way less discrimination is introduced among the analyte molecules, and the trap is more likely to capture all the compounds present in the gas stream.

In order to eliminate the heating coil, a piece of deactivated stainless steel tubing was used to hold the packing material. The stainless steel tubing conducts current and has a resistance high enough to heat to temperatures appropriate for analyte desorption.

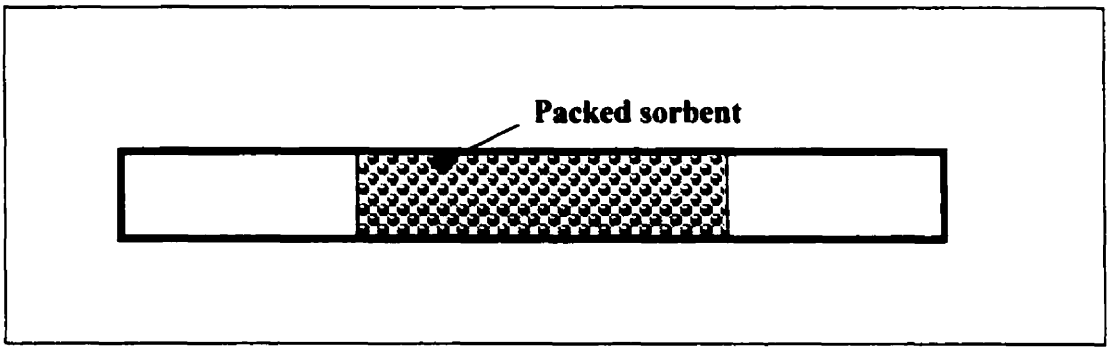
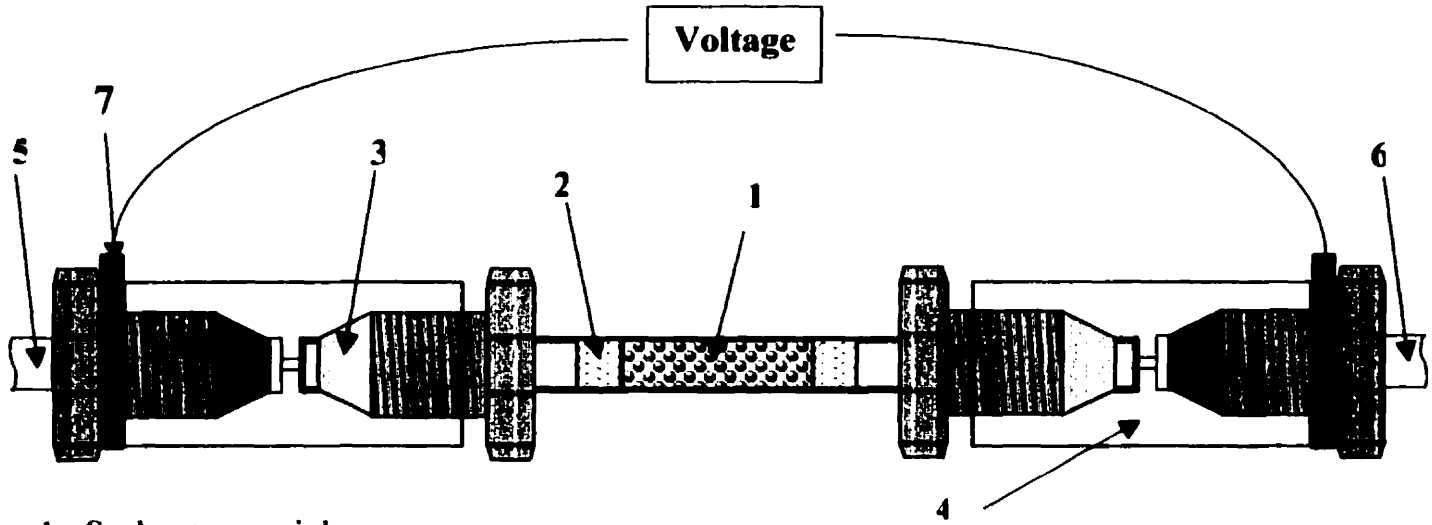


Figure 3- 5. Schematic representation of a packed sorbent trap

The new sorbent trap is more robust and it can be easily moved without the risk of breaking or affecting the desorption. At the same time, the trap is heated more efficiently, since the capillary tubing holding the sorbent is the heating element. In the previous design the heat had to be transferred from the heating coil to the walls of the trap, and then to the sorbent material.

The sorbent material has to be packed inside the tubing in such a way to allow the carrier gas to pass through and avoid a big pressure drop across the sorbent interface. A big pressure drop at the sorbent level would result in a high pressure on the inner side of the membrane, and thus the extraction process would be affected (it would result in a lower extraction efficiency).

The more sorbent present in the trap, the more efficient is the preconcentration of analytes. However, if the sorbent bed is too long, the injection band becomes broad and a bad separation would result, due to broad peaks. Thus, the length and density of the sorbent bed have to be optimized. At the same time, a compromise has to be made for the overall amount of sorbent in order to get proper preconcentration, but also good separation.



1. Sorbent material
2. Quartz wool
3. Gold plated ferrule
4. Valco connector
5. Stainless steel tubing connecting the membrane module to the sorbent trap
6. Stainless steel tubing connecting the sorbent trap to the GC column
7. Electrical connection to power supply

Figure 3- 6. Schematic description of the sorbent trap

Taking these factors into consideration, a 50 - 60 mesh particle size sorbent was used. Due to the use of this particle size and of a packing bed of about 1 - 1.5 cm long, almost no pressure drop resulted across the trap. The sorbent material was packed into a 5.5 cm long (o.d. = 0.73 cm) piece of deactivated stainless-steel tubing (MXT guard column) that was obtained from Chromatographic Specialties Inc. (Brockville, ON, Canada). The quartz wool used at the ends of the sorbent bed was obtained from Chrompack (Middelburg, The Netherlands). A schematic diagram of the sorbent trap is presented in Figure 3- 6.

The trap had to be connected to the membrane module and the chromatographic column. For this, two stainless steel Valco connectors (1/16 - 1/32) were used (Chromatographic Specialties Inc.). Gold platted stainless steel ferrules (Chromatographic Specialties Inc.) were used for the connection of the trap. These ferrules are softer than the pure stainless steel ferrules and sealed the trap (metal on metal), so that no gas leak was detected. Initially, pure stainless steel ferrules were used, but small leaks around the connection were detected. Graphite ferrules were also tried, since graphite is soft and can seal very well and it also conducts current. The ferrules proved to seal well until the trap was pulsed. During the electrical pulses the trap bends slightly, causing the graphite ferrules to deform after a few pulses.

On the other side of the Valco connectors ferrules that do not conduct electricity had to be used. Vespel-graphite ferrules (Chromatographic Specialties Inc.) were used in some applications, but the best choice was found to be the PEEK connectors with attached ferrules, supplied by Chrompack.

The way the trap is heated is very important. The analytes have to be released very fast from the sorbent and introduced into the chromatographic column. If the heating pulse is

not sharp, the sorbent material reaches the final temperature after a long period of time (a few seconds). In such a case the most volatile compounds are released at the beginning of the pulse, and the least volatile ones at the end of the pulse, which produces a broad injection band resulting in a bad separation. To avoid this, a capacitive discharge power supply was used. When such a power supply is used, the voltage maximum is reached within a few milliseconds, and thus the increase in the temperature of the trap is also very sharp. The power supply used in herein-described experiments was custom made and it contained a computer-grade 16000 μF/50 V capacitor. The electrical connectors that were used to connect the trap to the power supply were squeezed in between the nut and the body of the Valco connectors.

A special holder, designed to support the trap was constructed out of Teflon. The holder was attached to the side of the outer wall of the GC oven, and the column was taken out of the GC through the oven insulation, and connected directly to the trap. The holder was used only in the experiments in which the trap was not cooled.

For the experiments in which a Peltier cooler was used to enhance the trapping efficiency, a different holder was used. The cooler had to be placed on a heat sink and the sink had to be equipped with a fan for a faster release of the heat. On the top of the cooler, a small aluminum plate was placed, and fixed with two screws to the heat sink. This way the cooler was sandwiched in between the heat sink and the aluminum plate. The plate had a groove that accommodated the sorbent trap. Another aluminum plate, smaller than the bottom one, was placed on the top of the trap and fixed with two screws to the bottom aluminum plate. In order to avoid electrical contact between the trap and the aluminum plates, a very thin layer of glass wool was wrapped around the trap. The whole assembly was insulated with glass wool and pipe insulation, and was placed on the wall of the GC oven, to allow the connection of the

chromatographic column to the trap. By placing the trap on the wall of the GC, the whole column was allowed to sit in the oven. Thus, cold spots on the chromatographic column were avoided.

3.4 Characterization of the sorbent interface's performance

As the main role of the sorbent interface in MESI is to preconcentrate the sample prior to introduction into the chromatographic column, it is very important to know the extent to which the trap can preconcentrate the analytes present in the sample. In other words, it is very important to know the maximum trapping time that can be used in order to achieve the highest sensitivity, and also the shortest trapping time that would provide a useful preconcentration.

While in the sorbent trap, the analytes partition in and out of the sorbent. While they are in the carrier gas (out of the sorbent), they are carried along the sorbent trap for a short distance, until they are sorbed again. At the beginning of the trapping step no analyte reaches the chromatographic column. After a period of time, the analytes that have reached the sorbent interface firstly get to the end of the sorbent bed by partitioning in and out of the sorbent. Once they are at the end, the carrier gas takes them to the GC column. It is said that the analytes have "broken through" the trap. The volume of gas passing through the trap before the breakthrough of the analytes occurs is called "the break through volume".

The break through volume is a very important parameter of the sorbent trap. It depends on the flow rate of the carrier gas, the amount of sorbent material present in the trap and the temperature of the trap.

The higher the flow rate of the carrier gas, the faster the breakthrough volume is

reached. This means that at high flow rates shorter trapping times have to be used. However, as mentioned before, the extraction efficiency increases with the carrier gas flow. Thus, the best experimental parameters must be established for each application.

Placing more sorbent in the trap can increase the efficiency of the preconcentration step. However, the way the sorbent is placed in the trap is very important. If the sorbent particles are too small, the packing bed might be too tight and the carrier gas might not be able to pass through it. Or, if the gas can pass through it but the pressure drop across the sorbent trap is too large, the consequence would be a high pressure on the inner side of the membrane that would decrease the extraction efficiency. On the other hand, if the sorbent particles are too big, there would be a lot of space between the particles, hence the analytes in the gas phase would have to travel a longer distance to reach the sorbent. This would decrease the sorption efficiency and would increase the chance of having analytes that could channel through the sorbent particles without being retained. At the same time, if the particle size is too big, less sorbent can be packed in the same given volume. The length of the sorbent bed affects the chromatographic separation since a long bed would produce a wide injection band. It is not appropriate to use wider tubing for the trap in order to increase the amount of sorbent available for trapping for a given (optimal) length. This is because the release of the analytes from the trap is accomplished by heating the walls of the trap (the tubing). Thus, the sorbent particles that are closer to the walls can heat faster and more efficiently (reaching higher temperature), whereas the ones placed towards the center of the trap would get heated at a later time and reach lower temperatures. In such a case discrimination would occur for the analytes retained in different parts of the trap. This would again affect the separation. It is thus appropriate to choose narrower tubing.

As mentioned before, the analytes partition in and out of the sorbent material while passing through the sorbent trap. They cannot be completely retained by the sorbent at room temperature. However, at lower temperatures (below 0°C) the analyte partition coefficients between the sorbent and the gas phase become larger, generally several orders of magnitude higher than at room temperature.[3] In cases in which the concentration of analytes in the samples is very low and extended preconcentration is required, or if the ambient temperature is elevated, the sorbent trap can be cooled to increase the preconcentration efficiency.

A low sorbent temperature can be achieved by circulating a coolant around the trap. If dry ice (CO_2) is used the temperature can be lowered to -78°C, and to -196°C if liquid nitrogen is used. The trapping efficiency would be dramatically increased at such low temperatures. However, the use of these coolants is not practical, especially for field analysis, since they evaporate very rapidly and thus, have to be added frequently. As an alternative, semiconductor-based cooling devices have the advantage of ease of operation, maintenance of constant temperature, small geometry, and reliability.

All these aspects have been investigated in order to achieve a comprehensive understanding of the trap performance.

3.4.1 The preconcentration ability of MESI systems.

In order to prove that MESI can provide better sensitivity due to sample preconcentration, a syringe injection and a MESI sample introduction were compared using the same gas chromatograph.

Experimental setup

The analyses were performed using a Chrompack 2002 Micro-GC, equipped with two gas chromatographic modules. Each module consisted of an injector, two heated columns (an analytical column and a reference for the thermal conductivity detector) and a micromachined thermal conductivity detector. The injector was etched in a glass wafer and had very low dead volume. It contained pneumatically actuated valves, a micro sampling loop, and flow restrictors for the two columns. Experiments were performed using an unmodified module (4 m x 0.25 mm x 0.15 μm CP Sil 5 CB column) and a custom-made module containing the same type of column. In the custom-made module, two additional connectors allowed the hook-up of the MESI system after the built-in injector.

A Teflon holder was machined for the sorbent trap. The holder was designed in such a way to allow proper ventilation of the trap, since it is very important that the trap returns to the room temperature in the shortest possible time after the application of the heating pulse. Since the GC was portable, designed for field analysis, the trap was not placed on a cooler. The use of a Peltier cooler requires an additional 12 V power supply. Even though the power supply is small, any additional object that has to be carried in the field could be an inconvenience. Thus, the cooler should be used only if compounds at very low concentrations have to be analyzed. The trap holder was mounted perpendicularly to the wall of the GC module. This allowed the connection of the trap to the chromatographic column without having to take a piece of the column out of the thermal element that plays the role of oven in the portable GC.

For the desorption of analytes from the trap the same custom-made power supply was used, and the desorption pulses were controlled by the same Omron external timer. XAD-2 resin (Polystyrene divinyl benzene) purchased from Supelco (Oakville, ON, Canada) was used

as sorbent, and the sorbent bed in the trap was about 0.5 cm in length. Silicone polycarbonate membrane (SSP M-213) was placed in the membrane holder and used for analyte extraction.

A standard gas mixture of benzene, toluene and o,m,p-xylenes was obtained in the dynamic mode, using a standard gas generator and permeation tubes (KIN-TEK Laboratories, Inc., Texas City, TX, USA). The concentration of benzene and toluene was approximately 2 ppm (v/v), while that of the xylenes was approximately 1 ppt (v/v). A static generated gas mixture was obtained using a 2L SUMMA canister. The so-prepared mixture contained benzene, toluene, ethylbenzene, and o,m,p-xylene (4 ppt of each compound).

Hydrogen was used as carrier gas for the GC. For generating standard gas mixtures, air was used as diluting gas. All gases were of ultra high purity and were purchased from Praxair (Kitchener, ON).

The separation of analytes was performed isothermally at a temperature of 55°C, with the pressure set to 115 kPa for the carrier gas.

Results and discussion

A set of five chromatograms obtained with the MESI system described in the previous section is presented in Figure 3- 7. The trapping times were progressively longer. The time increment for each chromatogram was 30 seconds and it started with a trapping time of 1 minute for the lowest chromatogram and it ended with a 3 minute trapping time for the most upper trace. The standard gas mixture was generated dynamically for this experiment. The peaks correspond to: 1 = benzene; 2 = toluene; 3 = m,p-xylene; 4 = o-xylene. The concentration was 2 ppm for benzene and toluene and 1 ppm for the xylenes.

The small negative dips in the chromatograms correspond to the desorption pulses. The

thermal conductivity detector is sensitive to pressure variations. This is why negative dips in the baseline are produced during the heating of the sorbent interface when the carrier gas expands. The first peak in each group corresponds to water and air, which also permeate through the membrane and are trapped by the sorbent to some extent.

The response of the system was proportional to the trapping time. The longer the trapping time, the higher the peaks that were obtained. In the case of benzene, for a trapping time of 1 min the peak height was ~0.5 V, and it increased progressively to reach a height of ~1.5 V for a 3 min trapping time. Each separation was completed in 25 seconds.

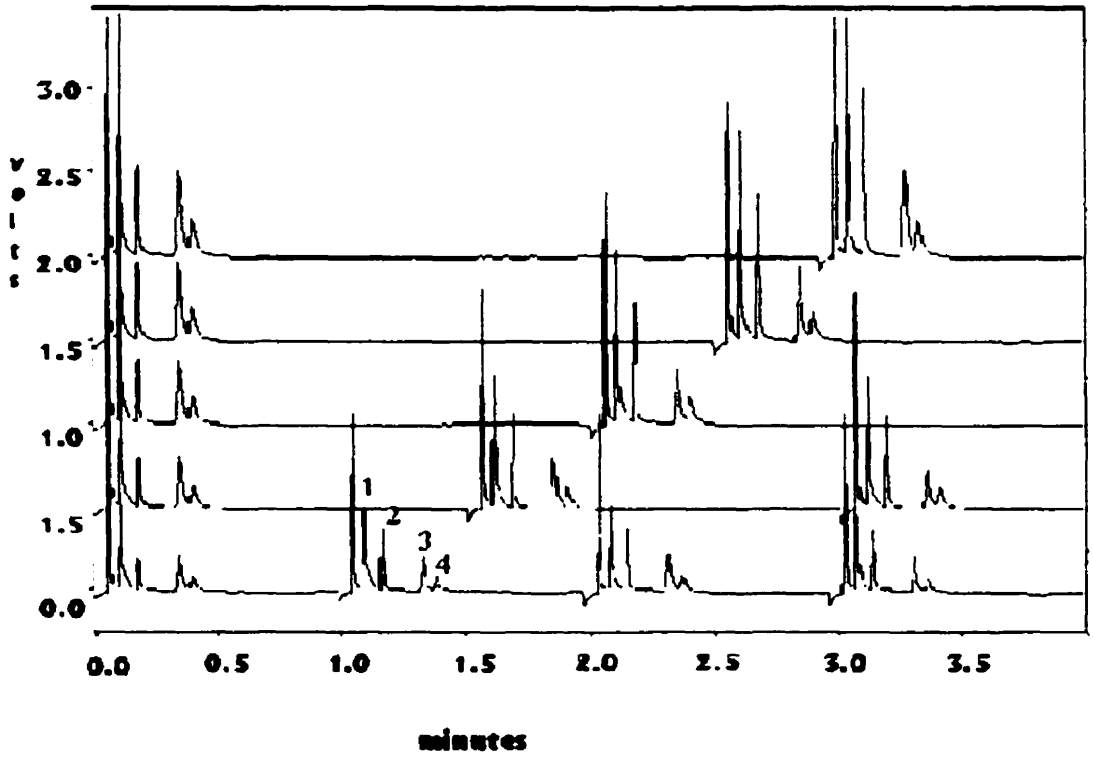


Figure 3- 7. Chromatogram obtained using progressively longer trapping time (from the lower to the upper trace: 1, 1.5, 2, 2.5, and 3 min respectively) for a mixture of benzene, toluene, and o,m,p-xylene. Peaks: 1 = benzene; 2 = toluene; 3 = m, p-xylenes; 4 = o-xylene.

The fact that the height of the peaks was proportional to the trapping time proves that for the examined time frame there was no breakthrough, despite the fact that the amount of sorbent available for trapping was very little (sorbent bed of only 0.5 cm).

In another experiment the results obtained with a regular microsampling loop injection were compared to the ones obtained by MESI sampling.

The Chrompack Micro GC had two channels. One of the channels was modified to accommodate the membrane and the trap and the other channel was used for the regular microsampling loop injection.

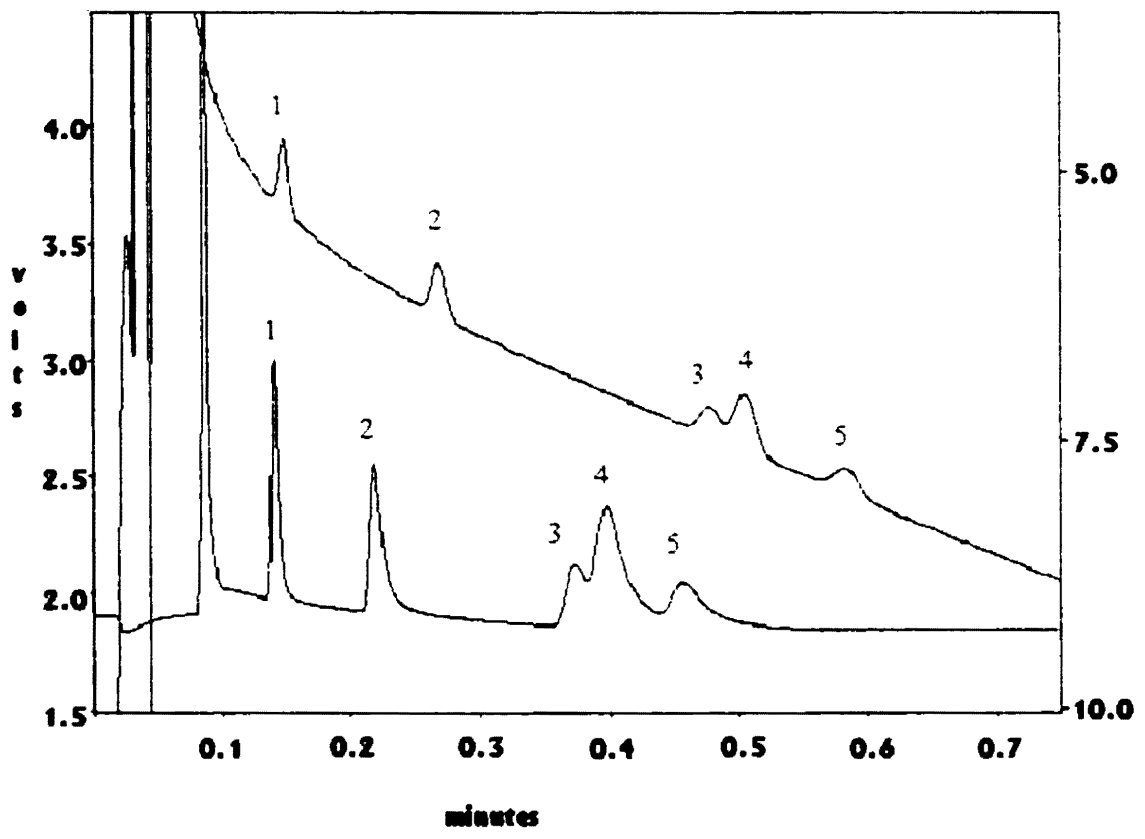


Figure 3- 8. Comparison of chromatograms obtained by regular gas injection (upper trace) and MESI injection (lower trace)

The standard gas mixture used in this experiment was obtained using the static mode and contained benzene, toluene, ethylbenzene and o,m,p-xylene (4 ppm for each compound). A trapping time of 1 min was used for MESI sampling. For the regular injection, a maximum injection time of 255 ms was used. The compared chromatograms are presented in Figure 3- 8.

The upper trace is the one obtained with regular gas injection while the lower one was obtained by MESI, using the modified second module from the same GC. For the upper trace the voltage scale was increased 100 times (see right y-axis) compared to the voltage scale for the lower trace. The settings for the normal injection correspond to the maximum sensitivity of the instrument. It is clear from the figure that for a trapping time as short as 1 min the sensitivity was increased by more than 2 orders of magnitude.

Thus, the MESI system provides significant sample preconcentration even for short trapping times. If the amount of sorbent placed in the sorbent trap is increased, longer trapping times can be used, resulting in a very much improved sensitivity. The fact that the separation was obtained in less than 30 seconds proves that the system can be used for continuous monitoring of VOCs. For that particular gas mixture (BTX), samples could be collected every 30 seconds and the sensitivity would be improved by approximately 500 times.

3.4.2 Carryover in the sorbent trap

The second role of the sorbent trap in a MESI system is to inject the analytes into the chromatographic column. The injector that the GC is equipped with can thus be bypassed or even completely removed from the system since it is not needed for sample injection.

Because of this important role - of introducing the analytes into the GC column - the trap must have a proper design. When the heating pulse is applied, the gas present in the

trap expands. The gas containing the desorbed analytes can go forward (towards the GC column), but can also be pushed backwards (towards the membrane module). This can happen because at the moment the pulse occurs, the pressure created by the fast expansion of the gas exceeds the pressure of the carrier gas. If a part of the carrier gas is pushed backwards, a part of the desorbed analytes are flushed back as well. Once the gas stops expanding, the carrier gas restarts its flow towards the column. The analytes that have been flushed back are forced to pass over the sorbent bed again. As a result of the fact the temperature of the sorbent drops very rapidly to its original value after the heating pulse ends, the analytes get sorbed again. It follows, that during the desorption pulse only a part of the trapped analytes are sent to the column and eventually reach the detector, to produce a signal, while others remain in the sorbent trap. There would thus be some carryover in the sorbent trap.

The carryover due to back flush can be overcome by placing a restriction in the trap, towards the membrane module. For example if the diameter of the tubing that connects the membrane module to the sorbent trap would be smaller than the diameter of the GC column, the carrier gas would follow the least restricted path, meaning that most of it would go towards the GC column. However, the tubing that connects the membrane module to the sorbent is relatively long and if its internal diameter would be smaller than 0.25 mm (the most common i.d. for a GC column), the pressure drop across this tubing would be too big, and would affect the extraction. Thus, a smaller restriction should be used.

Experimental design

As mentioned above, a different way of preventing the gas from back flush had to be found other than by connecting the membrane module to the sorbent interface via a small i.d. tubing.

A 0.5 cm long piece of fused silica capillary having an internal diameter of 100 μm has been found to provide efficient restriction to reduce back flush and not affect the flow of the carrier gas. The piece of capillary was immobilized in a piece of Teflon tubing having the same inner diameter as the outer diameter of the fused silica capillary (0.37 cm). In order to connect the Teflon tubing to the sorbent trap and immobilize the capillary in it, a ferrule was used. The ferrule squeezed the Teflon tubing against the capillary when it was placed in a Valco connector (Figure 3- 9).

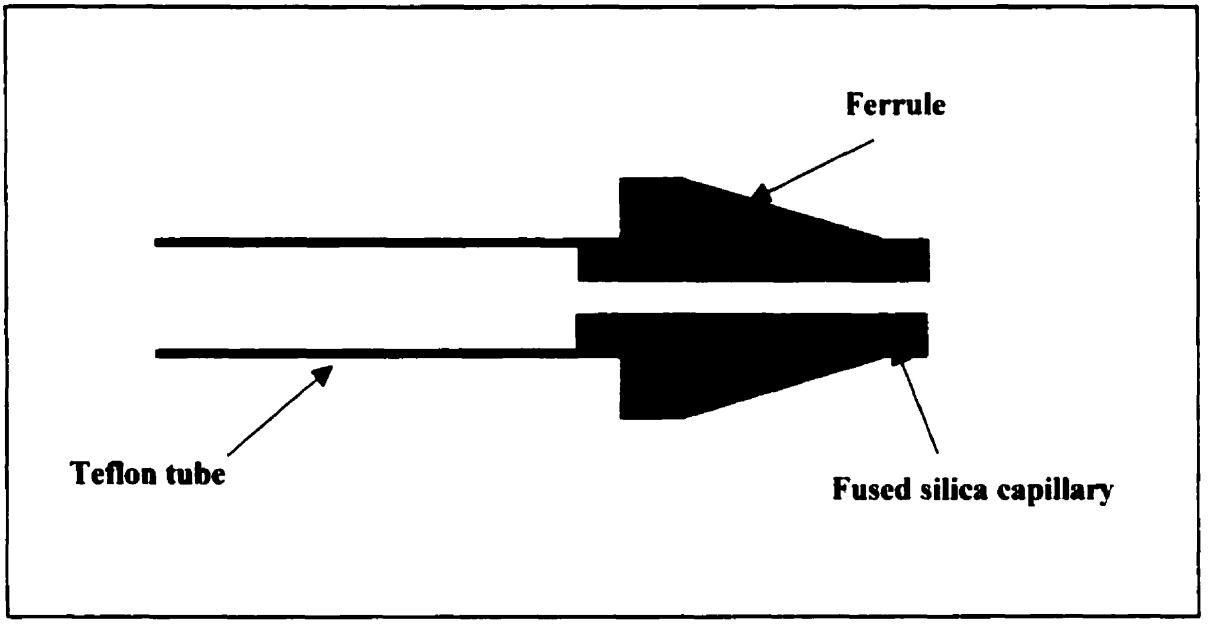


Figure 3- 9. Schematic representation of the restriction to the trap

A flat-sheet Silicone Polycarbonate Membrane (SSP-M213), of 25 μm thickness, purchased from Membrane Components (Ballston Spa, NY, USA) was used. This membrane material has been chosen because it is less permeable to oxygen than Poly(dimethylsiloxane). XAD-2 resin (Polystyrene divinyl benzene) purchased from Supelco (Oakville, ON, Canada) was used as packing material for the sorbent trap. The length of the sorbent bed was 1.3 cm. For the separation of the compounds, a CP-9002 gas chromatograph (Chrompack, Middelburg, The Netherlands) was used. The GC was equipped with a flame ionization detector (FID) and a 10 m, 0.25 mm i.d., 0.15 μm film thickness CP Sil 5 CB column (Chrompack).

The standard gas generator described in the previous chapter was used for the delivery of the standard gas mixture. Toluene and xylene of 99+% purity were purchased from Aldrich Chemical Company, Inc. The mixture contained 3.8820 g of toluene and 3.8706 g of xylene. Compressed air was used to dilute the mixture. The slowest delivery rate of the syringe pump was used and the diluting flow rate was 500 mL/min.

Ultra high purity nitrogen, helium, hydrogen and air required for the FID and the chromatographic column were purchased from Praxair (Kitchener, ON). A three stage Peltier cooler (Melcor Corp., Trenton, NJ) was used to decrease the temperature of the sorbent trap. For the desorption of analytes from the trap the same custom-made power supply was used, and the desorption pulses were controlled by the same Omron external timer.

The carrier gas flow in the GC column was 1.4 mL/min. The separation of analytes was conducted isothermally at 50°C. The desorption voltage was 50 V.

Results and discussions

The experimental setup was very similar to the one used to determine the memory introduced by the membrane module. However, if the membrane were exposed to analytes and bypassed after a while, the signal measured by the detector could come from the analytes that remained in the trap (trap carryover) but also from analytes that could have been adsorbed on the walls of the connectors (memory of the connectors). In order to be able to evaluate the trap carryover, the memory of the connectors had to be eliminated. To do so, the connectors were heated to approximately 80°C using heating tape.

The membrane module was placed in the sampling chamber of the standard gas generator, without being connected to the rest of the MESI system. When equilibrium was reached in the sampling chamber (constant concentration of analytes was delivered by the standard gas generator), the membrane was connected to the sorbent trap. The system was allowed to reach steady-state. When the extraction plateau was reached, the membrane was bypassed and the trap continued to be pulsed. The pulses were applied every minute.

For the case in which no restriction for back flush was placed in the trap, the signal recorded after bypassing the membrane is illustrated in Figure 3- 10. The first two peaks in the chromatogram were obtained with the membrane still connected. The following peaks represent the carryover in the trap. The first peak in each cycle is the toluene (T) peak and the second one is the xylene (X) peak.

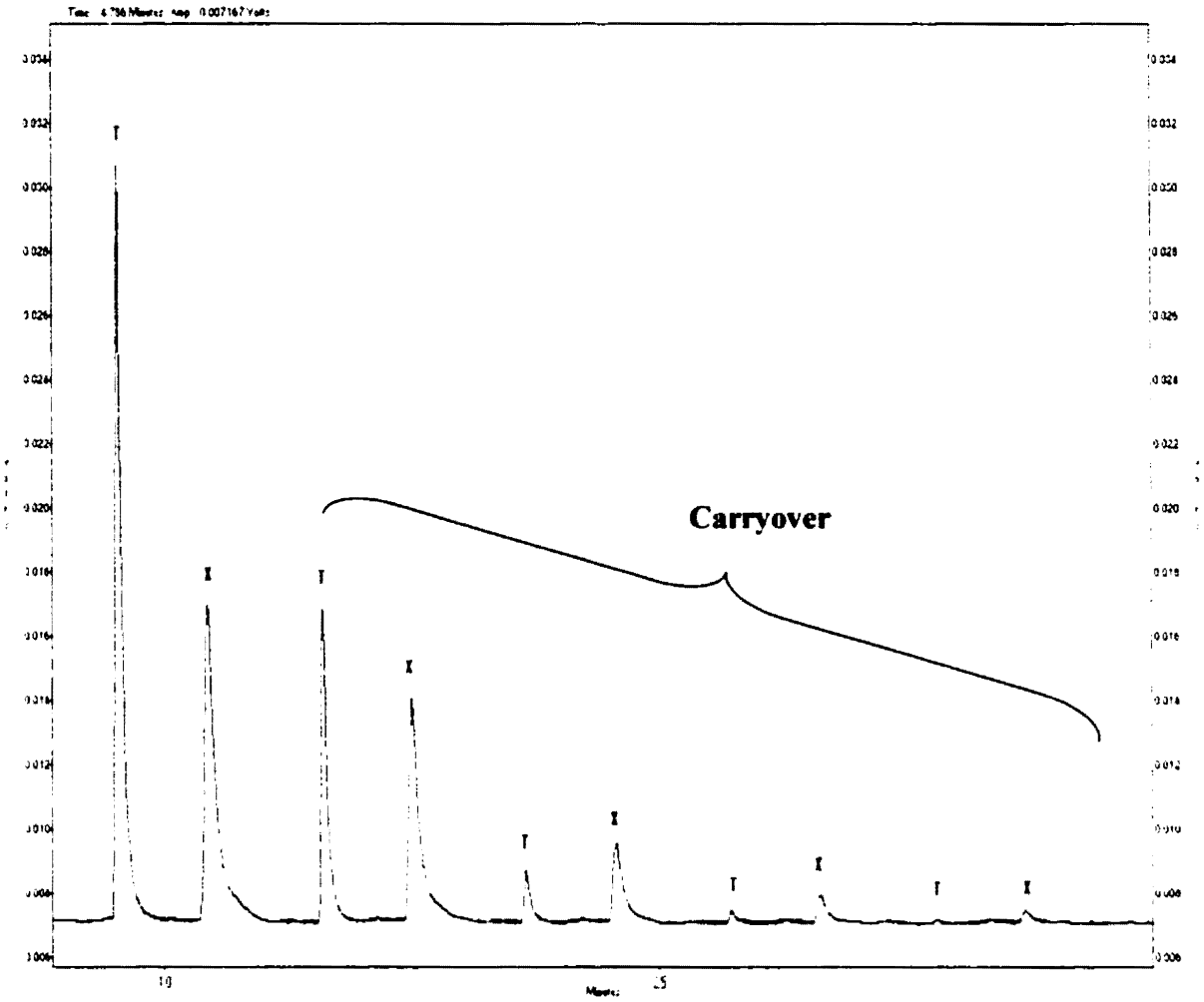


Figure 3-10. Chromatogram obtained for toluene and xylene carryover in the sorbent trap, when no restriction was used for back flush

It can be seen that even 3 minutes after the membrane was bypassed The fourth desorption in the chromatogram), toluene and xylene peaks can still be detected. The results are presented in Table 3-1 for toluene, and Table 3-2 for xylene. Due to the fact that the membrane was bypassed manually, some variation exists in the area peaks of the different runs considered in the experiment. The area peaks are presented as percentage of the last extraction peak in order to emphasize the carryover, and absolute values are presented in brackets.

Toluene

Desorption #	Area peak				Average	STD	%RSD
	Run 1	Run 2	Run 3	Run 4			
1	100 (55477)	100 (53725)	100 (35564)	100 (58894)	**100 (55365)**	**0.00 (2527**	**0.00 (5)**
2	41.06 (22780)	40.68 (21860)	40.32 (21515)	41.9 (24680)	**40.99 (22709)**	**0.59 (1418)**	**1.43 (6)**
3	7.15 (3965)	6.61 (3551)	5.76 (3076)	6.49 (3826)	**6.50 (3605)**	**0.50 (392)**	**7.62 (11)**
4	1.66 (924)	1.45 (780)	1.37 (730)	1.34 (788)	**1.45 (806)**	**0.12 (83)**	**8.59 (10)**

Table 3- 1. Carryover results for toluene analysis, obtained with the trap that had no restriction for back flush

Xylene

Desorption #	Area peak				Average	STD	%RSD
	Run 1	Run 2	Run 3	Run 4			
1	100 (36938)	100 (35916)	100 (35804)	100 (37351)	**100 (36502)**	**0.00 (660)**	**0.00 (2)**
2	70.86 (26177)	64.76 (24337)	69.82 (24998)	71.69 (26780)	**69.28 (25573)**	**2.94 (959)**	**4.29 (4)**
3	24.23 (8950)	20.99 (7542)	20.05 (7179)	22.06 (8241)	**21.83 (7978)**	**1.80 (679)**	**8.23 (9)**
4	7.53 (2784)	6.66 (2394)	6.23 (2230)	6.91 (2581)	**6.83 (2497)**	**0.54 (207)**	**7.95 (8)**
5	3.46 (1280)	2.71 (975)	2.98 (1067)	3.3 (1236)	**3.11 (1140)**	**0.33 (124)**	**10.74 (11)**

Table 3- 2. Carryover results for xylene analysis, obtained with the trap that had no restriction for back flush

As it can be seen for the two tables, the carryover in the sorbent trap after the first desorption was 40.99% for toluene and 69.36% for xylene. The peak areas were reported in percentage relative to the area of the peaks obtained with the membrane connected to the system.

In the second part of the experiment the 100 μm i.d. capillary meant to prevent back flush of analytes was connected to the front part of the trap. The carryover experiment for the toluene and xylene mixture was repeated.

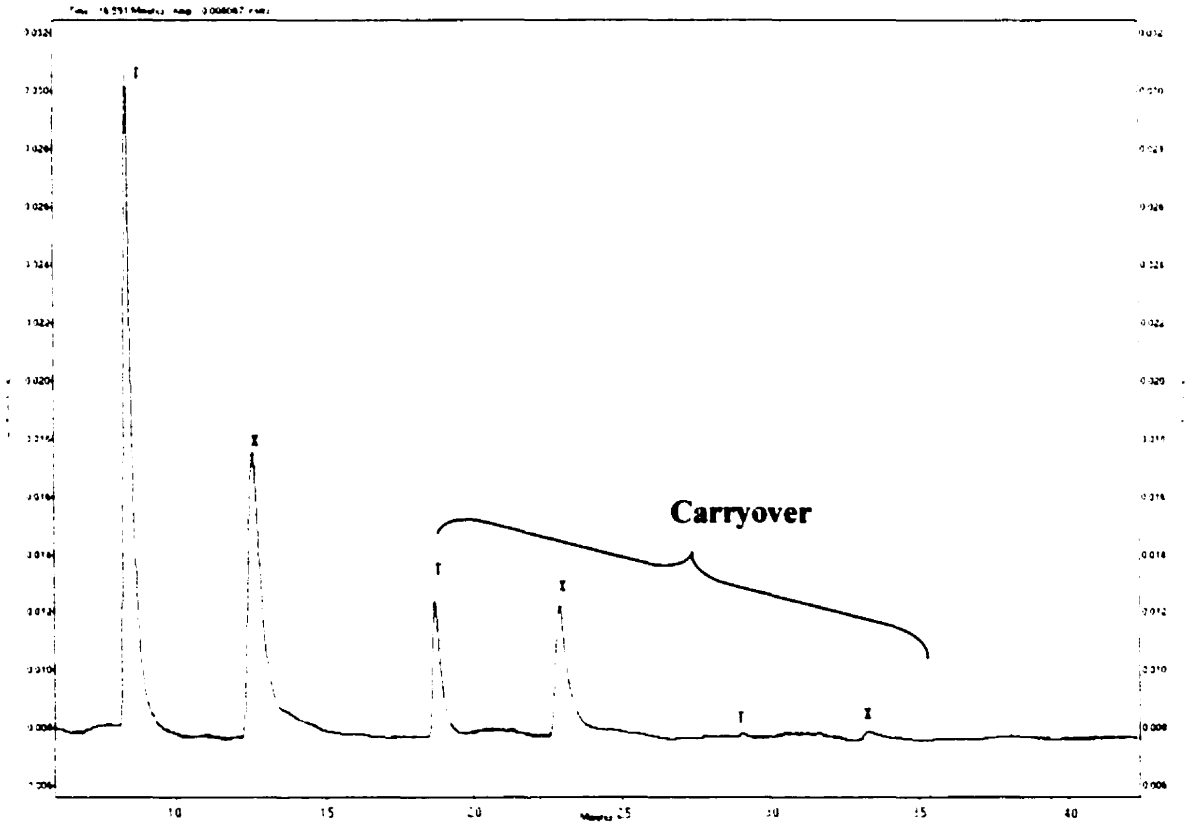


Figure 3- 11. Chromatogram obtained for toluene and xylene carryover in the sorbent trap, when restriction was used to prevent back flush

Figure 3- 11 presents one of the chromatograms obtained for the carryover measurements. The first two peaks were obtained having the membrane exposed to the gas mixture. The following peaks in the chromatogram represent the trap carryover.

The numerical results are presented in Table 3- 3 for toluene and Table 3- 1 for xylene.

Toluene

Desorption #	**% Area peak**				**Average**	**STD**	**RSD**
	Run 1	**Run 2**	**Run 3**	**Run 4**			
1	100 (54130)	100 (55929)	100 (56750)	100 (55274)	**100 (55521)**	**0 (1106)**	**0 (2)**
2	19.52 (10566)	19.62 (10973)	19.71 (11185)	19.55 (10806)	**19.60 (10883)**	**0.07 (262)**	**0.37 (2)**
3	unmeasurable	unmeasurable	unmeasurable	unmeasurable	**unmeasurable**	**unmeasurable**	**unmeasurable**

Table 3- 3. Carryover results for toluene obtained with the sorbent trap that had restriction for back flush

Xylene

Desorption #	**% Area peak**				**Average**	**STD**	**RSD**
	Run 1	**Run 2**	**Run 3**	**Run 4**			
1	100 (355678)	100 (369009)	100 (386794)	100 (371127)	**100 (370652)**	**0 (11042)**	**0 (3)**
2	37.73 (134197)	37.66 (138969)	38.88 (150385)	37.42 (138876	**37.44 (14067)**	**0.56 (5966)**	**1.42 (4)**
3	unmeasurable	unmeasurable	unmeasurable	unmeasurable	**unmeasurable**	**unmeasurable**	**unmeasurable**

Table 3- 4. Carryover results for xylene obtained with the sorbent trap that had restriction for back flush

When the restriction was placed in front of the trap. the carryover decreased for both. toluene and xylene. A comparison between the results obtained with and without the restriction to the trap is illustrated in Figure 3- 12 for toluene. and in Figure 3- 13 for xylene.

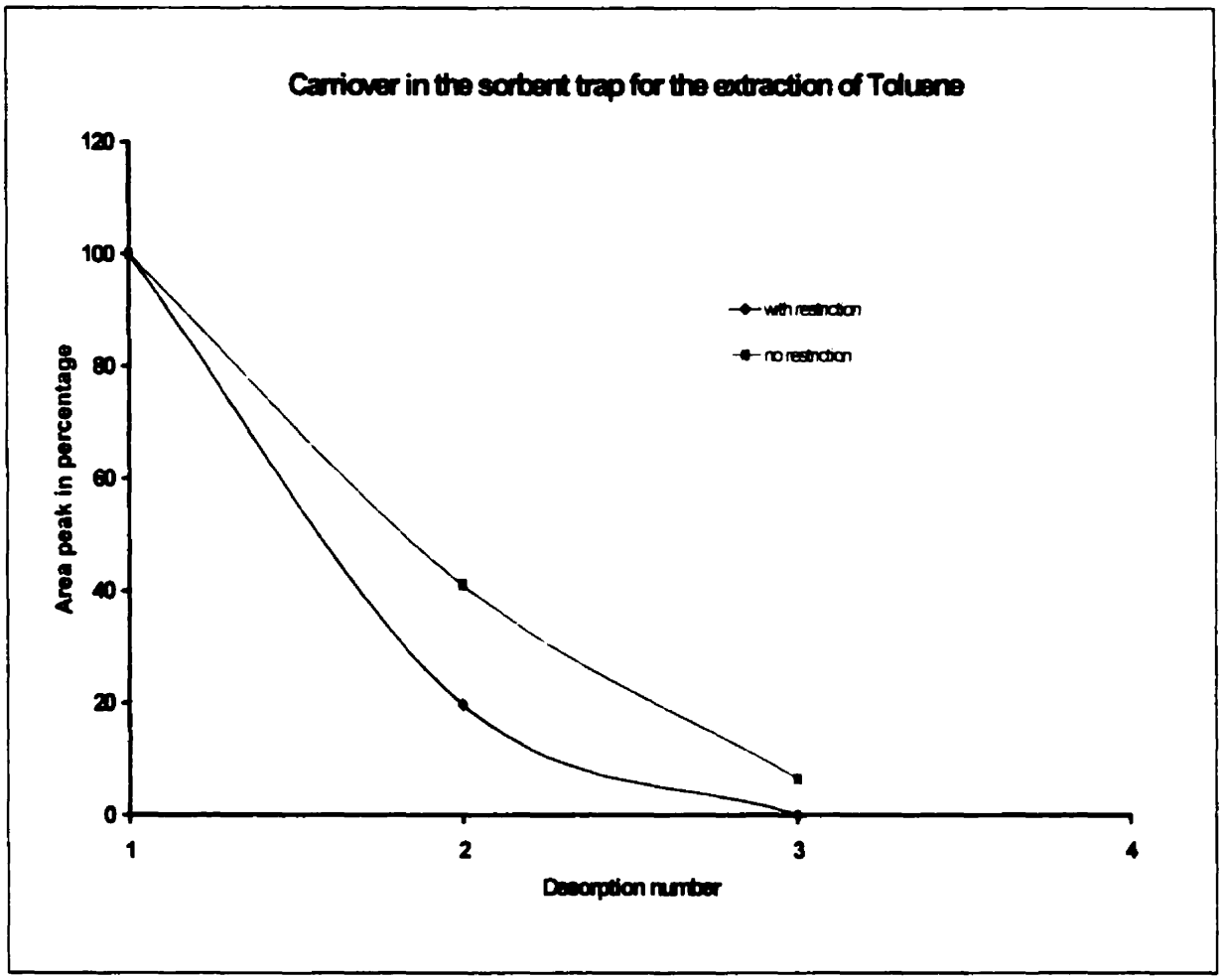


Figure 3- 12. Carryover results for toluene analysis with and without restriction to the sorbent trap

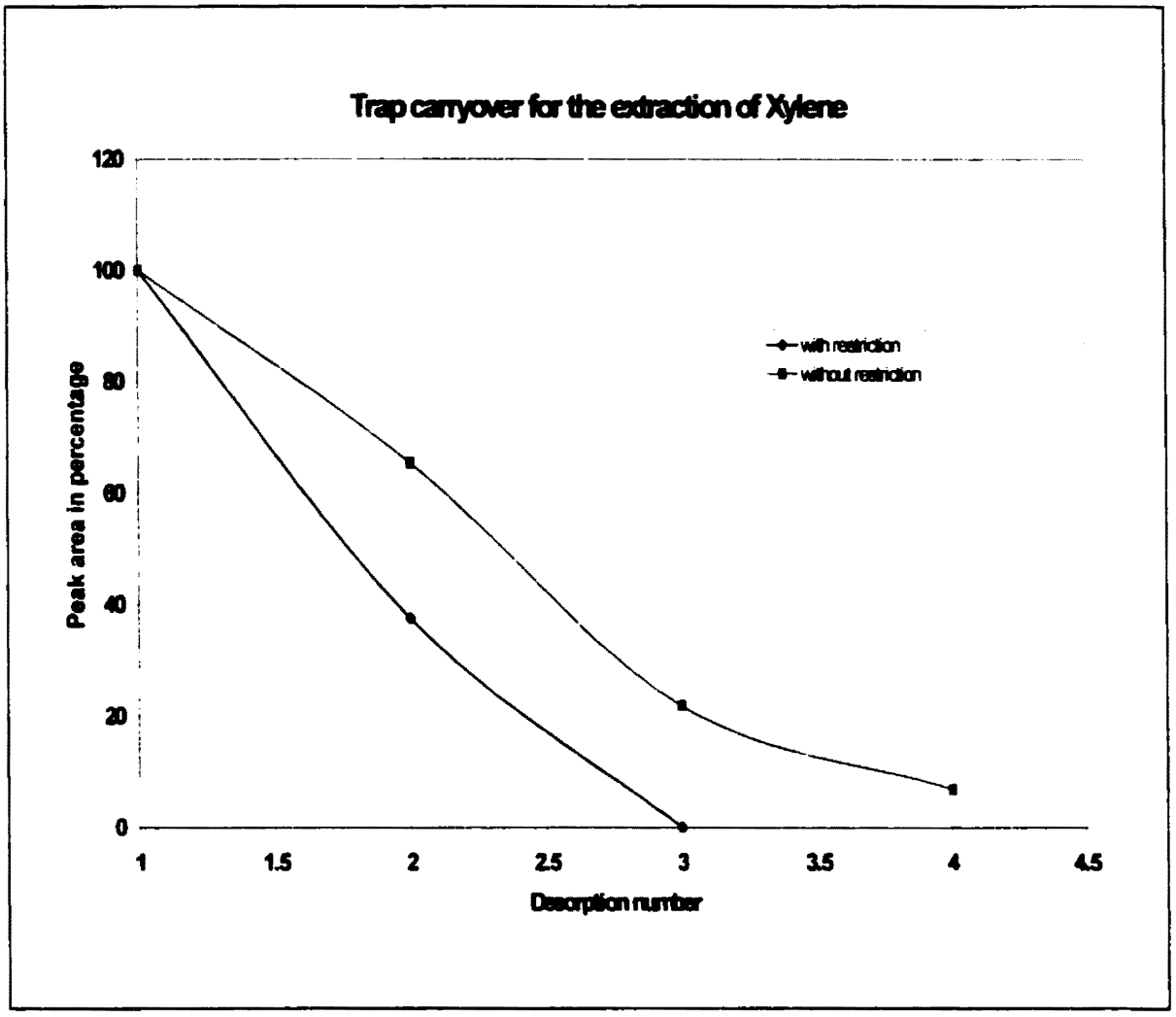


Figure 3- 13. Carryover results for xylene analysis, with and without restriction to the sorbent trap

The carryover decreased by 20 % for toluene and 28 % for xylene when restriction was placed in front of the trap. Thus, the presence of the small fused silica capillary in front of the trap prevented the analytes from being sent back towards the membrane. There is no proof though, that the restriction was 100% efficient, since a significant amount of analytes was

released from the trap during the second desorption pulse.

However, inefficient desorption is more likely to be the cause of carryover rather than an inefficient restriction for back flush. The heating pulse applied by the capacitive discharge power supply is very short (milliseconds). The temperature rises very quickly to its maximum and than returns relatively fast to its initial value. The maximum temperature is thus not maintained for a sufficiently long time. The particles of sorbent close to the trap wall can reach an optimum temperature for an efficient desorption. However, the sorbent particles from the middle of the trap do not reach the same temperature. Their lower temperature in comparison to the one of the marginal particles determines an inefficient (partial) desorption of the analytes sorbed in this region.

If the desorption voltage would be increased, the particles from the center of the trap would reach a higher temperature and thus, the desorption could be improved at this level. However, the marginal particles would, in this case, reach a temperature too high leading to the decomposition of the sorbent.

The problem could be solved if a square wave pulse could be applied by the power supply to the sorbent trap. In such a case the maximum temperature would be maintained for a longer time. That would allow the middle part of the sorbent to reach a temperature value close to the one of the sorbent periphery. Future research aims to solve this problem.

3.4.3 Improvement of the trapping efficiency

The amount of analytes that are preconcentrated is directly proportional to the trapping time used. The longer the trapping time, the lower is the concentration of analytes that can be detected. However, the breakthrough volume determines the longest trapping time that can

be used for a certain sorbent trap. The break through volume can be increased either by cooling the trap, or by increasing the amount of sorbent in the trap.

The use of a cooled trap is advantageous because it does not affect the separation of the analyzed compounds. However, for field analysis it might be inconvenient to have an additional power supply (for the cooler) that has to be transported in the field. Thus, for portable systems it might be more convenient to have traps containing a higher amount of sorbent.

Experimental setup

A CP-9002 gas chromatograph (Chrompack, Middelburg, The Netherlands) was used. The GC was equipped with a flame ionization detector (FID) and a 10 m, 0.25 mm i.d., 0.15 μm film thickness CP Sil 5 CB column (Chrompack). Ultra high purity nitrogen, helium, hydrogen and air required for the FID and the chromatographic column were purchased from Praxair (Kitchener, ON). XAD-2 resin (Polystyrene divinyl benzene) purchased from Supelco (Oakville, ON, Canada) was used as packing material for the sorbent trap. The sorbent bed was 1.3 cm long. Restriction was placed in front of the trap to prevent back flush. A flat-sheet Silicone Polycarbonate Membrane (SSP-M213), of 25 μm thickness, purchased from Membrane Components (Ballston Spa, NY, USA) was used. The connection between the membrane module and the sorbent trap was heated to about 80°C, using heating tape. For cooling the trap, a three stage Peltier cooler (Melcor Corp., Trenton, NJ) was used.

Benzene of 99+% purity was purchased from Aldrich Chemical Company, Inc. A standard stock solution was prepared by dissolving 45.5 μL benzene in water. During the

experiments, the membrane was placed in a 120 mL jar that contained 40 mL water. An appropriate amount of the stock solution was spiked into the 40 mL of water to give a final concentration of 50 ppb benzene. The sample was stirred during the experiments at a rate of 500 rotations per minute (rpm), using a 2.5 cm stir bar.

The carrier gas flow rate in the chromatographic column was 1.2 mL/min. The separation was isothermal at 50°C. The desorption voltage was 47 V for the experiments in which the trap was not cooled and 50°V for the ones in which the trap was cooled.

Results and discussions

The sorbent trap is supposed to retain all the analytes that pass through the sorbent bed until the breakthrough volume is reached. Thus, the only advantage that a cooled trap can offer is that longer trapping times can be used. In such a case, if the breakthrough volume is not reached, the amount extracted (for short trapping times) with the trap at room temperature should be equal to the amount extracted with a cooled trap. The experiment presented next was conducted in order to verify this.

The membrane was placed in the 120 mL jar that contained 40 mL of water. The water was stirred at 500 rpm. At time 0, benzene from the stock solution was spiked into the water, to obtain a 50 ppb concentration. The trapping started from time 0. Trapping times of 1, 2, 3, 4, and 5 minutes were used. In the first part of the experiment the trap was maintained at room temperature, and the amount extracted was measured for the trapping times mentioned above. In the second part of the experiment the trap was placed on the Peltier cooler and the experiment was repeated. The only difference was that the desorption voltage had to be

increased to 50 V to compensate for the lower temperature of the trap.

The results obtained during the experiment are presented in Figure 3- 14. The upper trace represents the amount extracted with the cooled trap. The lower trace was obtained with the trap at room temperature.

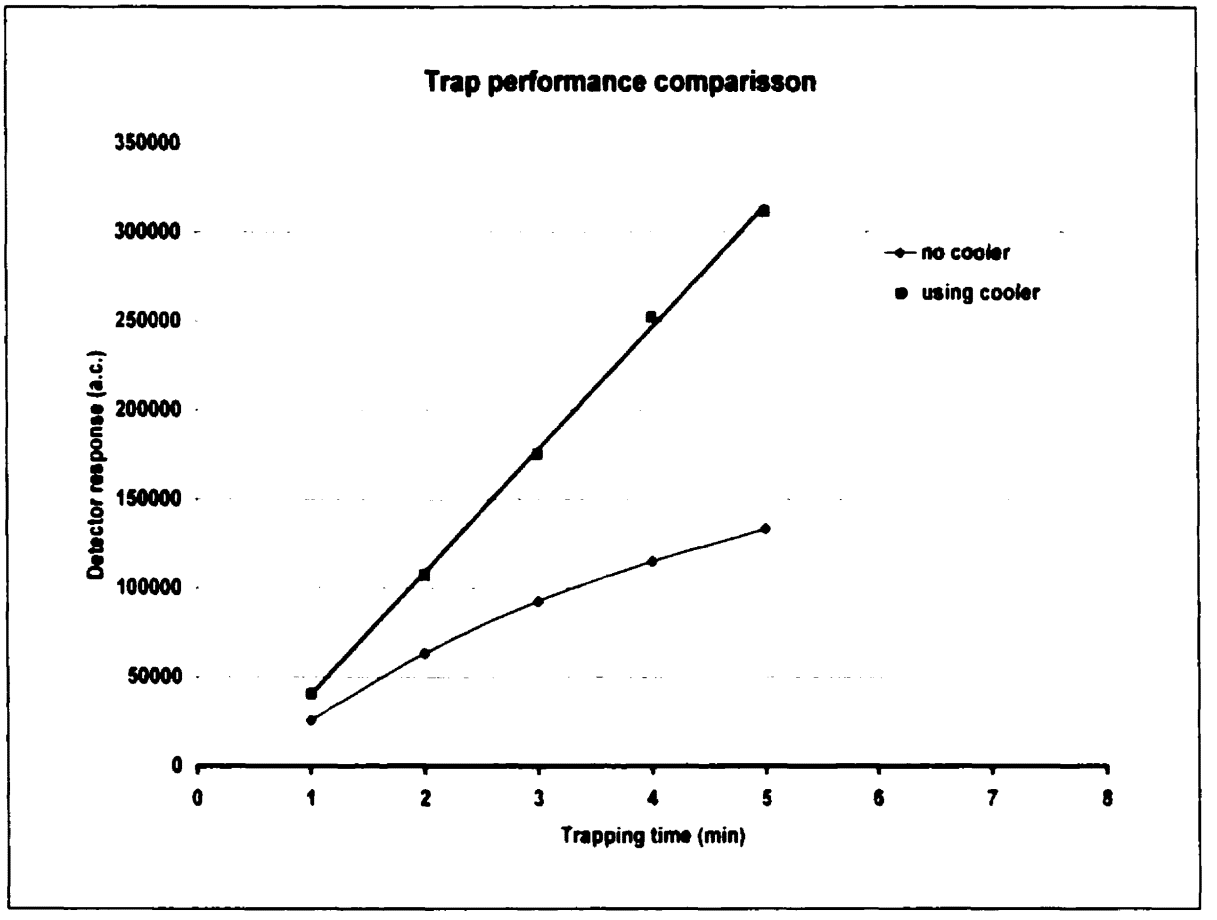


Figure 3- 14. Comparison of the trap performance at different temperatures

As it can be seen from the figure, the amount extracted with the cooled trap is higher than the one extracted with the trap at room temperature. The fact that the amount extracted when using the uncooled trap is increasing with the trapping time proves that the breakthrough

volume was not reached. However, in the case of the trap maintained at room temperature, the increase in the amount extracted is not perfectly linear, as it is for the cooled trap.

A possible explanation for this behavior is that the analytes are not completely retained by the sorbent due to channeling. The size of the sorbent particles being too large, a part of the analytes travel too far away from the sorbent surface and thus can not be retained. The solution to this problem would be to use smaller size particles. In all the traps used so far, the sorbent mesh was 50 - 60. In order to try to solve this problem, particles of smaller size were used (80 -100 mesh).

The sorbent bed acts as a heat sink in the sorbent trap. When the heating pulse is applied, the ends of the trap that do not contain sorbent reach higher temperatures than the middle of the trap, where the sorbent is placed. The more sorbent is placed in the trap, the bigger the difference between the temperature of the middle and the ends of the trap will be. Higher desorption temperatures will be needed to obtain efficient desorptions. At the same time, the heating pulse will have to be able to maintain the maximum temperature for a longer time (a few seconds maybe), so that the sorbent can be heated uniformly.

A new sorbent trap was used, packed with 80 - 100 mesh particles. The flow rate dropped by 0.5 mL/min after the trap was connected in the system and it had to be adjusted to the initial value by increasing the pressure. The first concern was that the trap would not be efficiently desorbed due to the limitations of the power supply. In order to verify this, the membrane was exposed to the same benzene solution. The carryover in the trap was measured. The sorbent trap was pulsed every minute until the extraction maximum was reached. The membrane was then bypassed and the trap continued to be pulsed every 90 seconds. One of the chromatograms obtained this way is presented in Figure 3- 15.

As expected, the desorption from this trap was very inefficient. It can be seen that two desorptions after the membrane was bypassed gave almost the same amount of benzene as the desorption obtained with the membrane exposed to the sample. The peaks started to decrease slowly, reaching about 50% of the initial value after 6 desorptions (9 minutes). Thus, with the existing power supply, a tightly packed trap can not be used.

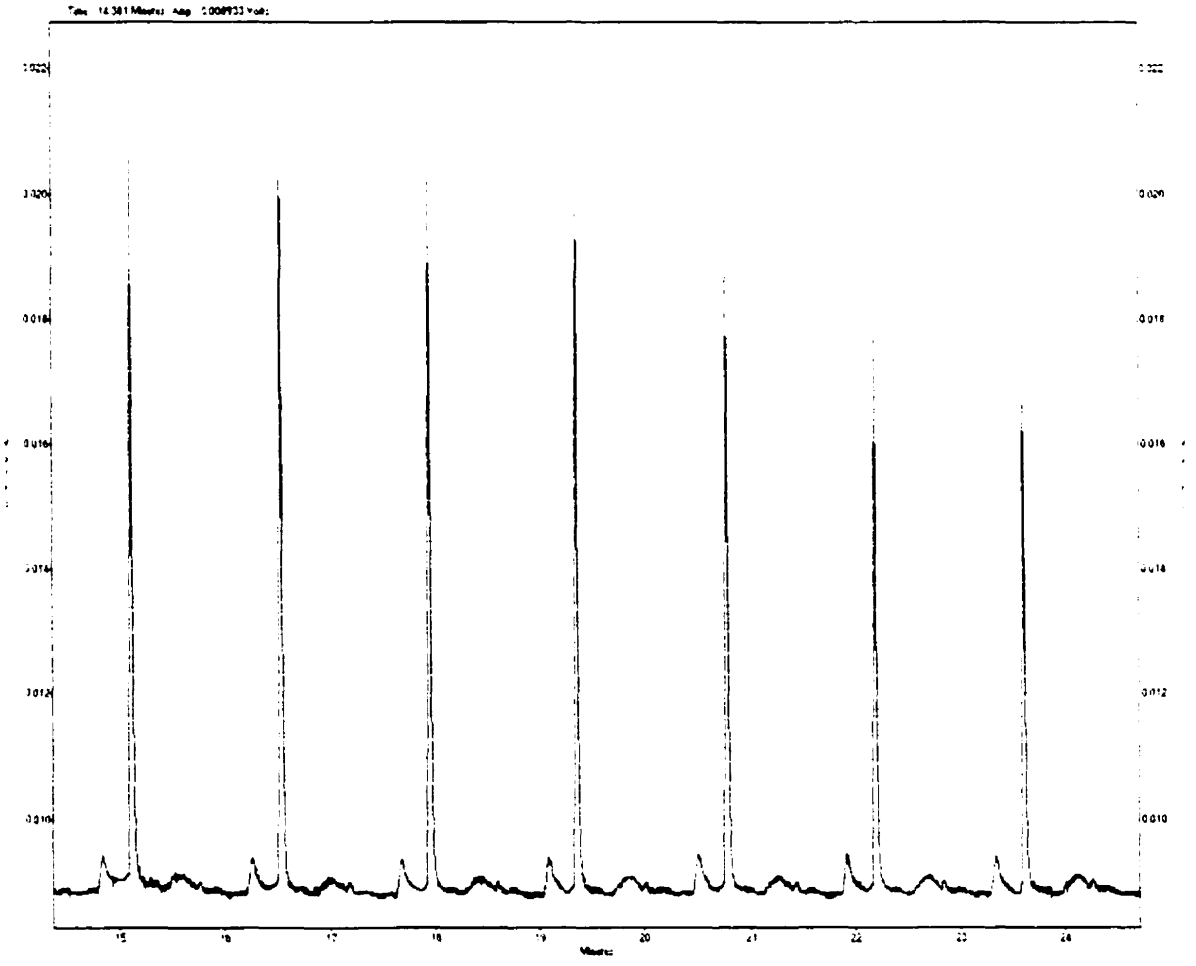


Figure 3- 15. Chromatogram obtained for benzene carryover in the tightly packed trap

Being unable to use a densely packed trap in the present MESI system the only way to improve the trapping efficiency is by cooling. Since longer trapping times are to be used in order to increase the sensitivity of the method, the trap performance was examined using trapping times as long as 27 minutes. The longest trapping time used with the trap at room temperature was 11 minutes. For the cooled trap, trapping times ranging from 15 to 27 minutes were used.

The same benzene solution was used and the experiments were conducted in the same way as the previous experiment, when the trap performance at different temperatures was examined. The results obtained are presented in Figure 3- 16.

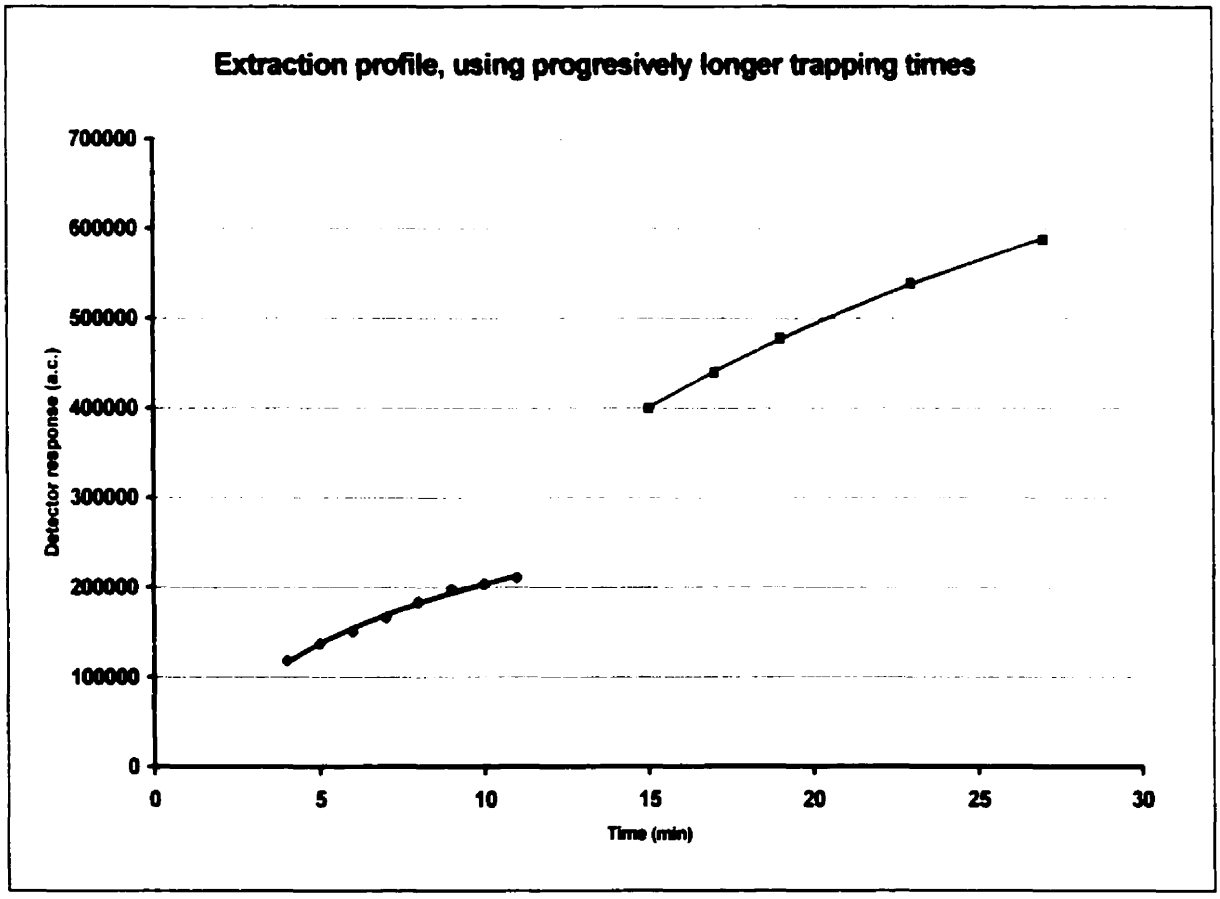


Figure 3- 16. Extraction profile using long trapping times

The extraction profile for the cooled trap is not perfectly linear anymore when long trapping times are used. However, the amount extracted at 27 minutes trapping time is bigger than the amount extracted using a 23 minutes trapping time. This indicates that the breakthrough volume has not been reached.

The experiments proved that the trapping efficiency is indeed improved by cooling. However, the nonlinear profile indicates that some losses occur, and that probably a more tightly packed trap should be used if a different power supply could provide an efficient desorption pulse for such a trap.

3.5 Summary

The sorbent trap is a very important part of a MESI system. It provides sample preconcentration, leading to improved sensitivity.

The packed sorbent trap allows for the use of longer trapping times. At the same time, the new sorbent trap is more conveniently designed for field analysis, since it has no movable parts. The stainless steel body of the new trap is also more resistant than the fused silica body of the old trap, and that makes it reusable for a very long period of time, without the risk of breaking it.

Even though the trap should retain all the analytes that pass through it until the breakthrough volume is reached, the present design fails to do so because of the large size of the sorbent particles. The use of a more tightly packed trap would solve the channeling problem, but a different power supply would have to be used in order to desorb the analytes efficiently.

The use of coolers improves the sorption efficiency of the trap. At the same time. longer trapping times can be used. Trapping times as long as 25-30 minutes can be used to improve the sensitivity of the system.

By placing a short (5 mm) piece of fused silica capillary having an inner diameter of 100 μm in front of the sorbent trap. the back flush of analytes is prevented during the desorption pulse. This reduces significantly the carryover in the sorbent trap.

The newly designed trap presents significant advantages versus the old version of the trap. However. future research could improve its design.

3.6 References

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CHAPTER 4

DETECTORS USED IN CONNECTION WITH MESI FOR ENVIRONMENTAL SAMPLE ANALYSIS

4.1 Introduction

Detectors are of great importance in analytical instrumentation. Analytical steps such as sample preparation, preconcentration, and separation are meant to facilitate the detection of the compounds in a matrix. The sensitivity of an analytical measurement ultimately depends on the detector.

Numerous methods have been described for the detection of organic vapors in the effluent of a gas chromatograph.[1, 2, 3] Based on the nature of their response, detectors can be classified as universal, selective or specific. These descriptions are applied loosely, as no single detector exactly meets the dictionary obligations of these appellatives. The flame ionization and thermal conductivity detectors respond to the presence of nearly all organic compounds in the gas chromatographic effluent and are considered to be general or (near) universal detectors. Other detectors respond only to the presence of a particular heteroatom (e.g., the flame photometric, thermionic ionization, or atomic emission detectors) and are considered to be specific detectors. These detectors are able to discriminate between some property of the organic compound of interest and an organic compound lacking that property. The response of detectors such as the electron-capture and photoionization detectors, is selective. These detectors are not elemental selective, but rather structure selective.

The choice of the detector is determined by the application. In many cases chemists face the challenge of having to analyze only a few compounds from a very complex matrix. In such a case it is desirable to use a selective detector, which responds to the target analytes and suffers little interference from the other compounds present in the matrix. However, there are cases in which all the compounds (or as many as possible) present in a sample have to be determined. A universal detector would be the choice in such a case.

Chemists rarely want to determine all the compounds present in a sample. Usually, analytical methods are developed to enable the determination of a group of compounds. If the target analytes are present in a complicated matrix, containing hundreds of other compounds, their determination can become very difficult. Separating the sample by chromatography before introducing it into the detector can facilitate the determination of the target analytes. However, sometimes chromatography can not resolve all the peaks for a very complex mixture. The use of an additional selective element in such a case would ease the analytical measurement. In a MESI system, selectivity is achieved by using the membrane for sample introduction.

Different detectors can be used for MESI applications. Since selectivity can be achieved by changing the chemistry of the membrane and the samples are separated using gas chromatography, universal detectors can be successfully used in conjunction with MESI. However, other aspects of the applications have to be considered when choosing the right detector. For example, since one of the major advantages of MESI is its use for field analysis, it is important to be able to couple the system to a detector that can be easily transported in the field.

A chromatogram provides information regarding the complexity (number of compounds), quantity (peak height or area) and identity (retention time) of the components in

a matrix. Of these parameters, the certainty of identification based solely on retention is considered very suspect, even for simple mixtures. When the identity can be firmly established, the quantitative information given by the chromatogram is very good. Thus, it is also very important to couple MESI to a detector that can identify the compounds.

The advantages and disadvantages of having MESI coupled to different detectors were examined. Gaseous and liquid samples were analyzed using a flame ionization detector (FID), a quadrupole mass spectrometer (MS), and thermal conductivity detector (TCD). The limits of detection, speed of analysis, capability of analyte identification, and applicability to various samples were compared.

4.2 Results obtained using a flame ionization detector

A nearly universal response to organic compounds, high sensitivity, long term stability, simplicity of operation and construction, low dead volume, fast signal response, and exceptional linear response range have contributed to the FID being the most popular detector in current use. Only some gases (e.g., He, Xe, H_2, N_2, NH_3, SO_2), certain nitrogen oxides (e.g., N_2O, NO), compounds containing a single carbon atom bonded to oxygen or sulfur (e.g., CO_2, CS_2), water and formic acid do not provide significant detector response. Minimum sample detectability corresponds to about 10^{-13} g carbon/s with a linear response range of 10^6 to 10^7.[4]

The response of the FID results from the combustion of organic compounds in a small hydrogen-air diffusion flame, which burns at a capillary jet. The carrier gas from the column is premixed with hydrogen and burned in a chamber through which excess air is flowing. A cylindrical collector electrode is located a few millimeters above the flame and the ion current

is measured by establishing a potential between the jet tip and the collector electrode. The performance of the detector is influenced by experimental variables, of which the most important are the ratio of air-to- hydrogen-to-carrier gas flow rates, the type of carrier gas, and individual detector geometry.

The fact that FID uses hydrogen and air for the flame and nitrogen as makeup gas, makes it very inconvenient to be used for field applications. Currently, researchers are trying to build a field portable FID. The new design tries to create a smaller FID that would not require the use of a makeup gas. At the same time, the air required for the flame is obtained from the atmosphere by using a pump. Thus, if hydrogen were to be used as carrier gas for the chromatographic column, only one gas cylinder would have to be transported in the field.

If the FID could be easily transported in the field, the only drawback of the detector would remain its incapability of identifying the analytes. As mentioned before, the identification of compounds based only on the retention times is not considered to be very accurate.

4.2.1 System requirements and limit of detection

Coupling MESI to a gas chromatograph equipped with a flame ionization detector was easily achieved, owing it to the fact that FIDs are very robust detectors. There were no special requirements for the system, which proved to be very reliable. The only concern was that, due to permeation through the membrane, the water present in the carrier gas would extinguish the flame. However, the problem was never encountered.

Sorbent trap on a Peltier cooler

Power supply

Membrane in a sampling jar

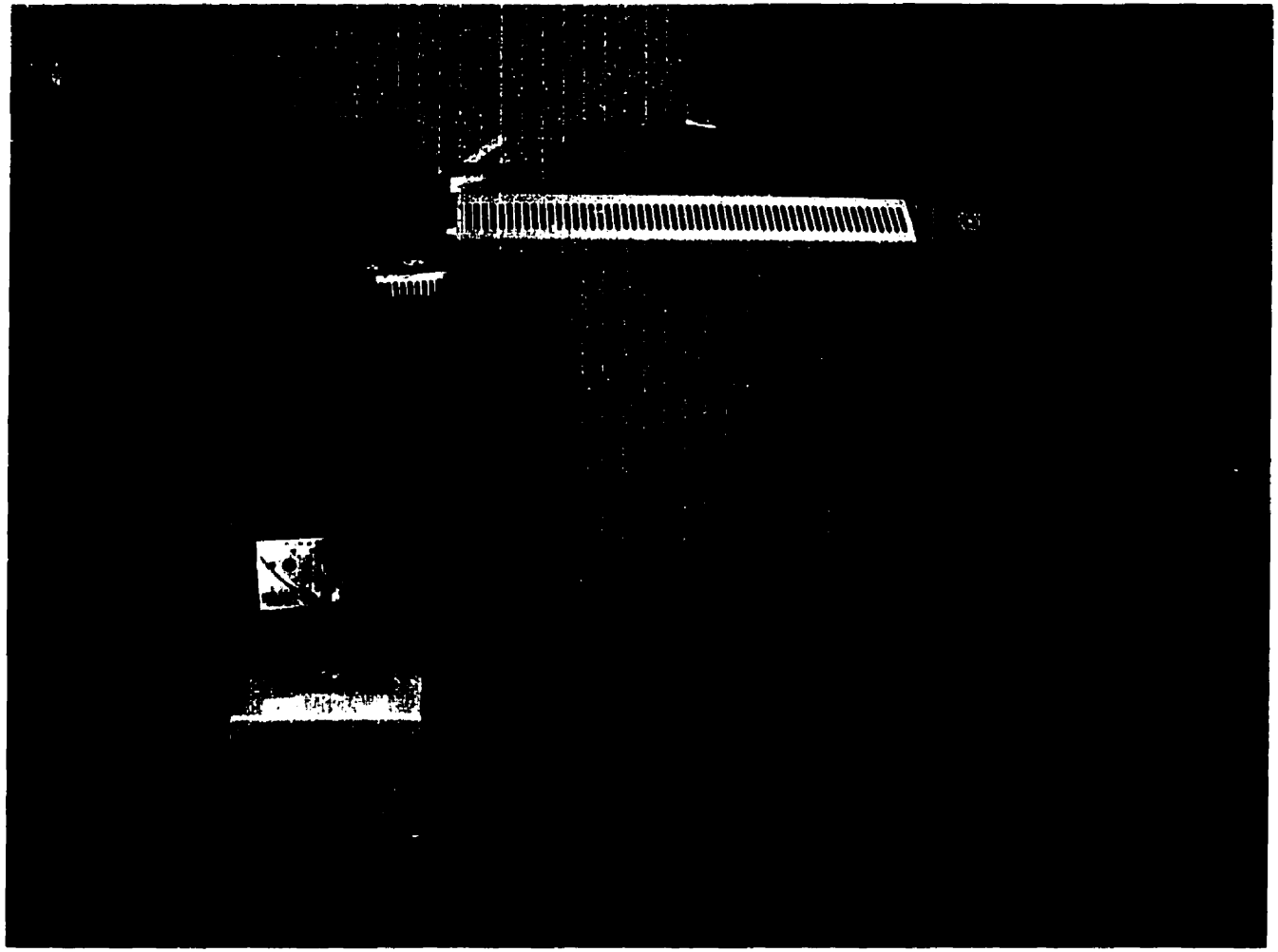

Figure 4- 1. MESI system coupled to a GC-FID

The MESI system was connected to a CP 9002 gas chromatograph (Chrompack), by mounting the sorbent trap on the wall of the GC (Figure 4- 1). The column was taken out of the oven through the insulation, and connected directly to the trap. There were no cold spots in the chromatographic column, since the trap was placed on the wall of the oven and the piece of the column required for connection was placed inside the trap assembly.

Since the system was designed for laboratory use, the trap was placed on a Peltier cooler. All the theoretical aspects of MESI were investigated using this MESI-GC-FID configuration.

Experimental setup

The gas chromatograph used in these experiments was a CP-9002 model (Chrompack, Middelburg, The Netherlands). The GC was equipped with a flame ionization detector (FID) and a 10 m, 0.25 mm i.d., 0.15 μm film thickness CP Sil 5 CB column (Chrompack). A flat-sheet Silicone Polycarbonate Membrane (SSP-M213), of 25 μm thickness, purchased from Membrane Components (Ballston Spa, NY, USA) was used for extraction. This particular membrane material has been chosen because it is less permeable to oxygen than Poly(dimethylsiloxane). XAD-2 resin (Polystyrene divinyl benzene) purchased from Supelco (Oakville, ON, Canada) was used as packing material for the sorbent trap. The length of the sorbent bed was 1.3 cm. A three stage Peltier cooler (Melcor Corp., Trenton, NJ) was used to decrease the temperature of the sorbent trap. The same custom-made power supply that was described in the previous chapter was used for the desorption of analytes from the trap, and the desorption pulses were controlled by the same Omron external timer. Ultra high purity

nitrogen, helium, hydrogen and air required for the FID and the chromatographic column were purchased from Praxair (Kitchener, ON).

A standard mixture containing benzene (B), toluene (T), ethylbenzene (E), and o-xylene (X) was analyzed in order to establish whether the system was working. A stock solution was prepared by dissolving BTEX of 99+% purity (Aldrich Chemical Company, Inc.) into HPLC grade methanol (Aldrich Chemical Company, Inc.). By spiking an appropriate amount of the stock solution in 40 mL water, a final concentration of 30 ppb for each compound was obtained. A 120 mL sampling jar was used, and the sample was stirred using a 5 cm stir bar at 500 rpm.

For the determination of the detection limit, a benzene solution was used. A stock solution was obtained by dissolving benzene of 99+% purity (Aldrich Chemical Company, Inc.) into deionized water. In order to obtain low concentrations (50 - 400 ppt), the stock solution was prepared by multiple dilutions. Appropriate amounts of the stock solution were spiked into 40 mL water to obtain concentrations of 50,100, 200 and 400 ppt of benzene in water. A 120 mL sampling jar was used, and the sample was stirred at 500 rpm.

The column temperature was set to 30°C for both sets of experiments. The carrier gas flow rate was 1.3 mL/min. The desorption voltage was 50 V. Trapping times of 6 min and 10 minutes were used.

Results and discussions

After the membrane and the trap were connected to the CP 9002 GC, the system was tested for leaks. Since there were so many connections in the system the risk of having leaks was very high.

In order to test the performance of the system, the BTEX mixture was analyzed. A chromatogram obtained for the 30 ppb BTEX mixture, using a trapping time of 6 minutes is presented in Figure 4- 2.

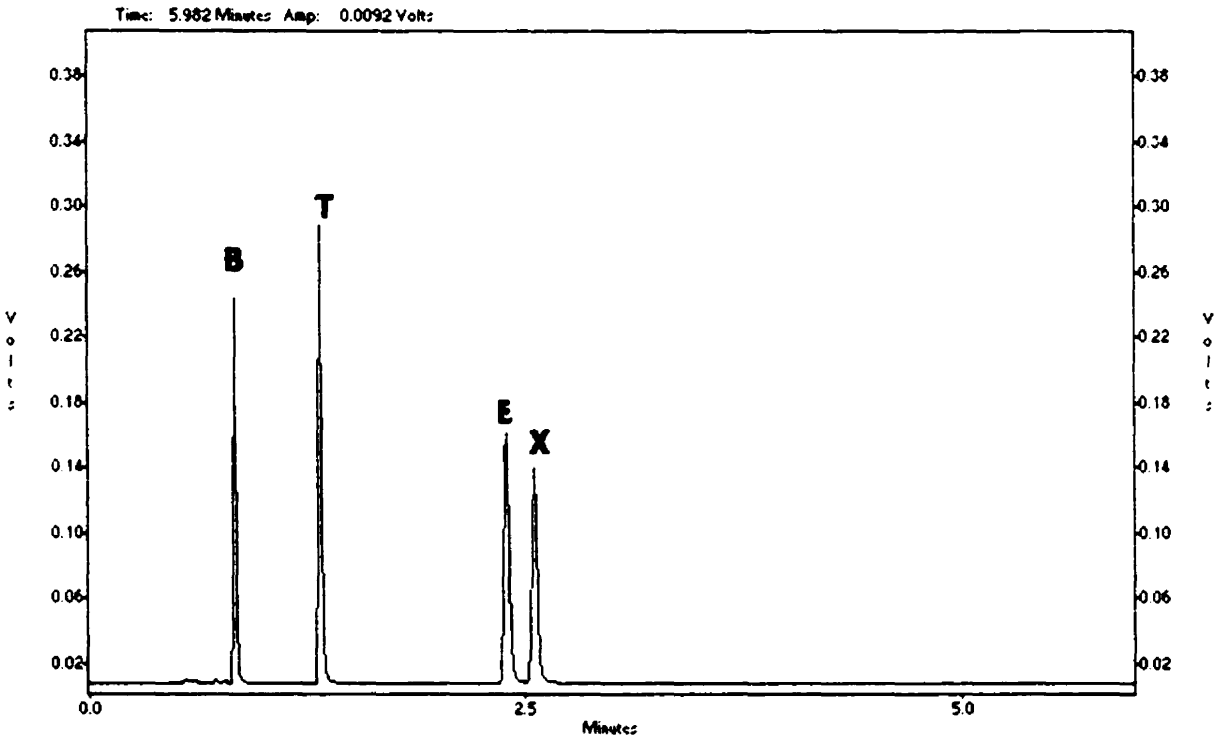

Figure 4- 2. Chromatogram obtained for a mixture of benzene (B), toluene (T), ethylbenzene (E), and o-xylene (X), using MESI connected to GC-FID

As it can be seen in the chromatogram, the peaks are very well defined and have a very good shape. A very fast separation of the compounds was achieved even at a very low column temperature (30°C), due to the fact that the column was short (only 10 m), and the film thickness was only 0.15 μm.

The baseline of the chromatogram is very stable, and the noise can not be seen, meaning that the detection limit of the system is much below the value of 30 ppb that was used for this experiment.

Benzene was chosen for measurement of the detection limit since the peak obtained for benzene was neither the biggest nor the smallest one of the BTEX peaks. Results were obtained for 50, 100, 200, and 400 ppt. The membrane was placed in the sampling vial containing 40 mL water. The water was stirred at 500 rpm, and then the stock solution was spiked into the jar, to obtain the desired concentration. The solution was allowed to mix for one minute after which the sampling started. Trapping times of 10 minutes were used. The peak height was measured in order to determine the limit of detection. The calibration curve is presented in Figure 4- 3.

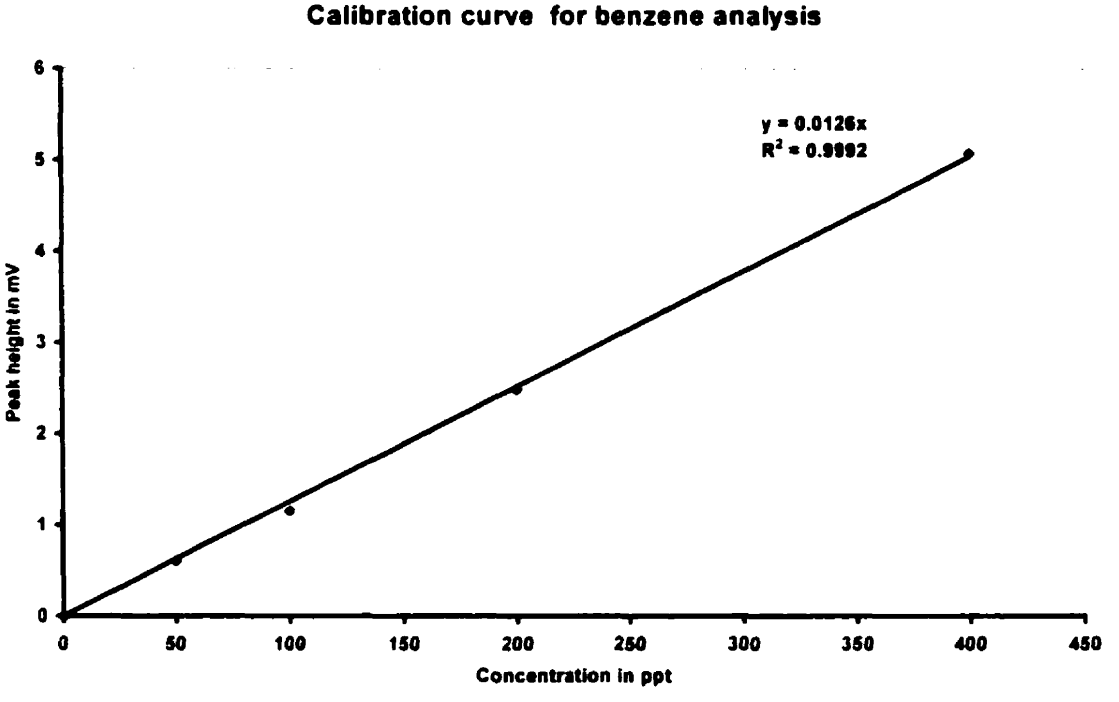

Figure 4- 3. Calibration curve for benzene analysis, using MESI-GC-FID

The limit of detection was calculated using the calibration curve and it was 10 ppt for benzene, with a noise level of $3.3*10^{-5}$ V. However, the system was not operated at its limits. The limit of detection could be improved by using longer trapping times. However, with such sensitivity, the system could be used for analysis of trace levels of VOC in various matrices.

4.2.2 Applications of the MESI-GC-FID system, for environmental samples analysis

Volatile organic compounds were analyzed in gaseous, liquid and solid samples. Headspace analysis was used since it protected the membrane from potentially dirty matrices. At the same time, the diffusion of analytes is faster in air than it is in liquid.

Tap water, cigarette smoke, and eucalyptus leaves were analyzed. The permeation of benzene and toluene through Latex gloves was also evaluated, as an application of MESI.

Experimental design

The same CP 9002 gas chromatograph was used for separation of the analytes. The column was a SPB-5 (Supelco, Oakville, ON, Canada), 30 m long, 0.25 mm i.d., and the film thickness was 0.25 μm. XAD-2 resin (Polystyrene divinyl benzene) purchased from Supelco (Oakville, ON, Canada) was used as packing material for the sorbent trap. The length of the sorbent bed was 1.3 cm. The trap was cooled using the same type of cooler as in the previously mentioned experiments. Silicone hollow fiber membrane (Baxter Healthcare Corp., McGaw Park, IL) of 330 μm thickness and 9 cm length was used for extraction. The previously described capacitive discharge power supply was used for analyte desorption. The

desorption voltage was set to 50 V. Helium (Praxair, Kitchener, ON) was used as carrier gas, and the flow rate was 1.5 mL/min.

For the experiments in which *tap water* was analyzed, water from one of the laboratory sinks, as well as water from Walkerton, ON was analyzed. The water was placed in a 600 mL sampling jar and 350 mL of sample was used. During extraction, the sample was stirred at a speed of 500 rpm, using a 7 cm long stir bar. The membrane was placed in the headspace and trapping times of 25 minutes were used for the laboratory water, and 15 minutes for the water from Walkerton. The temperature program used for the separation of the compounds present in water was:

30°C (5 min) $\xrightarrow{10°C / min}$ 200°C (10 min)

For the analysis of *eucalyptus leaves*, Dunnii-a trees were used. The trees were kept in the University green house. About 10 leaves were cut in small pieces and were placed into a 600 mL sampling jar. Fresh leaves (collected in the same day the analysis was performed) and old leaves (collected 2-3 days ahead, and kept in a closed jar) were analyzed. The membrane was placed above the leaves. The jar was kept at room temperature. Trapping times of 10 minutes were used. The temperature program used for the separation of the compounds was:

50°C (5 min) $\xrightarrow{10°C / min}$ 250°C (10 min)

Smoke from Dunhill cigarettes was analyzed by smoking the cigarette and exhaling the smoke into a 600 mL sampling jar. For each sampling, 2-3 puffs were collected. A part of

the smoke from each exhalation got into the sampling jar and a part escaped into the surrounding air. The membrane was placed in the sampling jar and static sampling was performed. Trapping times of 10 minutes were used. The temperature program used for the chromatographic column was the same as the one used for the separation of the compounds present in the tap water.

In order to evaluate the permeation of benzene and toluene through Latex gloves, a piece of glove was used instead of the membrane. The glove piece was immobilized in the membrane module. The results obtained by extraction via the Latex glove were compared with the results obtained when the same benzene and toluene mixture was analyzed using a SSP M-100 flat sheet membrane (Membrane Components, Ballston Spa, NY, USA) having a thickness of 50 μm.

Benzene and toluene of 99+% purity (Aldrich Chemical Company, Inc.) were dissolved in deionized water, to obtain a stock solution. Appropriate amount of the stock solution was spiked in 50 mL water to obtain a concentration of 100 ppb benzene and toluene in water. The membrane was placed in the sampling jar that had a volume of 120 mL. The sample was stirred at 500 rpm using a 5 cm long stir bar. Trapping times of 1 minute were used.

Results and discussions

Water analysis

The issue of water pollution was discussed in the first chapter of this thesis. Water obtained from two different locations was analyzed. The water obtained from Waterloo (one

of the sinks in the laboratory) was supposed to reflect the contamination level of average quality tap water. However, the analysis of the water obtained from Walkerton was intended to show the high level of contamination of water that, according to the present regulations, is still considered drinkable.

In May 2000 the tap water from Walkerton, ON became contaminated with a deadly strain of E. coli bacteria (O 157:H7) and was prohibited from domestic use for an extended period of time. A water sample was collected for analysis from a Walkerton house sink in December 2000, about a week after the water was declared drinkable.

The chromatogram obtained for the water sample from Waterloo is presented in Figure 4- 4. Since identification of the compounds was not possible because a flame ionization detector was used, we can only assume that one of the main peaks in the chromatogram is chloroform (due to the heavy chlorination of the water, chloroform should be present in large amount). The results obtained for the analysis of the water from Walkerton are presented in Figure 4- 5.

By comparing the chromatograms obtained for the two samples, it can be seen that some of the peaks are present in both chromatograms. Even though the sample from Walkerton was preconcentrated for only 15 minutes and the one from Waterloo for 25 minutes, it can be seen that the difference in the peak height is very big. The overlaid chromatograms of the two samples are presented in Figure 4- 6. The upper trace was obtained for the Walkerton sample, and the lower trace for the Waterloo sample.

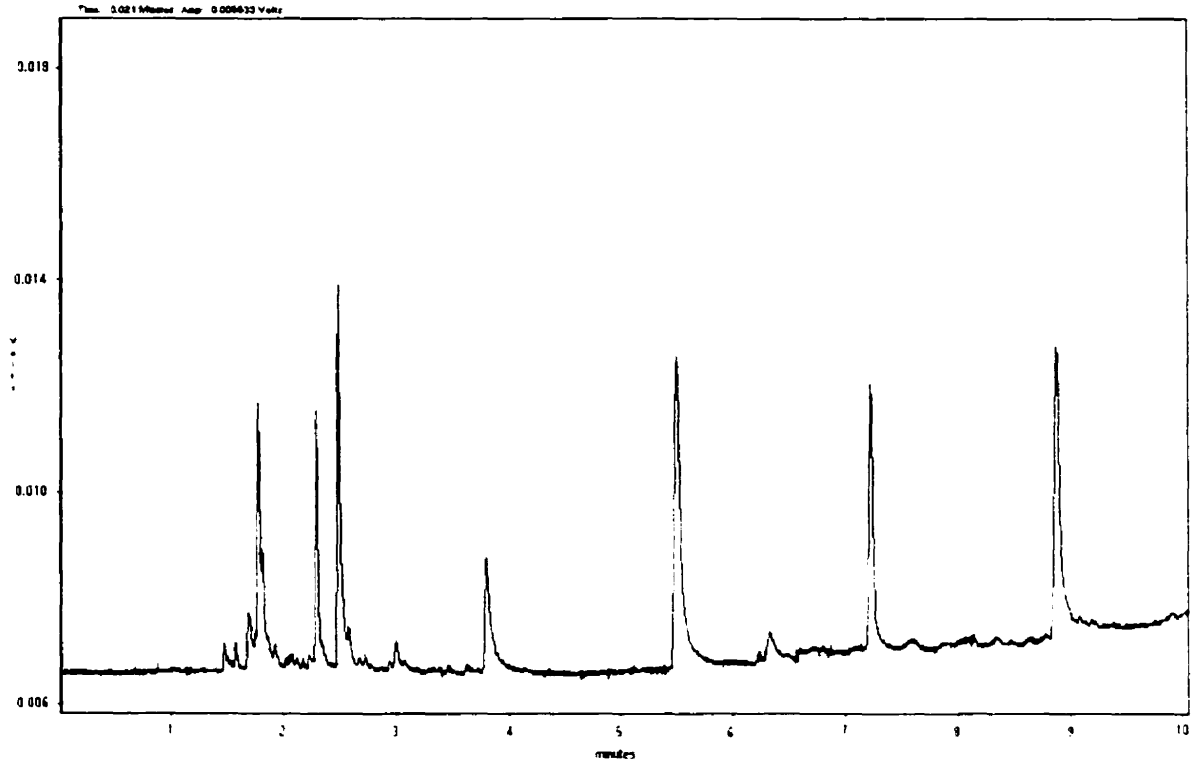

Figure 4- 4. Chromatogram obtained for the analysis of a Waterloo water sample

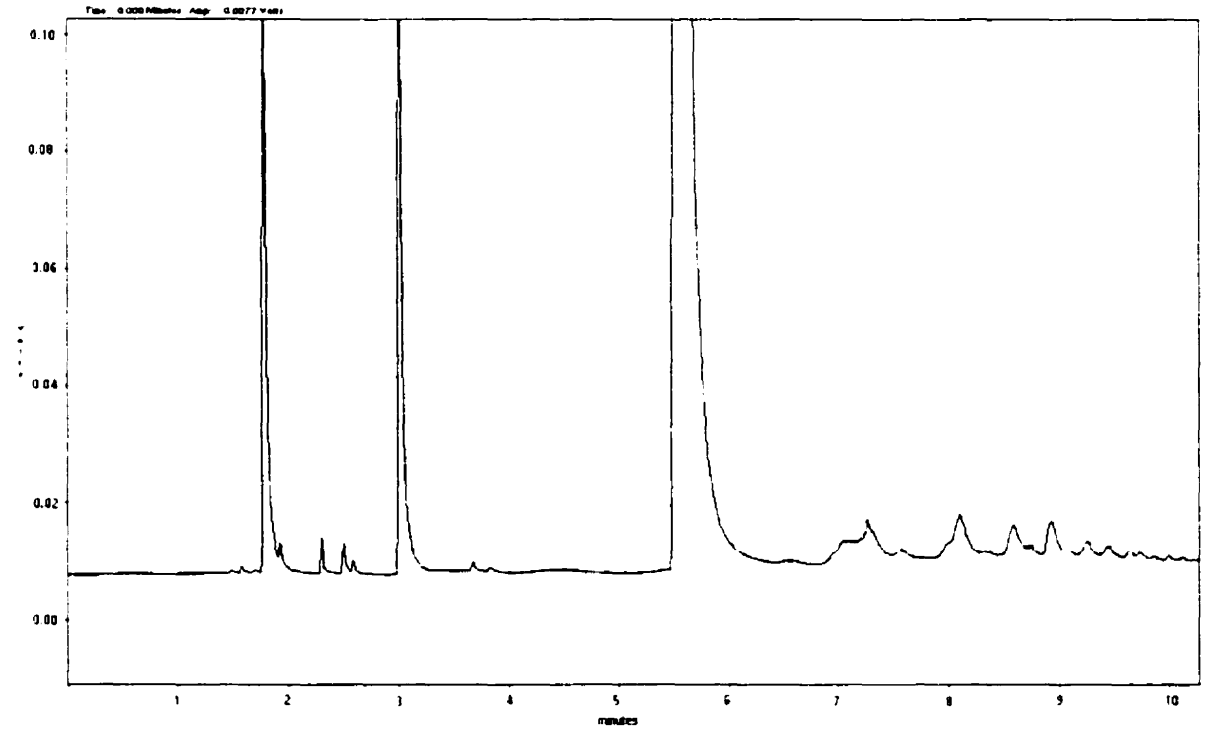

Figure 4- 5. Chromatogram obtained for the analysis of a Walkerton water sample

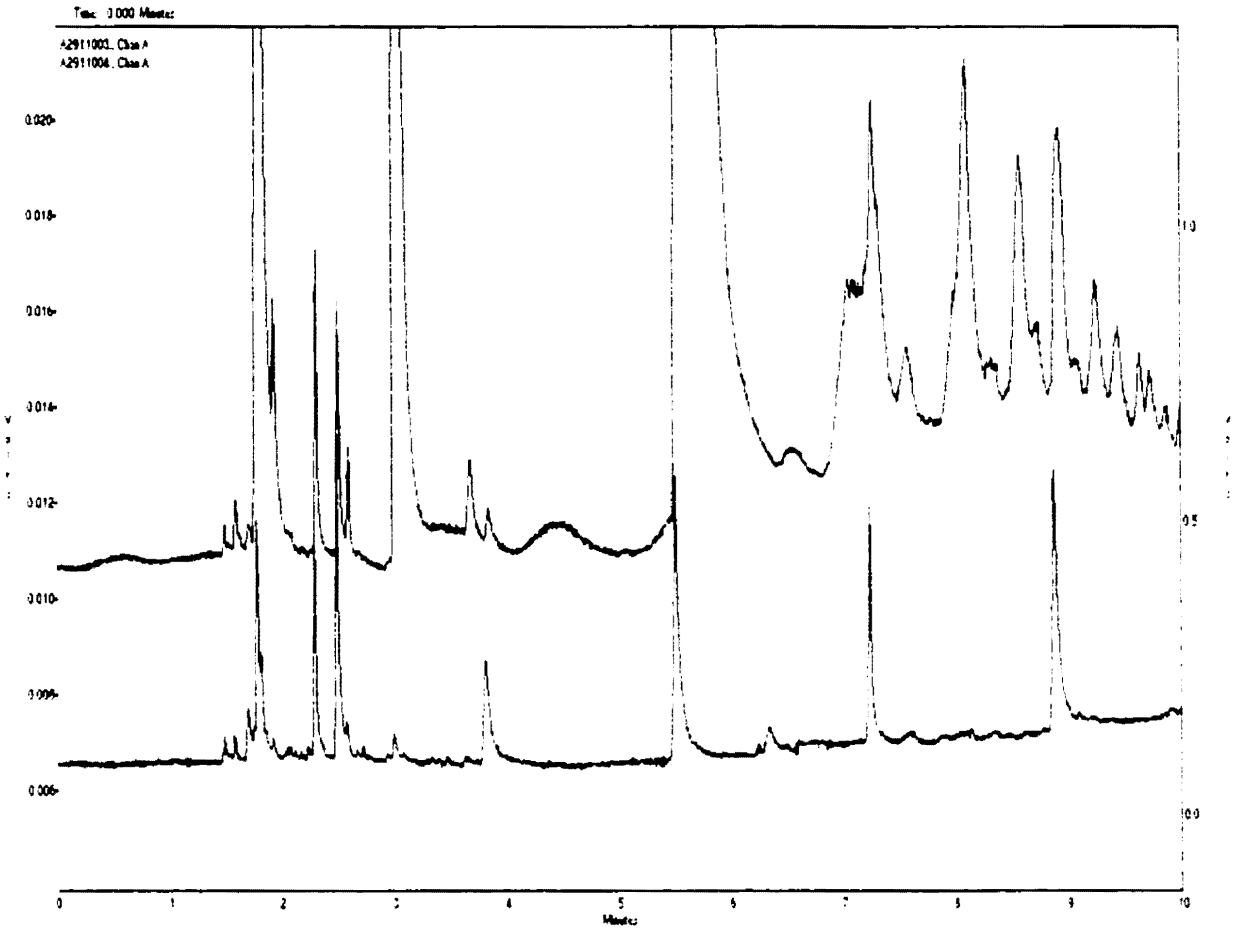

Figure 4- 6. Chromatogram comparison: upper trace = water sample from Walkerton; lower trace = water sample from Waterloo

The membrane used for the analysis of the two samples had a very big surface area, and the trapping times were relatively long. A universal detector was also used. Thus, the chromatograms contained many peaks. However, if only certain peaks are of interest, the selectivity can be increased by modifying the chemistry of the membrane, increasing the thickness of the membrane wall, decreasing the trapping time, or by using a selective detector.

Eucalyptus leaves

There is a great number of species of Eucalyptus trees yielding essential oils, the foliage of some being more odorous than that of others, and the oils from the various species differing widely in character. It necessarily follows that the term Eucalyptus oil is meaningless from a scientific point of view unless the species from which it is derived is stated.

The Eucalyptus industry is becoming of economic importance. The oils may be roughly divided into three classes that are of commercial interest: (1) the *medicinal* oils, which contain substantial amounts of eucalyptol (also known as cineol); (2) the *industrial* oils, containing terpenes, which are used for flotation purposes in mining operations; (3) the *aromatic* oils, such as *E. citriodora*, which are characterized by their aroma.

Eucalyptus leaves were chosen in order to test the applicability of MESI for the analysis of fragrances. Fresh and old eucalyptus leaves were analyzed, and the results were compared.

The fresh leaves were analyzed within hours from the time they were collected. Pieces of about 1 cm^2 were obtained by cutting the leaves. Because of the strong smell, a 10 minute trapping time was used. However, the results obtained by MESI sampling were compared with the results obtained by SPME sampling. The SPME and MESI measurements were conducted simultaneously, using the same samples.

In the SPME experiment (performed by another member of our research group), the same trapping time of 10 min was used. A PDMS fiber (having a coating thickness of 100 μm) was used. The temperature program for the chromatographic column was the same as the one used in the MESI experiment. However, the flow rate of the carrier gas was slower (1

mL/min). A Varian 3500 GC was used for sample separation. All the other experimental conditions were the same as for the MESI experiment. The SPME fiber was placed in the same sampling chamber as the membrane, and extraction using the two techniques was performed simultaneously. The comparison with SPME is mentioned because SPME is a well-established technique used worldwide, and extensive work in eucalyptus leaves analysis was conducted in our laboratory using this technique.

The old eucalyptus leaves were collected a few days before they were analyzed. The leaves were cut in small pieces the same day they were collected, and placed in a jar. The jar was covered with aluminum foil and was maintained at room temperature until the leaves were analyzed. The purpose of the experiment was to determine potential differences (the compounds emitted and difference in their concentration) between old and fresh leaves.

One of the chromatograms obtained by MESI sampling of fresh eucalyptus leaves is presented in Figure 4- 7. As a comparison, the chromatogram obtained for the same sample, using SPME extraction is presented in Figure 4- 8. As it can be seen from the chromatograms, two groups of compounds are present. The first group represents the very volatile compounds present in the eucalyptus oil, and the second group represents the less volatile group. If the temperature of the sample would have been elevated and a longer trapping time would have been used (to allow permeation through the membrane and extraction by the fiber), a third group containing less volatile compounds (that are released by the leaves at elevated temperatures) would have been present in the chromatogram.

In Figure 4- 7, the peaks from the first group are much smaller than the ones in the second group. However, the first group of peaks seems very small because the peaks from the second group are in fact extremely big. The peaks from the 0 - 5 minutes region of the

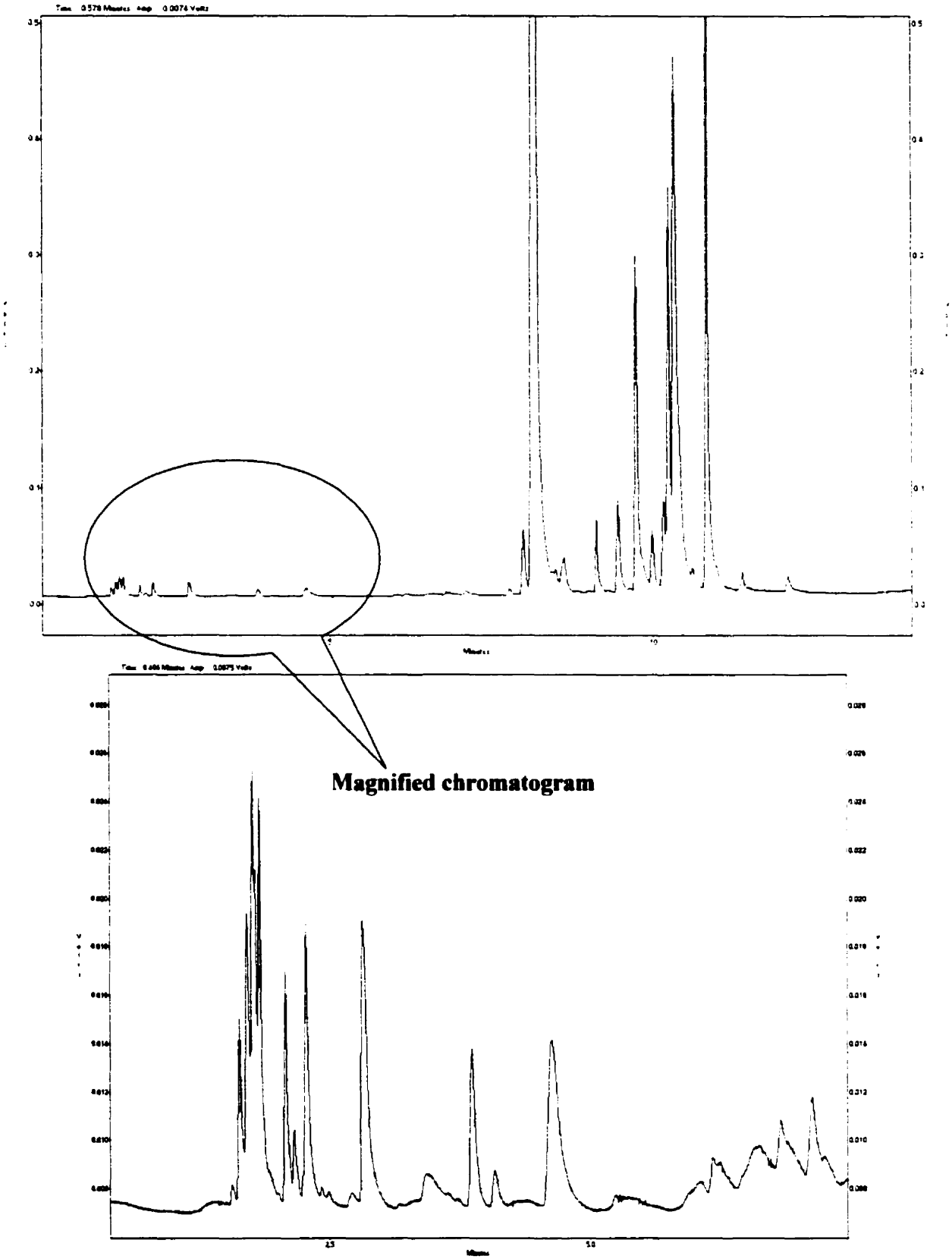

Figure 4- 7. Chromatogram obtained by analyzing fresh eucalyptus leaves using MESI

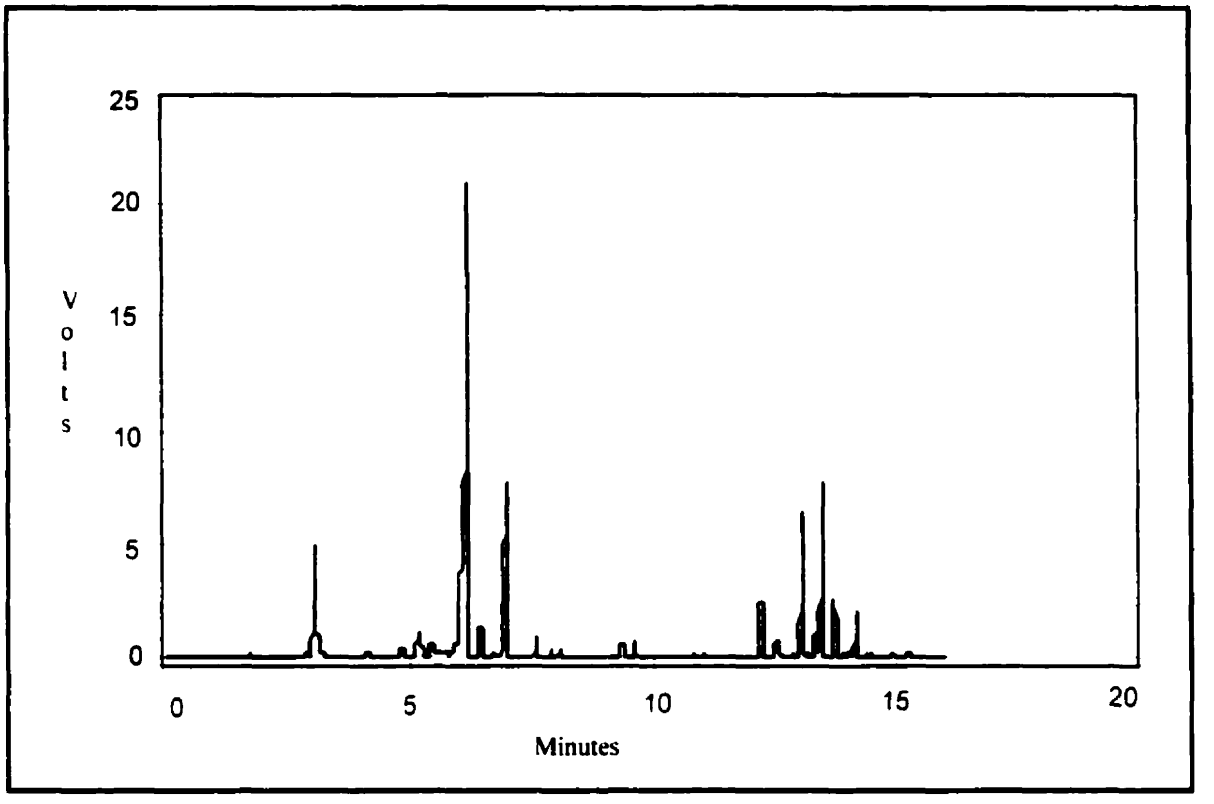

Figure 4- 8. Chromatogram obtained by analyzing fresh eucalyptus leaves using SPME

chromatogram can be seen better on a magnified scale. The average peak height was 0.02 for the peaks in the first group and 0.5 for the peaks in the second group, at a scale of a maximum 1 V. The difference in the peak height is not observed in the chromatogram obtained for the SPME experiment. This fact indicates that, even though the two groups of peaks can be seen using both sampling techniques, there is a significant difference between the two sets of results.

The difference in the height of the peaks from the two groups could be explained in two ways. Owing it to the fact that some of the compounds from the second group are present in very high concentration, either the permeation through the membrane was affected (the

chemistry of the membrane was modified because of the presence of a high concentrations of compounds in it at a certain time), or displacement occurred at the surface of the sorbent.

When old eucalyptus leaves were analyzed (Figure 4- 9) by MESI the two sets of peaks were of comparable heights. Due to the fact that the experiments were performed on different gas chromatographs, the chromatogram scales are different (for the MESI and SPME experiments). However, when the signal to noise ratios of the two sets of results were compared (peak to peak noise levels were 7*10-5 V for MESI and 0.1 V for SPME), MESI

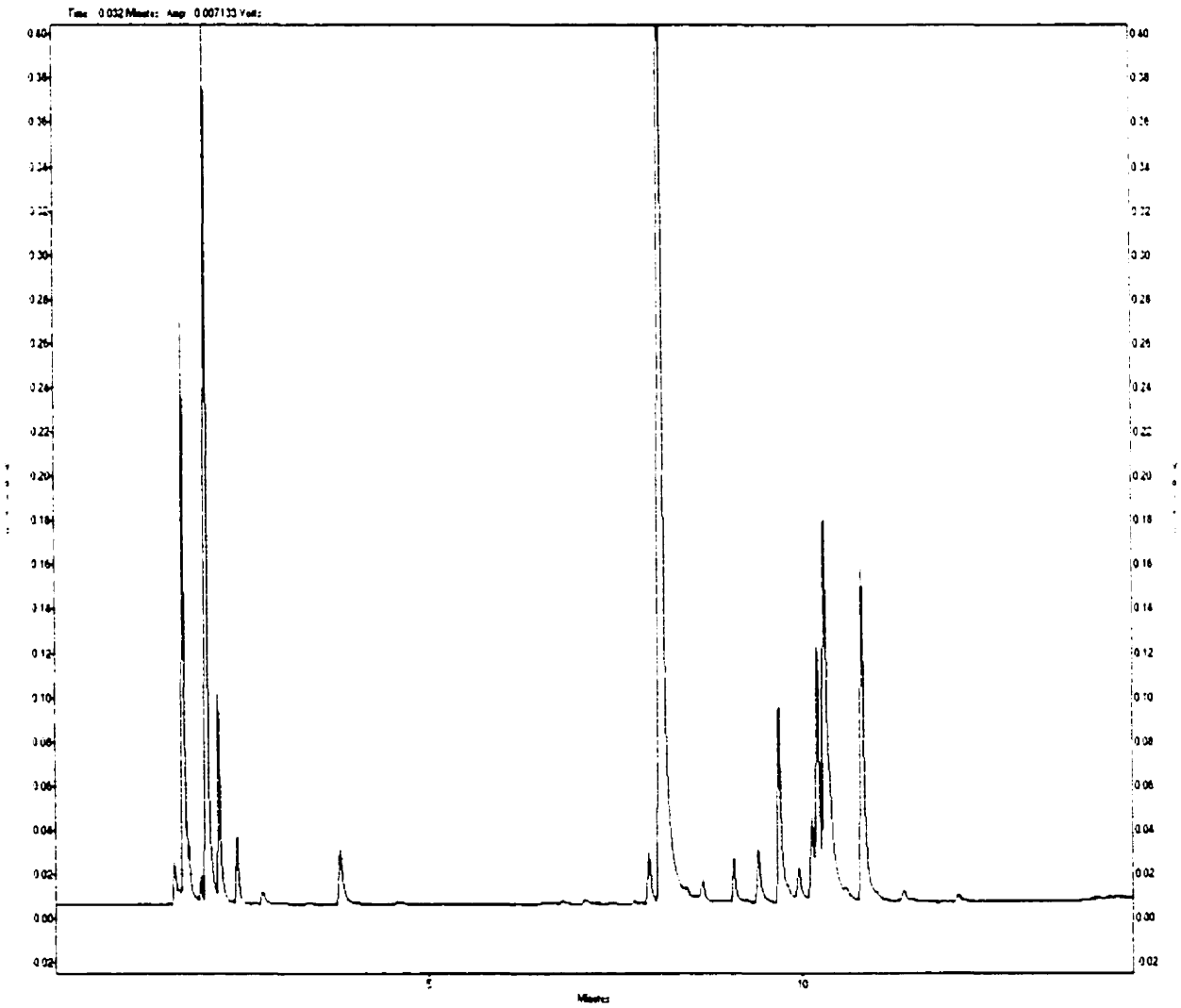

Figure 4- 9. Chromatogram obtained by analyzing old eucalyptus leaves using MESI

proved to be more sensitive.

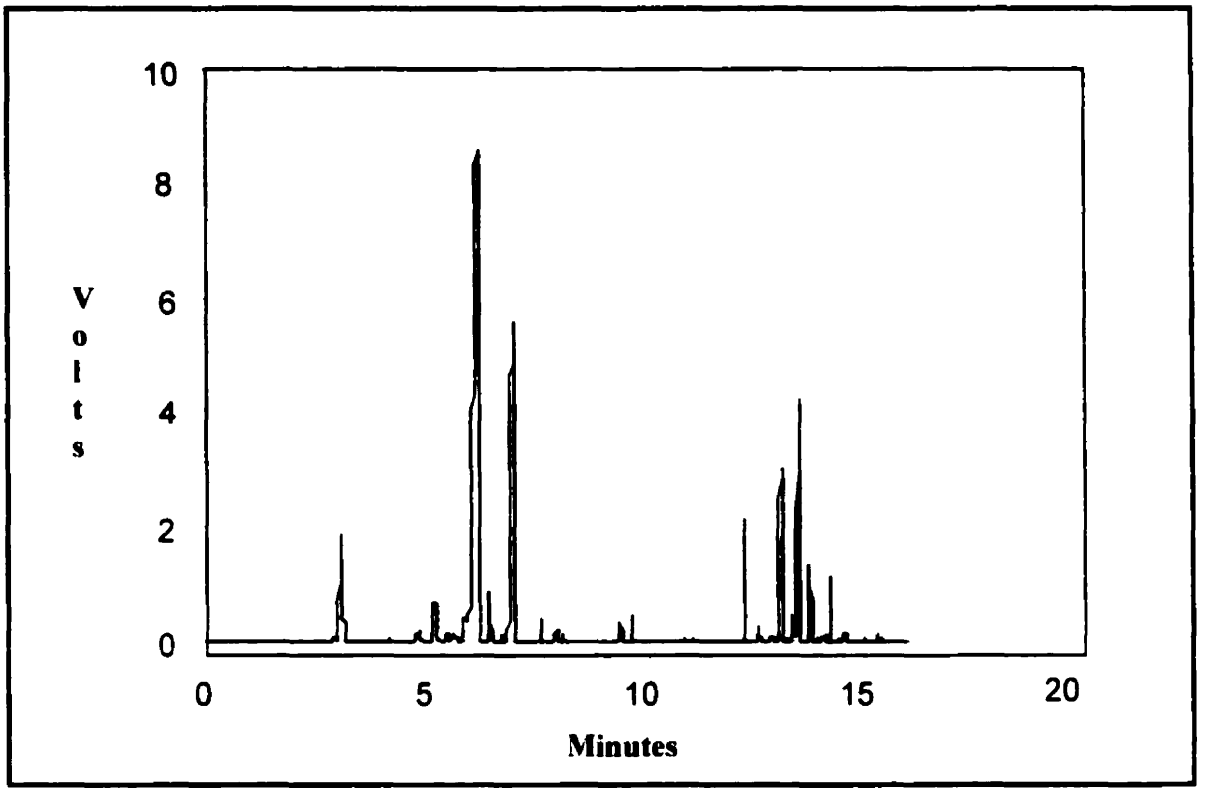

Figure 4- 10. Chromatogram obtained by analyzing old eucalyptus leaves using SPME

Thus, there are in fact differences between the fragrance of fresh leaves and the fragrance of old leaves. Since the peaks were not identified and quantitative measurements were not performed, the only conclusion that can be drawn from the experiment is that over time, the leaves lose some of their original fragrance (reflected in smaller peaks).

The experiments proved that MESI could be applied for the analysis of fragrances, and the method could compete with state of the art sampling techniques, like SPME.

Cigarette smoke analysis

Over 300 non-smokers die each year in Canada from lung cancer caused by tobacco smoke. In addition to lung cancer, involuntary smoking has been linked to cancer of the sinuses, brain, breast, uterine cervix and thyroid, leukemia, and lymphoma.[5]

Second-hand smoke is made up of exhaled smoke from smokers. It also comes from smoldering cigarettes, cigars or pipes. This smoke contains many of the same harmful chemicals that a smoker inhales. Some of these chemicals are even stronger in smoke from a cigarette sitting in an ashtray than they are when the smoke is inhaled. Tar, nicotine, acetaldehyde, isoprene, benzene, toluene, phenol, and formaldehyde are some of the nasty compounds present in the cigarette smoke.[6]

Thus, it is very important to be able to determine the concentration of toxic compounds present in cigarette smoke. MESI could provide continuous monitoring of the air contaminated with such compounds.

Dunhill cigarettes were chosen for the experiment since they are considered to be strong, based on the tar content. The collection of the smoke in the sampling chamber tried to simulate the conditions to which a second hand smoker would be exposed. A relatively long trapping time (10 min) was used in order to get a complex chromatogram, since the experiment tried to prove that MESI could be used for continuous monitoring of toxicants in air.

The chromatogram presented in Figure 4- 11 was obtained using the above-described conditions. As it can be seen in the chromatogram, a very large number of volatile compounds were present in the sample. The peaks could not be very well separated since the

number of compounds eluting in a very short time interval was too large. The measurement of certain (target) compounds could be achieved only by introducing selectivity in the system.

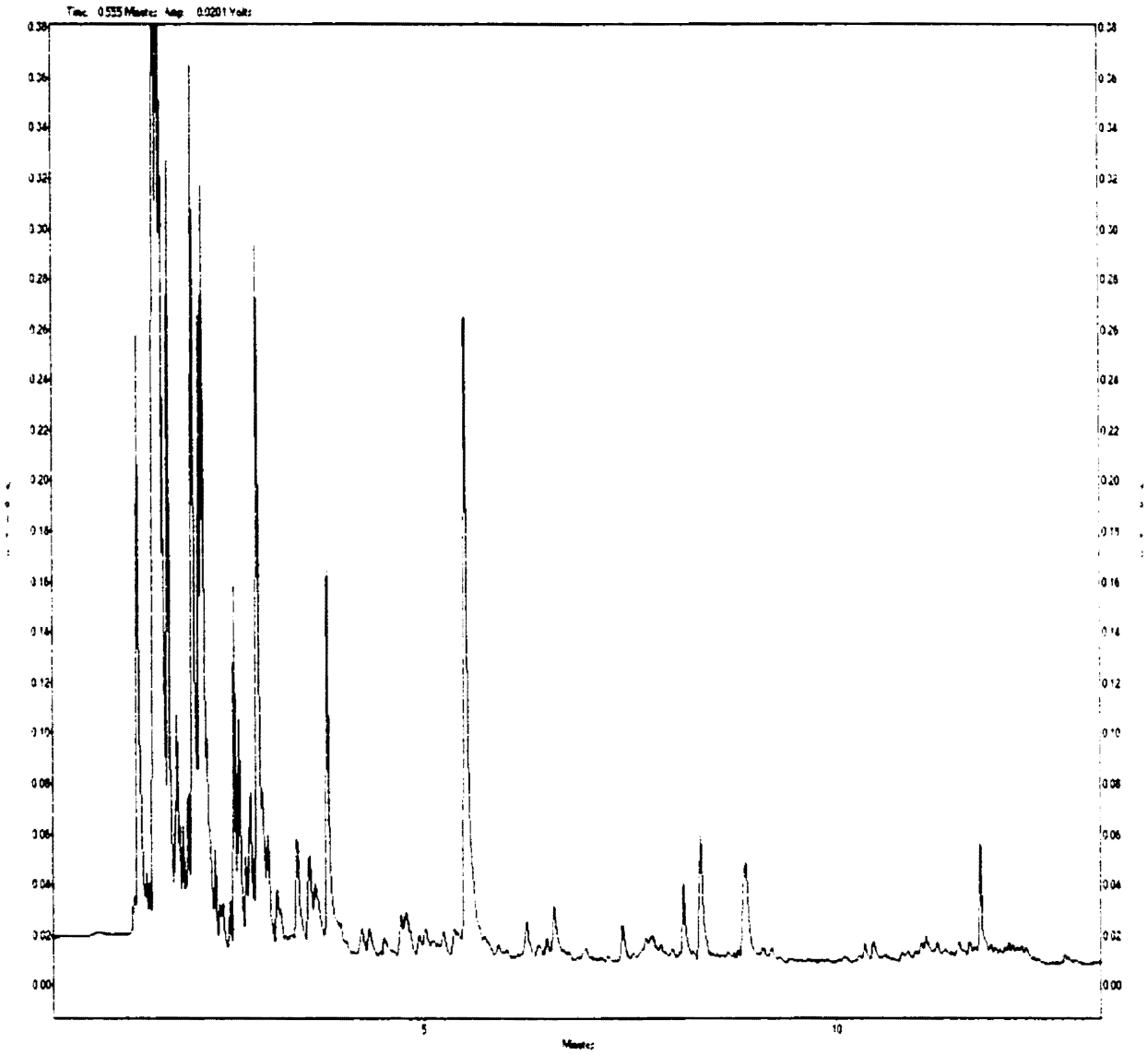

Figure 4- 11. Chromatogram obtained for cigarette smoke analysis

Permeation of organic solvents through gloves

Gloves are supposed to protect chemists from getting in contact with chemicals. However, the polymeric materials that the gloves are made out of are not perfect barriers, and thus some compounds would permeate through them, while others would not.

Each company that commercializes gloves is supposed to provide charts describing the permeation of the most commonly used chemicals through each type of gloves. However, the charts provide information about a restricted number of chemicals. At the same time, the methods used for measuring the permeation of the chemicals through the glove material are time consuming and can not detect low amounts of chemicals. Thus, even though some compounds can be very toxic even in small amounts, because of the incapability of measuring their permeation through the polymeric material, the charts provided by manufacturers would state that the gloves are perfectly safe to be used for working with those compounds.

MESI could be applied for the determination of the permeation of some of the compounds through different glove materials. A piece of glove could be placed in the membrane module, replacing the membrane. By exposing the glove to a chemical and recording the response of the detector, the permeation through the glove could be evaluated. The methods would be very sensitive, and would be able to detect small amounts of chemicals that cross the glove material. The analysis time would also be much shorter than the time spent using the conventional methods.

In order to test the applicability of MESI for such determinations, Latex gloves were used. A piece of a Latex glove was placed in the membrane module and then exposed to a solution containing 100 ppb of benzene and toluene. The response of the detector was recorded. The sorbent trap was pulsed every minute. One of the chromatograms obtained this way is presented in Figure 4- 12. It can be seen that both toluene and benzene could permeate through the membrane, and it only took 5 minutes to obtain a significant signal.

In order to have an idea about the magnitude of the permeation, the results obtained using the Latex glove were compared with the ones obtained using the most permeable membrane that was available (50 μm SSP M-100) membrane. The chromatogram from

Figure 4- 13 presents the results obtained using the silicone membrane for the analysis of the same benzene and toluene mixture.

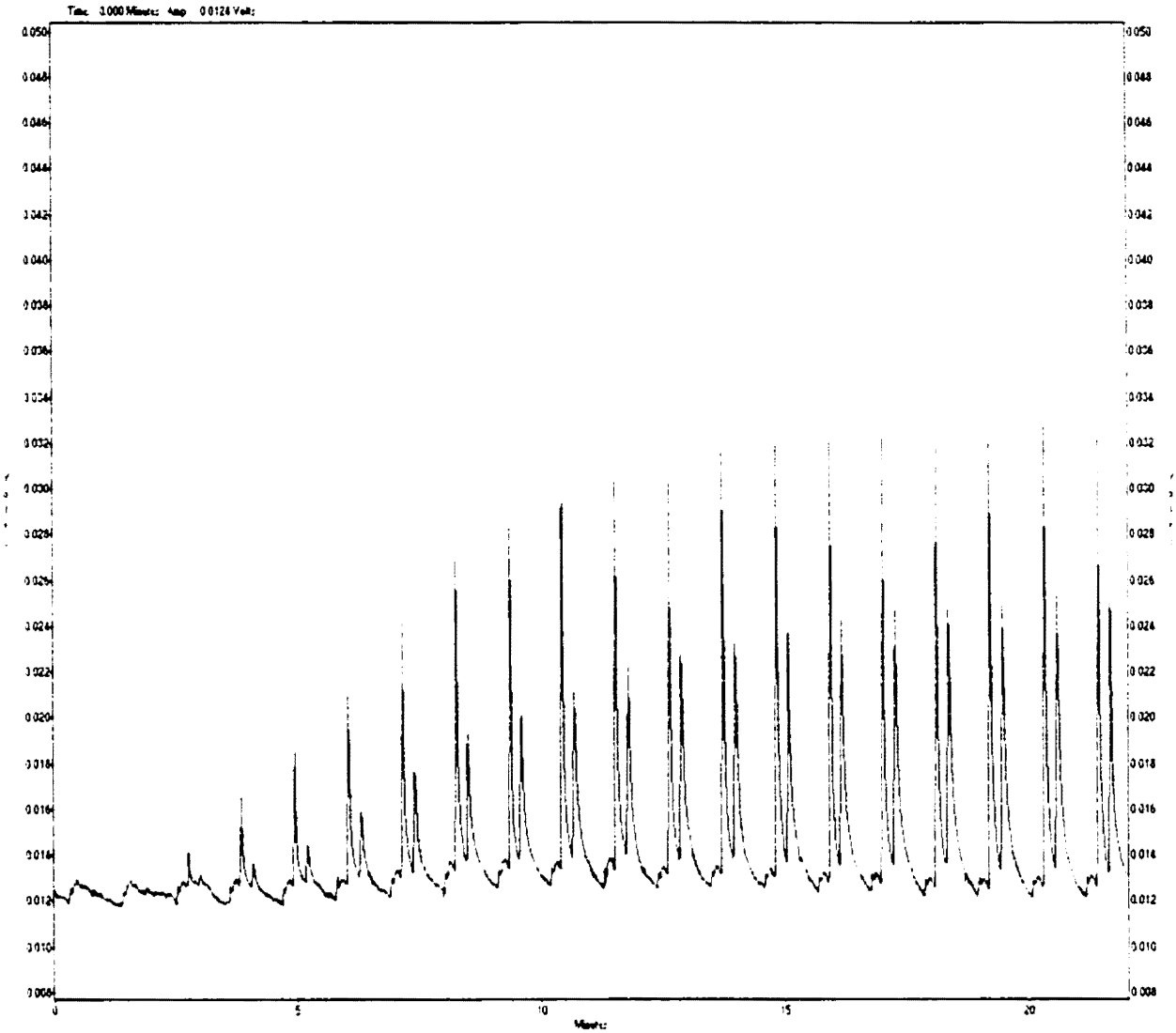

Figure 4- 12. Chromatogram presenting the permeation of benzene and toluene through Latex gloves

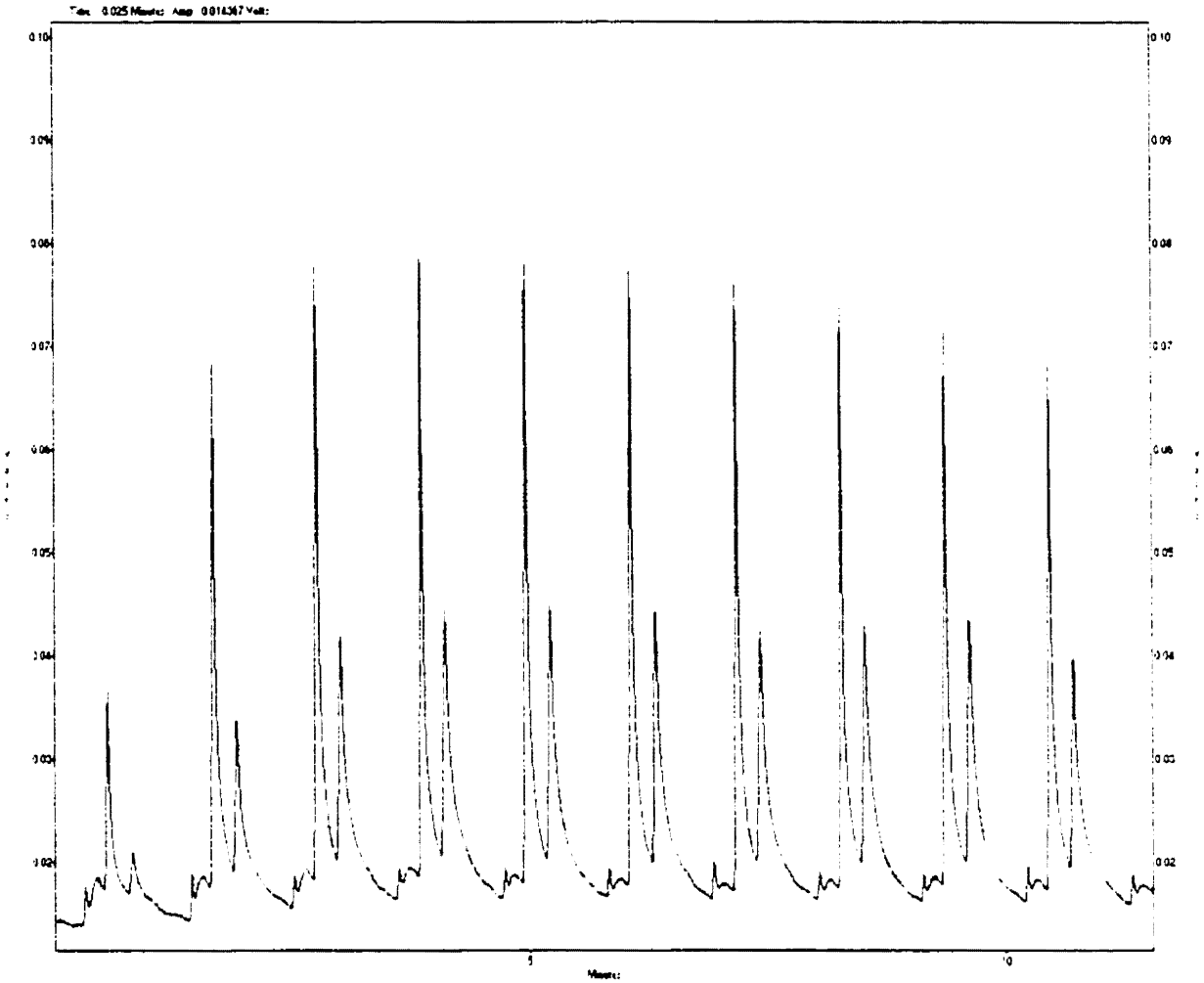

Figure 4- 13. Chromatogram presenting the permeation of benzene and toluene through silicone membrane

By comparing the results it can be seen that the permeation of benzene and toluene through the Latex material is comparable to the permeation through the silicone membrane. The amount that permeated through the glove in one minute (at steady state) was about 40 % of the amount that permeated through the silicone membrane, in the same conditions.

The experiment proves the applicability of MESI for evaluation of the permeation of chemicals through various polymeric materials. The technique is inexpensive and very rapid, and could provide information about low permeation rates.

4.3 Results obtained using a quadrupole mass spectrometer

Gas chromatography-mass spectrometry (GC-MS) hyphenation enables the identification of the compounds eluting from the chromatographic column. A mass spectrum is a histogram of the relative abundance of individual ions having different mass-to-charge ratios (m/z) generated from a sample of, in most cases, neutral molecules. The mass spectrum is a molecular fingerprint, providing information about the molecular weight and structure of the sample.

Mass spectrometers are sophisticated instruments and the processes of ionization, separation of ions in vacuum according to their m/z ratio, and ion detection are complex.

The ionization of organic molecules may be achieved in many ways: those of principal importance for use with chromatographic inlets include electron impact, chemical ionization, atmospheric pressure ionization, fast atom bombardment, thermospray and electrospray.

Ions leaving the source of the mass spectrometer must be separated according to their mass-to-charge ratio prior to detection. This is achieved by imposing an external electric or magnetic field in the ion beam to affect dispersion (resolution). The resolving power of a mass spectrometer is a measure of its ability to distinguish between two neighboring masses. The quadrupole mass filter consists of four parallel hyperbolic rods. Diagonally opposite rods are electrically connected to radio frequency and direct current voltage. For a given radio

frequency/direct current voltage ratio. only ions of a specific m/z value are transmitted by the filter and reach the detector. Ions of a different m/z ratio then the one of the transmitted ions are deflected away from the principal axis of the system and strike the rods. To scan the mass spectrum. the radio frequency voltage and the ratio of the alternating current/direct current (AC/DC) voltage are held constant. while the magnitude of the AC and DC voltages are varied.

Problems arise in interfacing column chromatographic techniques to mass spectrometry from the difference in the flow rates required for the two instruments and the desire to generate information about the sample without interference from the mobile phase. The most favorable case occurs for gas chromatography where the mobile phases commonly used do not influence the spectra. and the sample. being in the vapor phase. is compatible with the widest range of ionization techniques. The primary incompatibility in this case is the difference in operating pressure for the two instruments. The column outlet in gas chromatography is typically at atmospheric pressure. while the pressure in the source of the mass spectrometer is in the range of 2 to 10-15 Torr for chemical and electron impact ionization. respectively. Thus. the interface must be capable of providing an adequate pressure drop between the two instruments and should also maximize the throughput of the sample while maintaining a gas flow rate compatible with the source operating pressure.

When coupling a MESI system to a GC-MS. the fact that water and air permeate through the membrane has to be taken into consideration. Error messages could be received from the software. indicating a leak in the system. Thus. it is desirable to use membranes that are less permeable to air and water. when using a mass spectrometer detector. There are no other special requirements for coupling MESI to a GC-MS.

MESI was coupled to a GC-MS in order to enable identification of the compounds present in different samples. The system was used to analyze tap water, fume hood air and snow from a parking lot.

Experimental setup

A Hewlett Packard (HP) 5890 gas chromatograph coupled to a HP 5970A mass spectrometer was used for analysis. The column was a SPB-5 (Supelco, Oakville, ON, Canada), 30 m long, 0.25 mm i.d., and the film thickness was 0.25 μm. The sorbent trap was packed with XAD-2 resin (Polystyrene divinyl benzene) purchased from Supelco (Oakville, ON, Canada). The length of the sorbent bed was 1.3 cm. The trap was cooled using the same type of Peltier cooler as in the previously mentioned experiments. Silicone Polycarbonate Membrane (SSP-M213), of 25 μm thickness, purchased from Membrane Components (Ballston Spa, NY, USA) was used. The MESI system was connected to the HP 5890 gas chromatograph in the same way it was connected to the CP 9002 GC. Al the gases required for the chromatographic column and for the detector were purchased from Praxair (Waterloo, ON).

Tap water from one of the laboratory sinks was analyzed. Dynamic sampling was performed. For this, the water from the sink was allowed to flow continuously through plastic hose to a sampling chamber and from the chamber to the drain. The sampling chamber was made out of glass and had a volume of 600 mL. About 250 mL of tap water were present at any time in the sampling chamber. The membrane was placed in the headspace of the water and the sampling procedure tried to simulate sampling from a water stream. In order to keep the membrane away from the water matrix, a "cap system was used". The cap was custom-

made out of Teflon and it accommodated the membrane. Trapping times of 20 minutes were used for the analysis, and the separation was conducted isothermally at 50°C. The flow of the carrier gas (He) through the chromatographic column was 1 mL/min. Desorption voltage of 50 V was applied from the same custom-made capacitive discharge power supply that was mentioned in the previous experiments. To avoid interference from water masses higher than 40 were analyzed.

Air from one of the fume hoods in which waste containers were deposited was analyzed. The containers were closed and no laboratory activity was performed in the fume hood at the time sampling was performed. Passive sampling mode was used, since the GC-MS system was not portable. The sampling chamber (600 mL glass jar) was placed without the lid in the fume hood. The chamber was allowed to sit in the fume hood for 30 minutes. The membrane module was disconnected from the system, and together with the lid of the sampling chamber was transported to the fume hood in order to seal the chamber. Once the fume hood air was trapped inside the chamber, the membrane was reconnected to the MESI system and trapping began. A 15 minutes trapping time was used and the desorption voltage was 50 V. The flow of the carrier gas (He) through the chromatographic column was 1 mL/min. Isothermal separation at 50°C was performed. Only masses higher then 40 were analyzed.

Snow from a parking lot was collected and transported to the laboratory. The snow was placed into a 600 mL sampling chamber made out of glass. The chamber was closed with the membrane inside. However, the membrane was not connected to the MESI (no gas flowed

through the membrane module). The snow was allowed to melt. When all the snow transformed to water, the membrane was connected back to the MESI system and trapping of the volatile organic compounds emitted by the snow began. A trapping time of 20 minutes was used, after which a 50 V desorption voltage was applied to the trap to release the analytes. The compounds were separated isothermally at 50°C. Helium was used as carrier gas at a flow rate of 1 mL/min.

Results and discussions

The mass spectrometer used for these experiments was old and we were aware of its poor sensitivity. However, it was very important to identify the peaks from the real samples, using MESI extraction even if the sensitivity was not the desired one, rather then just getting chromatograms with many peaks (as when FID was used), without knowing their identity. Thus, the limit of detection was not determined.

Tap water

Tap water from one of the sinks present in the laboratory was analyzed. Dynamic sampling was experimented. A new sampling technique meant to facilitate the membrane extraction process from liquid sample was implemented. The membrane was placed inside the plastic cap and it was exposed to the water sample. A schematic representation of the cap membrane design used for sampling flowing water is presented in Figure 4- 14. The cap created a headspace on the top of the flowing water, keeping the membrane away from a potentially dirty matrix. If a dirty water sample would be analyzed, the particles present in it could get stuck to the membrane, minimizing the extraction surface. A second reason, for

using the cap design, was the diffusion of the analytes through air, which is faster than the diffusion through water. By having the cap on the top of the water, the headspace created is constantly supplied with fresh sample and the compounds are not wasted in the air, but rather extracted by the membrane. The cap is placed on the top of the flowing water (just touching the water), and due to the fact that the water is flowing, the sample is naturally mixed.

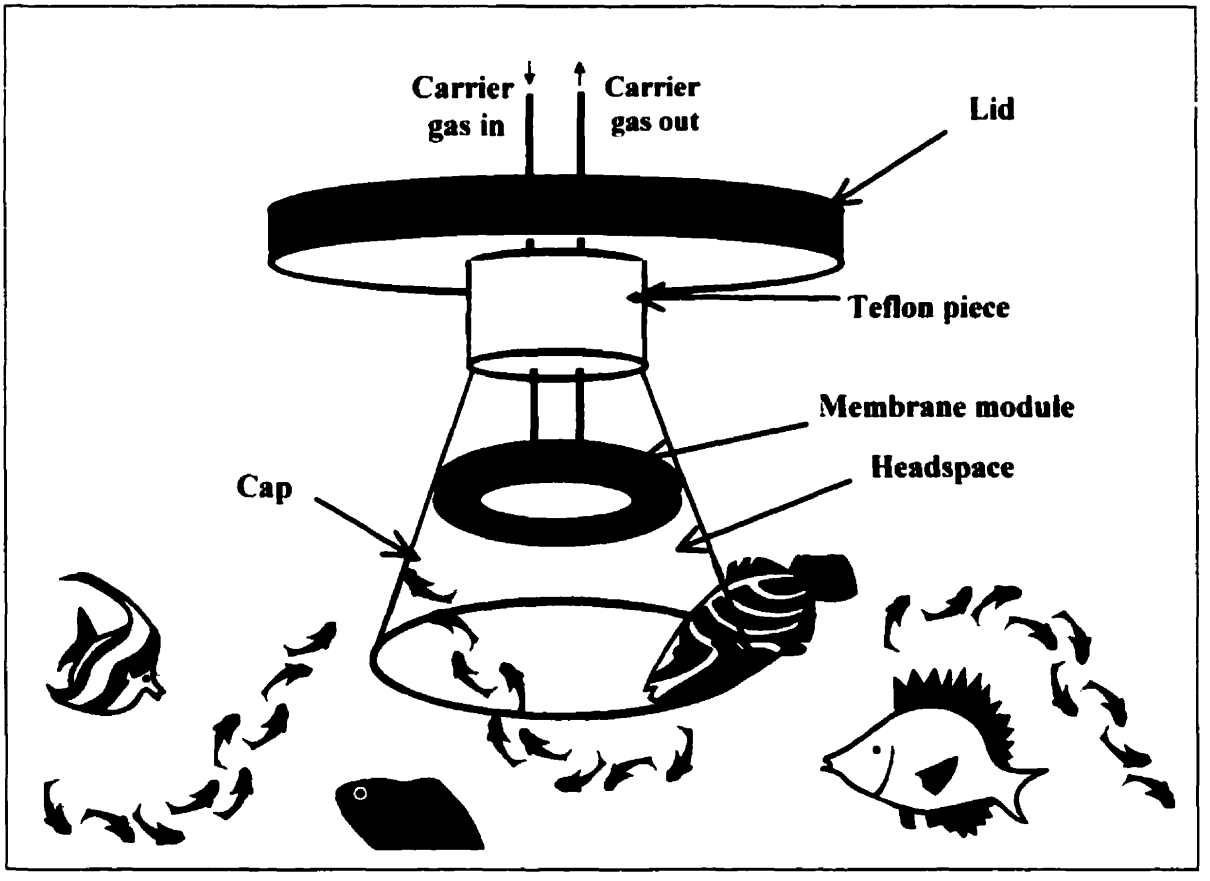

Figure 4- 14. Stream water sampling, using the MESI cap design

One of the chromatograms obtained by sampling the tap water is presented in Figure 4- 15. It is very obvious that this MS detector did not provide the same sensitivity as the previously examined FID. However, it was very important to identify the three peaks

obtained. As expected, one of the peaks was chloroform. One of the other compounds present in the chromatogram was bromodichloromethane (another trihalomethane). Toluene was also present in high concentration, and this might be due to contamination of the water pipes.

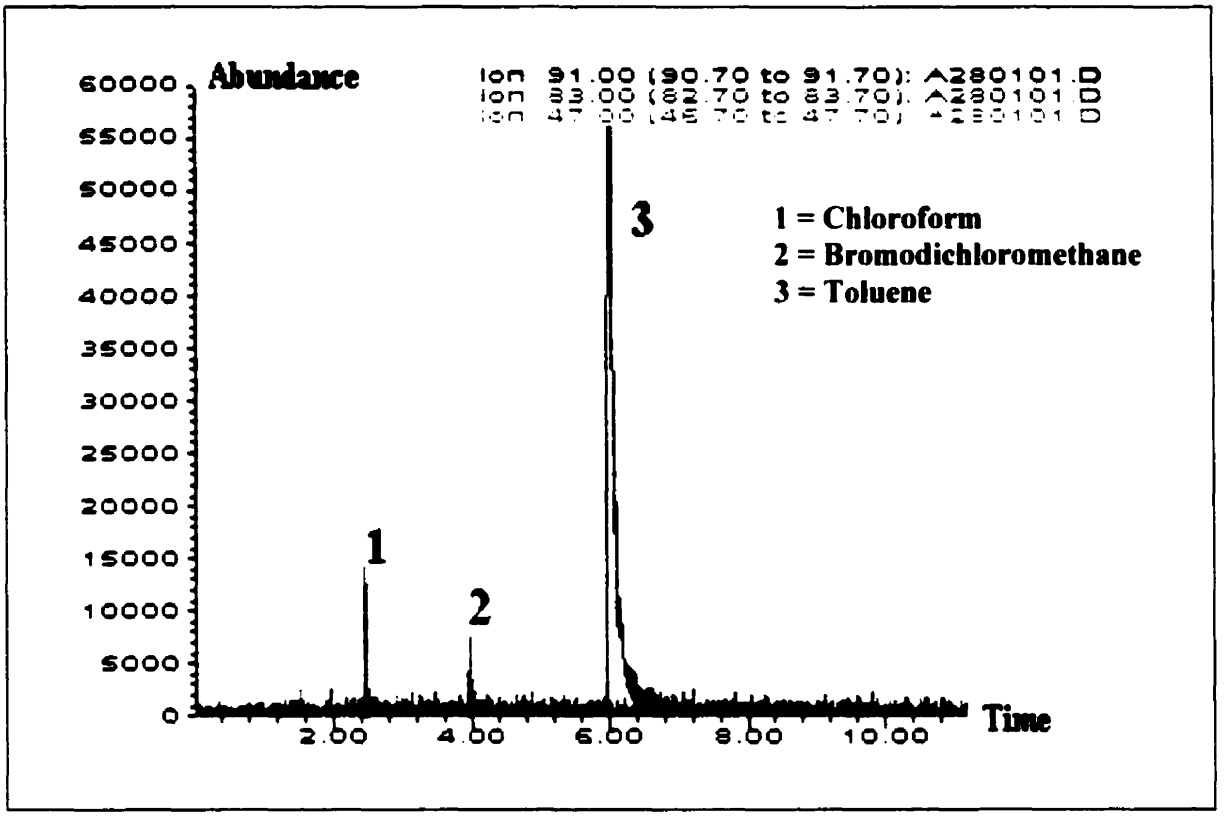

Figure 4- 15. Chromatogram obtained by analyzing tap water by MESI-GC-MS

Nonetheless, the experiment proved that MESI could be coupled to a GC that uses a mass spectrometer detector, and it can be applied for the analysis of real samples. Since portable GC-MS systems are commercially available, such systems could be successfully used for continuous monitoring of water from rivers, or wastewater from chemical plants.

Fume hood air

Air from a fume hood was analyzed to simulate the application of MESI for the analysis of air from contaminated areas. Even though passive sampling of the fume hood air

was used. the experiment was meant to prove that MESI is capable of capturing chemicals present in air. and that by coupling MESI to a mass spectrometer detector. the compounds could be identified.

The chromatogram presented in Figure 4- 16 illustrated the results obtained for fume hood air analysis.

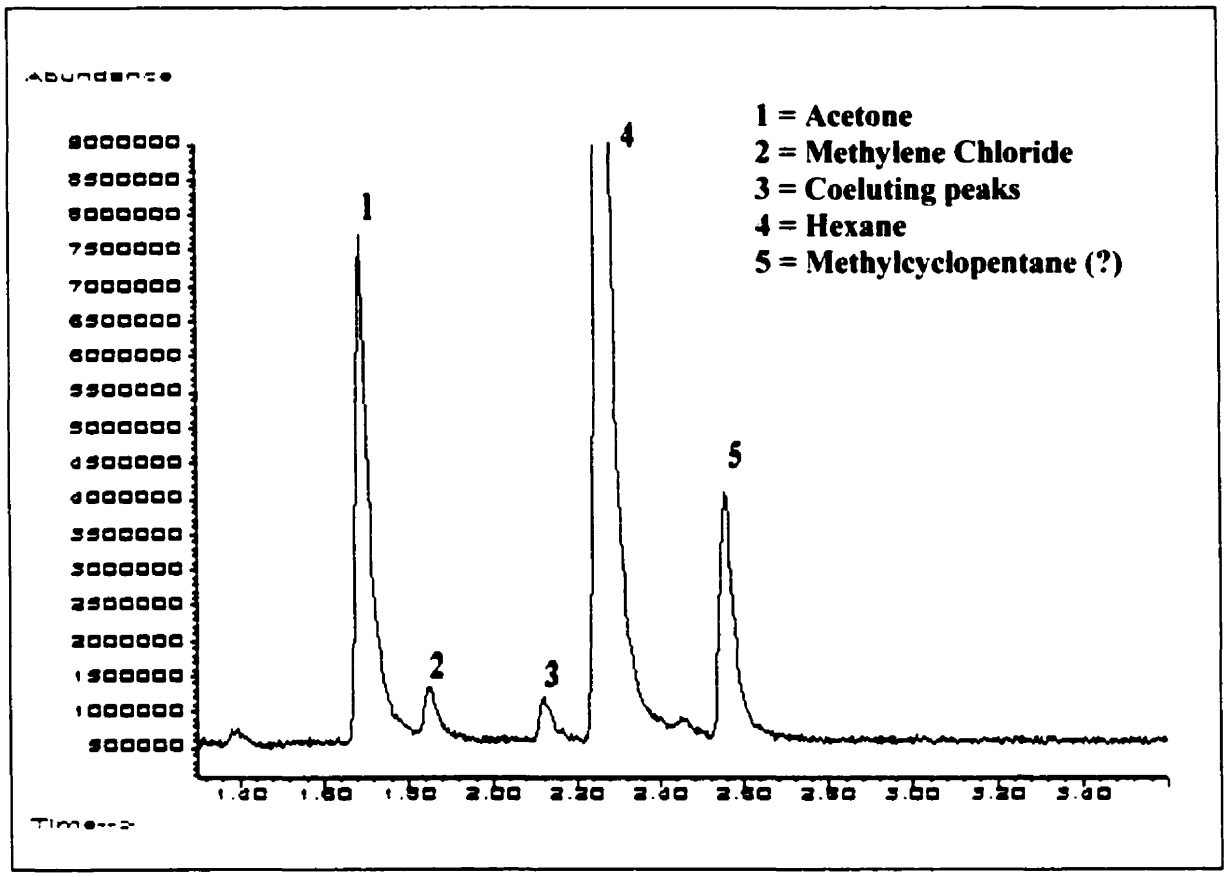

Figure 4- 16. Chromatogram obtained by analyzing fume hood air using MESI-GC-MS

The chromatogram from Figure 4- 16 reflects the laboratory activity. The compounds identified from the air sample (acetone. methylene chloride. and hexane) are constantly used in our laboratory, and were probably present in the waste containers (that were not properly sealed). The third peak in the chromatogram resulted from the coelution of two compounds

and due to the low signal to noise ratio the peaks could not be identified. The last peak in the chromatogram was identified as methylcyclopentane. However, the match of the spectra was poor, and the compound is not commonly used in our laboratory.

If MESI would have been connected to a portable system, the air in the fume hood could have been monitored over an extended period of time. In a similar way, the membrane could be placed into a stack and the composition of the released air could be measured.

Parking lot snow

Snow from a parking lot has been sampled because volatile compounds that are released by cars could get trapped and concentrated on the ice. As fresh layers of snow build on the top of the old ones, the chemicals that are trapped on the bottom layers do not get released until the snow melts. Since in many parts of Canada there is a lot of snow during the winter, big cities are at risk of getting a high concentration of volatile organic compounds in the air, once the snow starts to melt (especially if the melting process occurs rapidly).

Thus, a snow sample was collected from a parking lot, and it was analyzed. The snow sample was relatively fresh (it snowed the night before sampling), and thus it could not collect a high amount of analytes. The snow was sampled after melting and the results are presented in Figure 4- 17. Benzene, toluene, ethylbenzene and xylene were identified in the sample.

The process could be better assessed if a MESI system would be placed in a parking lot, and the air would be monitored during the winter and spring period. The variations in the concentration of VOCs in the air could be correlated with the variations in the temperature and the amount of snow present on the ground.

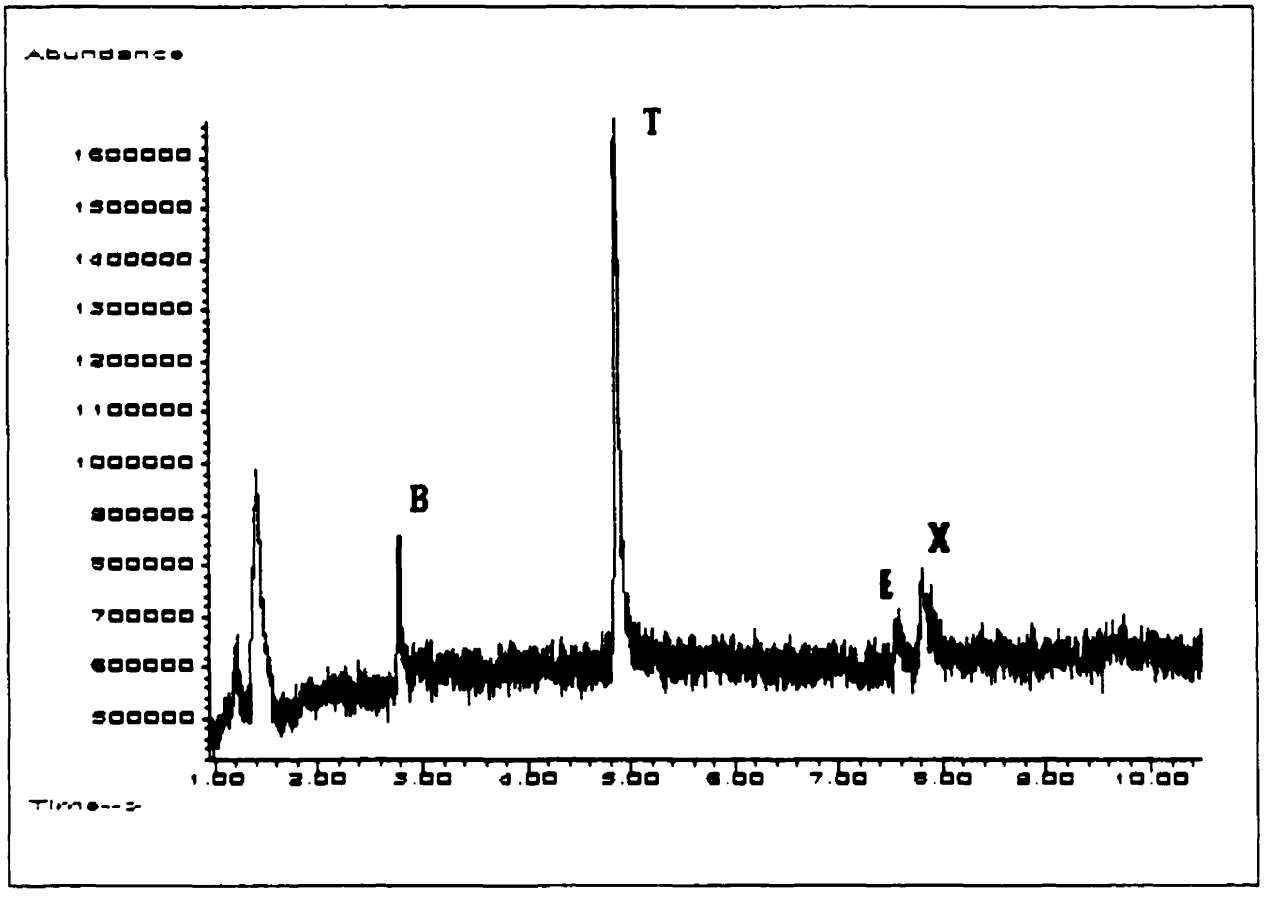

Figure 4- 17. Chromatogram obtained by analyzing snow from a parking lot, using MESI-GC-MS

Even though the results of the analyses presented herein do not benefit from the great sensitivity of regular mass spectrometers, it was proven that MESI could be coupled to a more demanding detector than FID, without encountering problems.

4.4 Field portable design of MESI

As the analysis time is an important issue, the design of a small, field portable gas chromatograph, capable of performing high-speed separations, is very important. Most often, short capillary columns operated at high carrier gas velocities are used in high-speed GC analyses. Also, a very narrow injection band is required in order for a good separation to be achieved. The width of the injection band has to be small compared to the contributions to band broadening due to diffusion, interfaces, detector volume and electronics.[7]

Some important aspects must be taken into consideration when a field-portable gas chromatograph is used. Most of the commercially available field-portable GCs have limited applications, being almost exclusively designed for gaseous samples and isothermal separations. The inconvenience of transporting additional gas tanks into the field makes the choice of detectors quite narrow. Thermal conductivity detectors and photoionization detectors are typically used. The response of a photoionization detector is limited to the volatile compounds that undergo photoionization.

The thermal conductivity detector (TCD), also known as the hot-wire or katharometer detector, is a universal, nondestructive, concentration-sensitive detector, that responds to the difference in thermal conductivity between pure carrier gas and carrier gas containing organic vapors. The TCD is generally used to detect light hydrocarbons and compounds which respond only poorly to the FID. For many general applications it has been replaced by the FID, which is more sensitive, has a greater linear response range, and provides a more reliable signal for quantitation.

In a typical TCD, the carrier gas flows through a heated thermostated cavity that contains the sensing element, which is either a heated metal wire or a thermistor. With pure

carrier gas flowing through the cavity, the heat loss from the sensor is a function of the temperature difference between the sensor and cavity and the thermal conductivity of the carrier gas. When an organic compound enters the cavity, there is a change in the thermal conductivity of the carrier gas that results in a change in the temperature of the sensor. The change in the temperature of the sensor results in an out-of-balance signal, which is amplified and sent to the recorder. Carrier gases of low molecular weight and high thermal conductivity are required to maximize the response of the detector and to maintain a large linear response range. Consequently, helium and hydrogen are predominantly used.

Thermal conductivity detectors are conveniently used for field applications since they do not require additional gases for operation. However, even though micro-thermal conductivity detectors are much more sensitive than the regular size TCDs, they do not reach the sensitivity of a flame ionization detector. The sensitivity can be improved though, by sample preconcentration. Most often cold trapping followed by thermal desorption is used, but this requires additional complicated instrumentation. MESI could provide the required sample preconcentration for a TCD, and if separation would be accomplished using a small GC, the system could be conveniently used for field analysis.

4.4.1 System requirements and limit of detection

MESI was connected to a Chrompack 2002 Micro-GC. The gas chromatograph was equipped with a micro-TCD, and a short chromatographic column (4 m) that enabled fast separation. A heater controlled the temperature of the column. The schematic representation of the standard Chrompack 2002 Micro-GC is presented in Figure 4- 18.

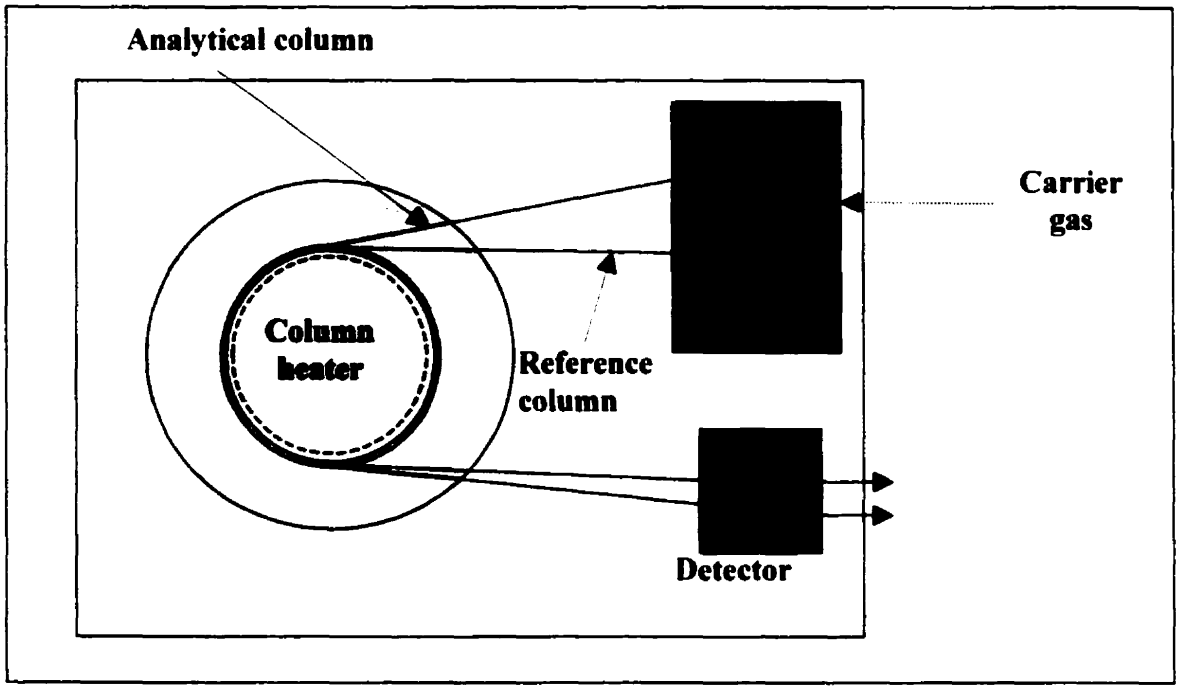

Figure 4- 18. Schematic representation of the Chrompack 2002 Micro-GC

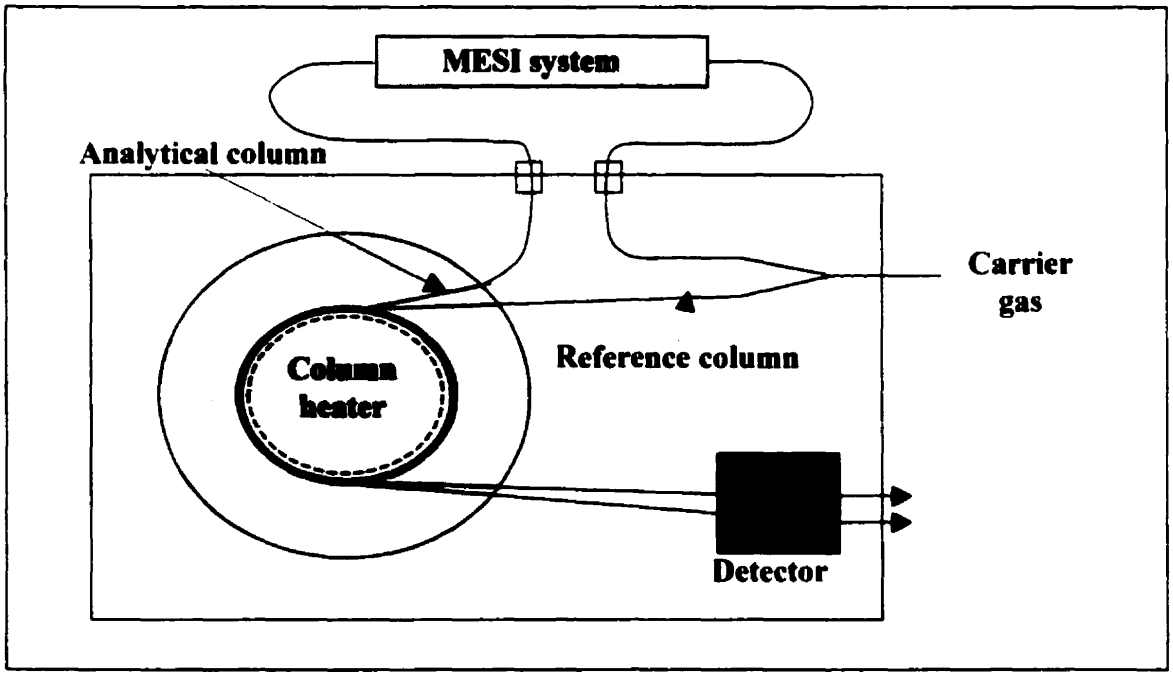

Figure 4- 19. Schematic representation of MESI system coupled to a Chrompack 2002 Micro-GC

In order to couple MESI to the Chrompack micro-GC, the injector was replaced by a "Y" splitter. One end of the splitter was connected to the gas flow controller. The other two ends of the splitter were used to connect the MESI system (the membrane module and the sorbent interface), and the reference column. A schematic representation of the modified GC is presented in Figure 4- 19.

The chromatograms presented in Figure 3-7 from the previous chapter were obtained using the above-described system. The analysis was performed by exposing the membrane to a standard gas mixture that was prepared using compressed air of reduced humidity. However, it can be seen in the chromatograms that there is interference from the water and air (the first peak in each chromatogram). For this reason, real samples, containing an elevated water concentration could not be analyzed. Due to the short length of the chromatographic column, the peaks produced by some organic compounds would elute on the tail of the water peak, making the analysis of low concentration samples impossible.

In order to minimize this unwanted interference, a water trap was placed in the system between the membrane module and the sorbent trap. In this way, the water that permeated the membrane was prevented from entering the sorbent trap. A 10 cm long, poly(methaacrylate) tube having an inner diameter of 3.5 cm was used to build the water trap. Two rubber stoppers were placed at the ends of the plastic tube, to ensure the sealing. A piece of Nafion tubing, having a length of 70 cm was placed inside, and connected to the carrier gas line using two Valco connectors and Vespel ferrules. Holes were drilled in the rubber stoppers in order to accommodate the connectors. The cylinder was then filled with 70 grams of activated molecular sieve, that surround the Nafion tubing. The water that penetrates the Nafion tubing could also be removed from the water trap by flushing the tubing with gas. However, an extra cylinder is required for this purpose. As the whole system was designed for field applications,

the water trap also had to meet the requirements of a portable device. For this reason molecular sieve was chosen for the water trap. The dimensions of the trap could be modified, depending on the time that the trap is required to function without being replaced.

Water can penetrate the Nafion tubing. Thus, when the carrier gas passes through the trap, the moisture permeates the Nafion membrane and gets retained by the molecular sieve present on the other side of the membrane. To some extent, very polar compounds could also be lost in the water trap.

Experimental setup

After the introduction of the water trap into the system the membrane could be exposed even to aqueous samples, without a significant interference from water. A BTEX standard mixture was prepared in methanol (HPLC grade), using BTEX components of 99+% purity. An appropriate amount of the standard solution was spiked in 40 mL water to obtain a final concentration of 20 ppb (w/w) for each compound. The sampling jar had a volume of 120 mL. The solution was stirred at 500 rpm using a 5 cm long stir bar. Silicone Polycarbonate Membrane (SSP-M213), of 25 μm thickness, purchased from Membrane Components (Ballston Spa, NY, USA) was used for sampling. XAD-2 resin (Polystyrene divinyl benzene) purchased from Supelco (Oakville, ON, Canada) was used as packing material for the sorbent trap. The length of the sorbent bed was 1.3 cm. The trap was kept at room temperature. A trapping time of 6 minutes was used. The same capacitive discharge power supply was used to pulse the trap, at a discharge voltage of 47 V.

For the determination of the limit of detection (LOD), aqueous solutions containing different concentrations of toluene were analyzed. A standard stock solution was prepared by

dissolving toluene (99+% purity) in water. Appropriate amounts of the stock solution were spiked in 40 mL water to obtain final concentrations of 1, 3, 10, 30 and 90 ppb toluene in water. The sampling jar had a volume of 120 mL, and the membrane was placed in the headspace of the sample. During extraction, the sample was stirred at 500 rpm, using a 5 cm long stir bar. Trapping times of 4 minutes were used.

All the chemicals required for the experiments were purchased from Aldrich Chemical Company, Inc. Helium purchased from Praxair (Waterloo, ON) was used as carrier gas at a flow of 4 mL/min. The column temperature was maintained at 50°C.

Results and discussions

The chromatogram obtained for the analysis of 20 ppb BTEX solution in water is presented in **Figure 4- 20**. The first peak in the chromatogram is the air peak, followed by the peak of water and methanol. The next four peaks are as follows: 1 = benzene, 2 = toluene, 3 = ethylbenzene and 4 = o-xylene. It can be seen that in this chromatogram obtained with the new system, the peak of water (and methanol) is as small as 0.5 V. In the experiments performed without the water trap and having the membrane exposed to a standard gas mixture containing very little moisture, the water peak was 1.5 V high after 3 min concentration time.

As it can be seen in the chromatogram, the peaks are separated, and the size of the water peak decreased significantly to a value of about 0.5 V. The difference is significant since previously the membrane could not be exposed even to ambient air because the tail of the water peak would entirely cover the analyte peaks of concentrations lower than a few hundred ppb.

Thus, the water trap could be used successfully to eliminate the interference produced by the moisture present in the sample. Losses of polar compounds in the water trap could be compensated for by using longer trapping times.

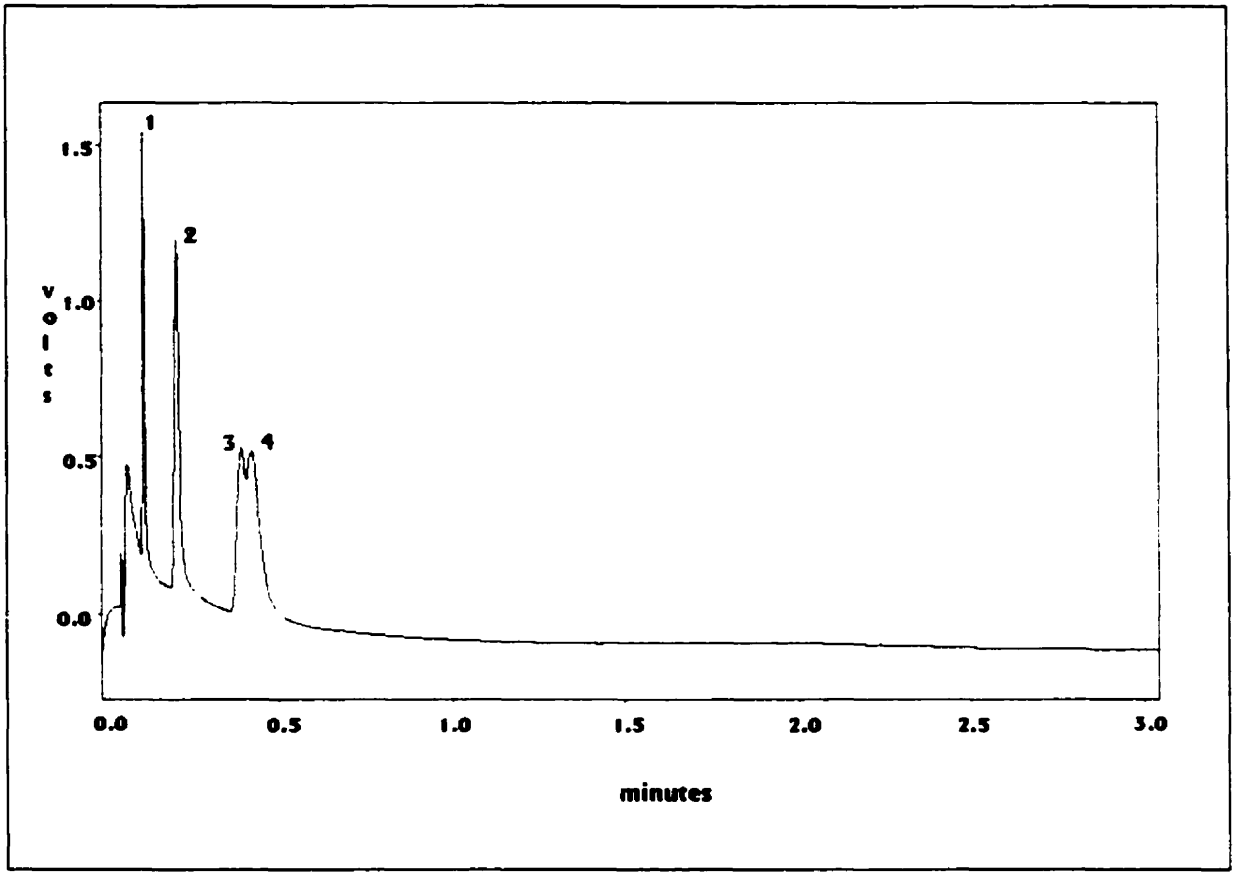

Figure 4- 20. Chromatogram of BTEX, obtained after the introduction of the water trap in the system. Peaks: 1 = benzene; 2 = toluene; 3 = ethylbenzene; 4 = o-xylene

For the determination of the detection limit, the calibration curve was constructed for toluene concentrations in water of 1, 3, 10, 30 and 90 ppb. The calibration curve is presented in Figure 4- 21.

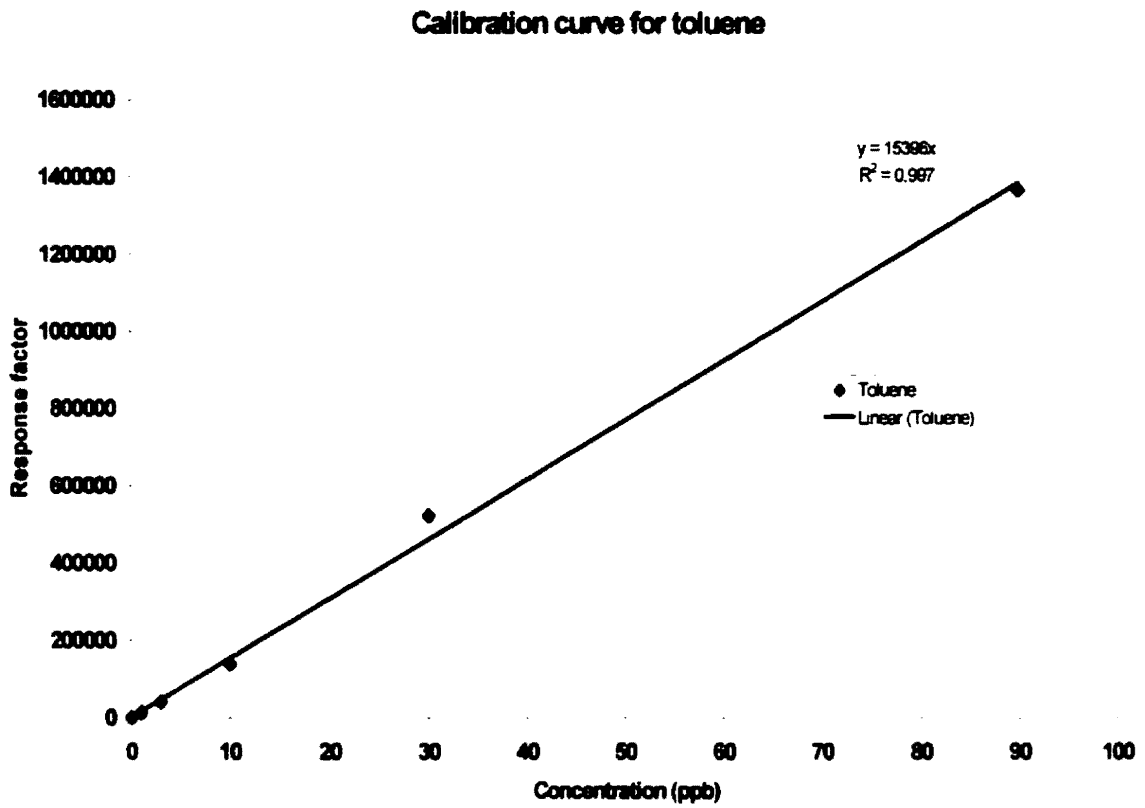

Figure 4- 21. Calibration curve for toluene obtained with MESI - Micro GC

The peak heights were used to calculate the detection limit. With a noise level of $2.2*10^{-4}$ V, having a standard deviation of $4*10^{-5}$, the detection limit was calculated to be 60 ppt. Taking into consideration that the detector used in the experiments was a TCD, the detection limit can be considered to be very good.

4.4.2 Tap water analysis using the MESI - Micro GC system

In order to compare the performance of the TCD detector in conjunction with MESI, with the performance of the other detectors used so far, tap water from one of the sinks in the laboratory was analyzed.

Experimental setup

The system used for the experiments was the same as the one described in the previous section. Water from one of the sinks in the laboratory was collected in a sampling jar and was analyzed. The volume of the sampling jar was of about 600 mL and 350 mL of sample were placed in the jar. The sample was stirred at a rate of 500 rpm. The membrane was placed in the headspace of the sample. A 6 minute trapping time was used. The column temperature was 50°C and helium having a flow rate of 4 mL/min was used as carrier gas.

The performance of two traps, both packed with XAD-2 resin (Polystyrene divinyl benzene purchased from Supelco, Oakville, ON, Canada) was compared. The sorbent bed was of 1.3 cm long for one of the traps and of 0.5 cm long for the other.

Different packing materials were evaluated. For this, Tenax-TA, PDMS and PSDVB sorbents were used. The Tenax-TA and the PSDVB (XAD-2 resin) were purchased from Supelco (Oakville, ON, Canada). The particles of PDMS were obtained by crushing a piece of silicone hollow fiber membrane (Baxter Healthcare Corp., McGaw Park, IL) of 330 μm thickness, under liquid nitrogen. The particles obtained were packed into a piece of stainless steel tubing to obtain the trap. The sorbent bed length for the three traps was 1.3 cm.

The cup membrane system described earlier in this chapter was used to sample an aqueous mixture of benzene, toluene, and ethylbenzene. Two standard stock solutions were prepared in methanol. The first solution contained benzene and toluene, and the second solution contained benzene, toluene and xylene. The stock solution was spiked in 40 mL water to obtain a final concentration of 100 ppb (w/w) for each compound. The sample was stirred at 500 rpm during sampling. Headspace sampling was performed.

Results and discussions

The chromatogram obtained by analyzing the tap water is presented in Figure 4- 22 (lower trace). The small peak in the chromatogram was believed to be chloroform. In order to verify this, the tap water was spiked with chloroform. The experiment was repeated, and the goal was to see an increase in the peak height, when standard chloroform solution was added to tap water. For this, a concentrated chloroform solution was prepared by spiking 1μL of pure chloroform in 100 mL deionized water. The tap water was reanalyzed, using the same concentration time. 30 μL of the concentrated chloroform solution were added to the tap water, and the solution was again analyzed using a 6 min trapping time. The chromatogram obtained in this way is presented in the upper trace of Figure 4- 22. It can be seen that the height of the peak presumed to be chloroform increased after the tap water was spiked with the standard chloroform solution. The experiment was repeated several times, using different amounts of the chloroform solution and the peak of interest increased proportionally each time. Thus, it was concluded that the unknown peak was probably chloroform.

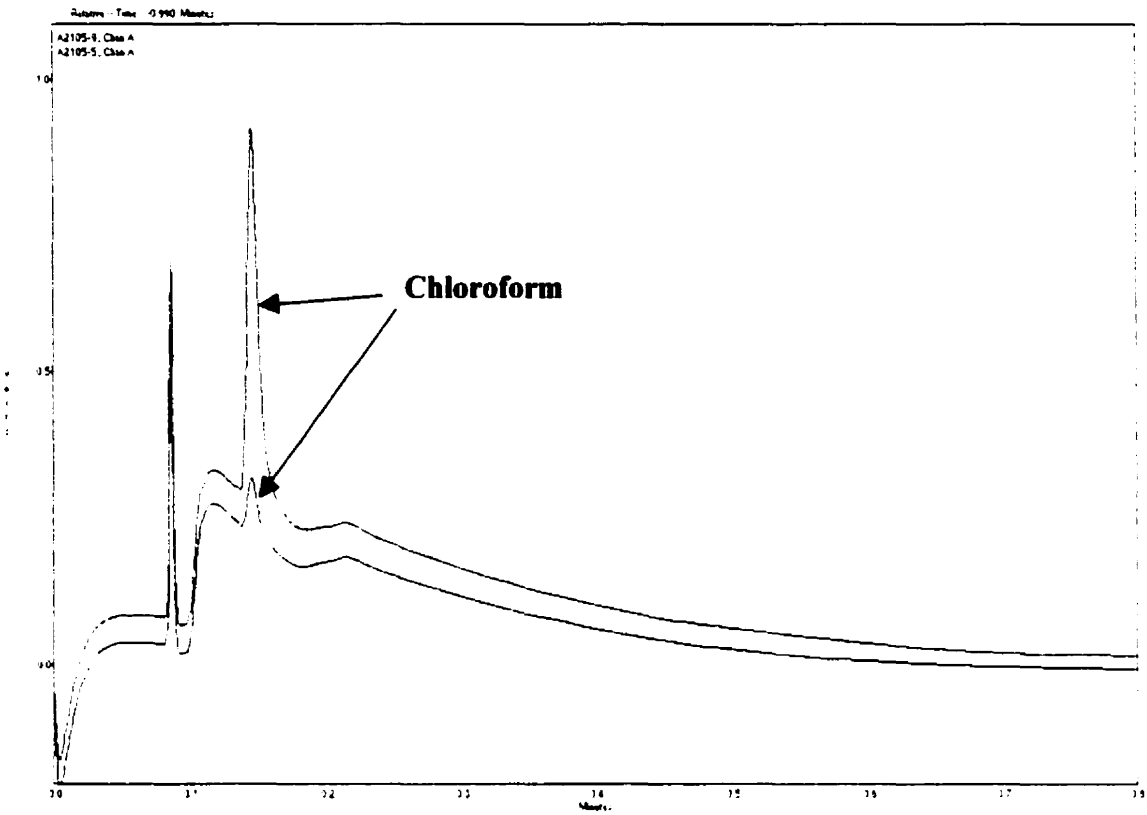

Figure 4- 22. Overlaid traces presenting the chromatogram obtained for the analysis of tap water (lower trace), and analysis of tap water spiked with chloroform (upper trace)

However, even though chloroform could be found in the tap water, it can be seen that the peak is partially covered by a "wide tailing peak" (WTP). Experiments conducted for the determination of the WTP nature concluded that the WTP disappeared every time the membrane was bypassed (no sample would get into the carrier gas line). Figure 4- 23 presents the overlaid chromatograms obtained for the system blank.

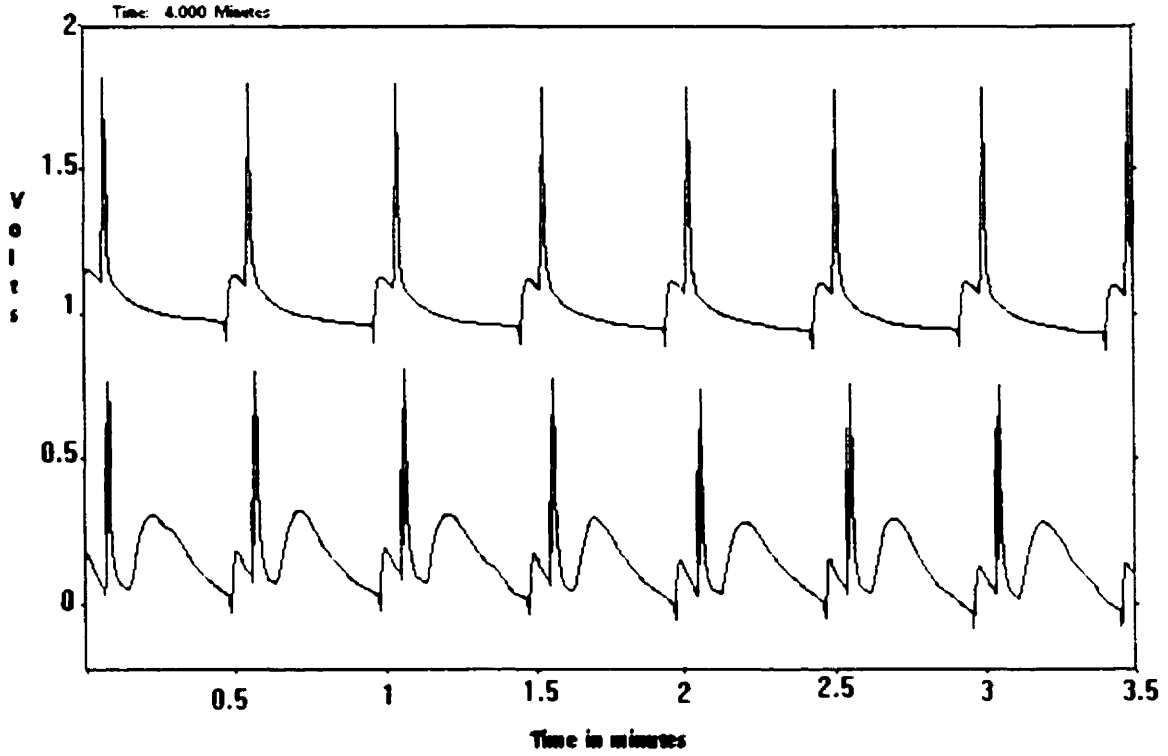

Figure 4- 23. Chromatograms obtained for the system blank. Upper trace: membrane not connected to the system. Lower trace: membrane connected to the system and exposed to clean air.

When the membrane was bypassed (upper trace of Figure 4- 23), the WTP was not present in the blank. However, when the membrane was connected in the system and exposed to clean air, the WTP was visible in the blank (lower trace in Figure 4- 23). Because the membrane was exposed to clean air, it was believed that the WTP was produced by oxidation of the sorbent material.

Two traps packed with PSDVB particles were used to determine a possible correlation between the amount of the sorbent and the size of the WTP. One of the traps had a sorbent bed length of 1.3 cm. and the second trap had a shorter sorbent bed. of only 0.5 cm. The blanks were recorded for both traps, having the membrane connected to the system and exposed to air. The results are presented in Figure 4- 24.

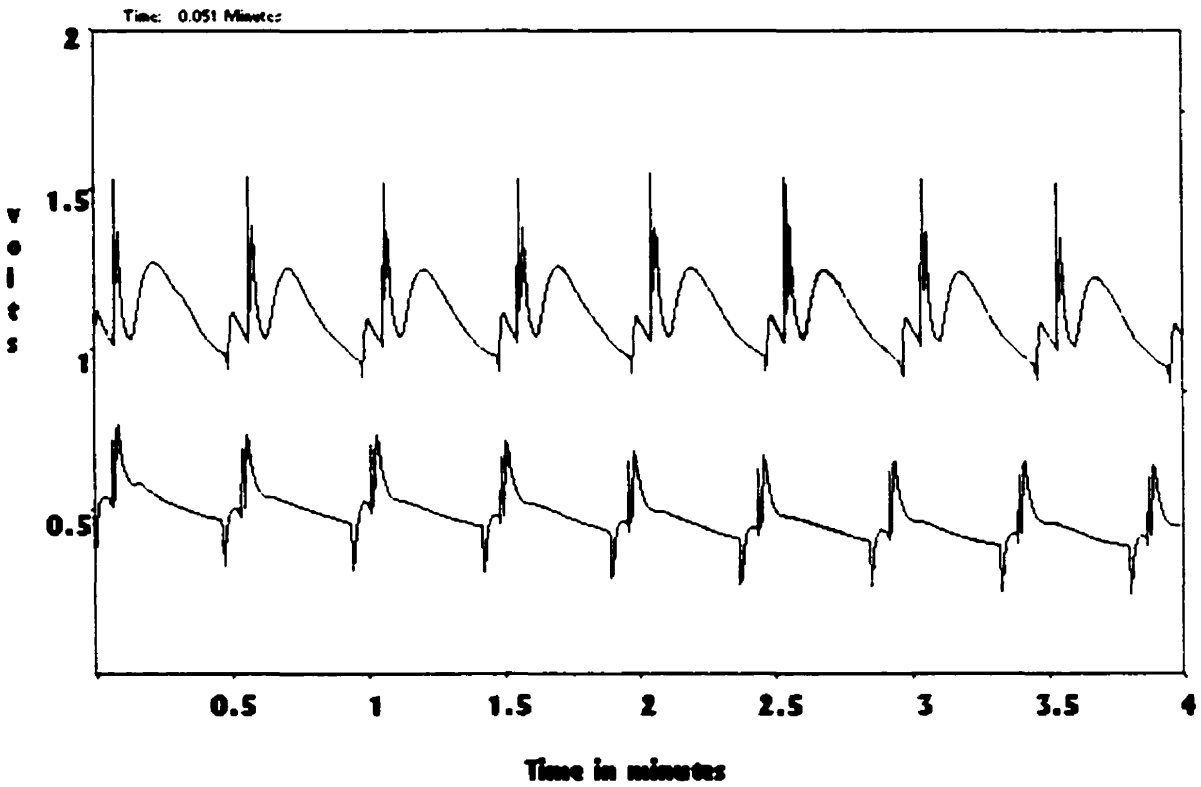

Figure 4- 24. Comparison of the blanks obtained with two traps, packed with different amounts of PSDVB sorbent. Sorbent bed length: upper trace = 1.3 cm; lower trace = 0.5 cm

From the results obtained, it appears that the amount of sorbent present in the trap had a strong influence on the size of the WTP. The cause of this could be the oxygen that

permeates the membrane. During the heating pulse, due to the high temperature, the oxygen might degrade the sorbent material. The resulting oxidation products could be reflected in the chromatogram as a WTP.

Different sorbent materials were tested in order to verify the presence of the WTP. Tenax-TA, PDMS and PSDVB sorbents were used. The membrane was connected to the system and the blank was recorded for the three traps. The results are presented in Figure 4-25.

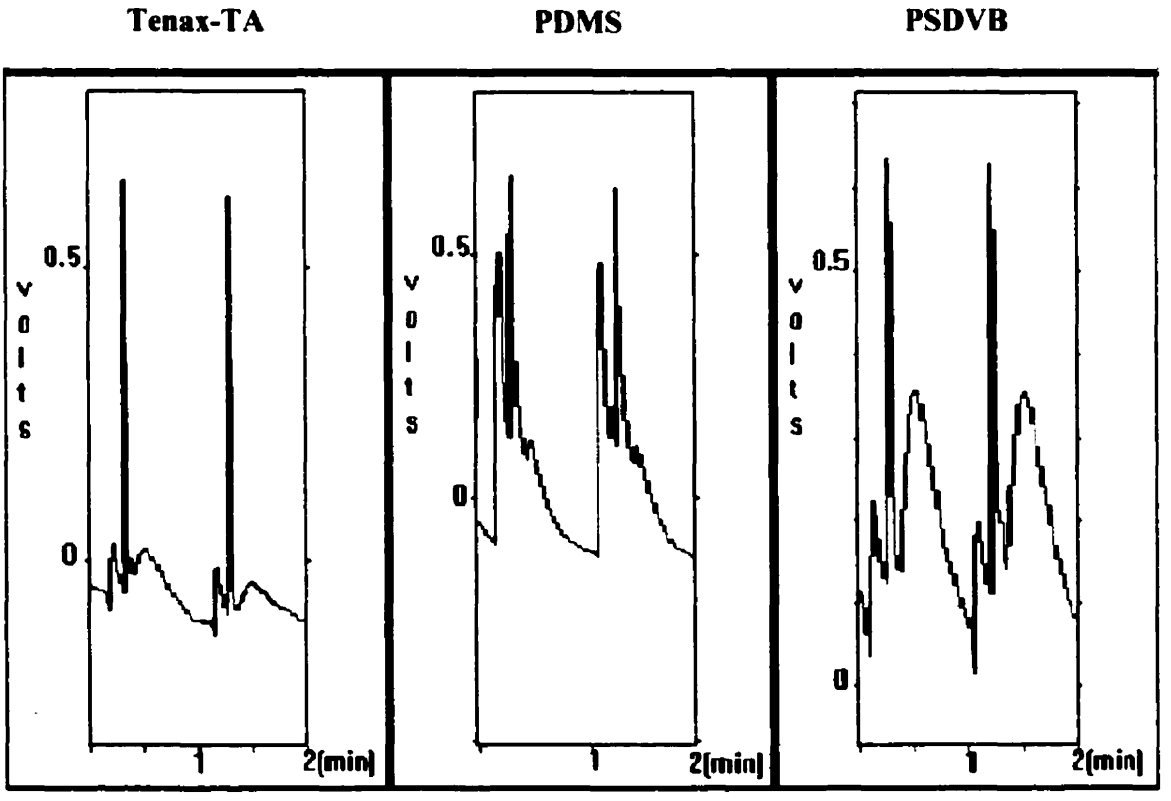

Figure 4- 25. Comparison of the blanks obtained using traps packed with different sorbent material

Due to the fact that the traps were packed manually, the amount of sorbent present in them could not be controlled very well. The length of the sorbent bed was measured. However, the particle size was different. The size of the PDMS sorbent particles could not be controlled during the crushing of the hollow fiber membrane. Furthermore, the particle size was much bigger than the one of the other two sorbents that were used in the experiment. Thus, the variations of the size of the WTP can be attributed also to the variations in the amount of sorbent present in the trap. However, the experiment tried to determine if only the PSDVB traps produced the WTP. As it can be seen in Figure 4- 25, all three traps produced the WTP, the size of it being considerably smaller in the case of the PDMS trap. However, due to the experimental limitations, it could not be concluded whether the oxygen that permeated through the membrane affected the PDMS sorbent less then it affected the other sorbents.

It was observed that the WTP would disappear when the membrane was exposed to helium, and decreased significantly when dipped in water. This fact strengthens our belief that oxygen is responsible for the presence of the WTP in the chromatograms. The use of a mass spectrometer could probably confirm the identity of the WTP. At the same time, we thought of a way to apply the cap membrane design to eliminate this inconvenience.

The cap design, as well as the MESI-Micro GC system were designed for field analysis, the combination could be advantageously used to prevent oxygen from permeating through the membrane. The helium that circulates through the reference column of the GC is not contaminated, and it is wasted after exiting the column. Thus, a connection has been made to transfer the helium from the end of the reference column to the cap in which the membrane was located. During sampling, the cap is maintained in an upright position, with the membrane facing down. Thus, if the air that surrounds the membrane is replaced by helium,

an inert atmosphere is created around the membrane. The oxygen can not reach the membrane, and the helium remains trapped in the cap, for as long as the trap is maintained in an upright position. Thus, helium is needed to purge the membrane until all the air is replaced, after which the helium flow could be stopped. A schematic representation of the cap design purged by helium is illustrated in Figure 4- 26.

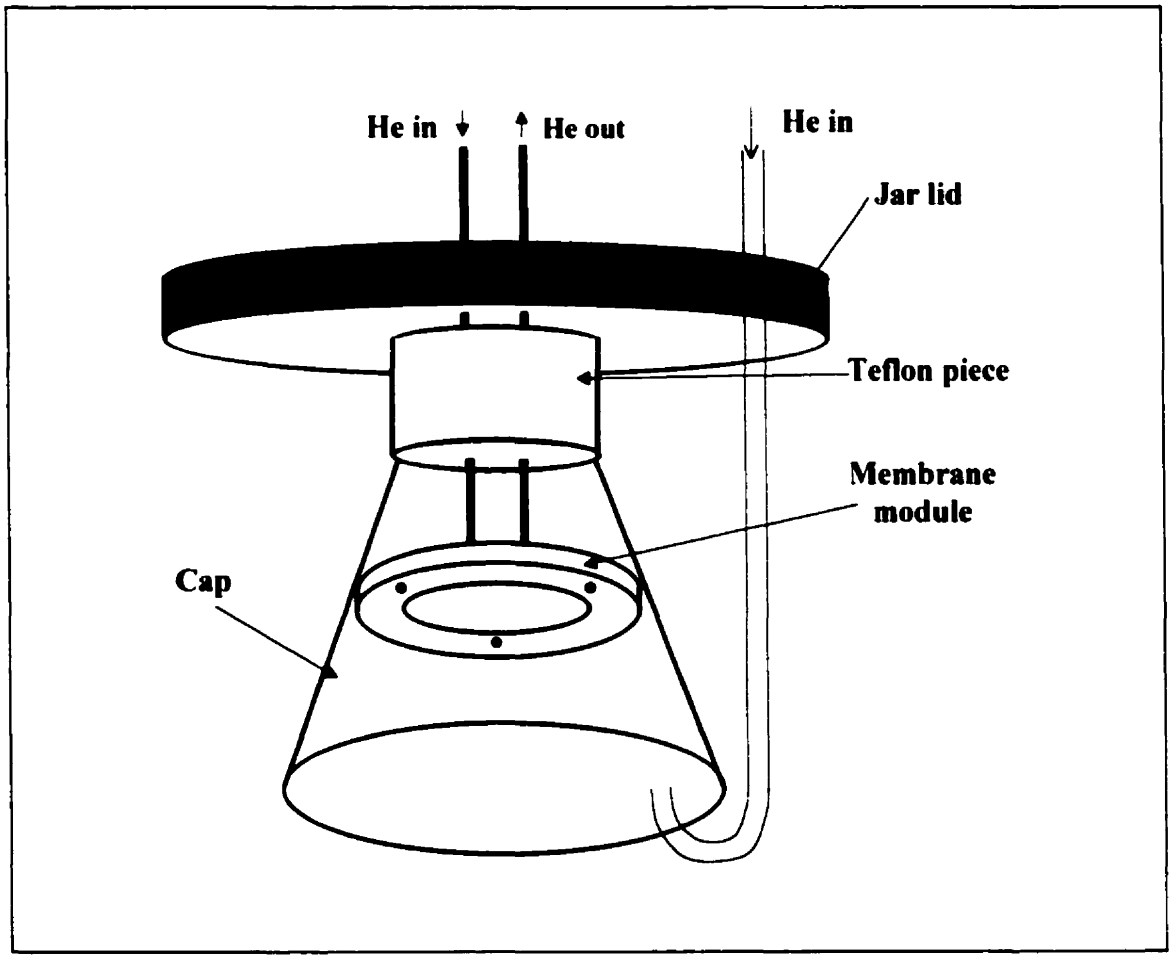

Figure 4- 26. Schematic representation of the cap membrane design purged by helium

A benzene and toluene mixture was analyzed using a PSDVB trap (packing bed of 1.3 cm). The concentration of the B and T in the mixture was 100 ppb (w/w). The membrane was

immobilized in the cap, but it was not purged with helium. The trapping time was 1 minute. In another experiment, a mixture of benzene, toluene and xylene (100 ppb for each compound) was analyzed using the same experimental setup, but having the membrane purged with helium. The baseline of the TCD was monitored as it dropped during purging. When the baseline value reached a steady value the helium flow was interrupted and the membrane was exposed to the sample. The trapping time was 2 minutes. The results obtained for the two experiments are illustrated in Figure 4- 27. The upper trace represents the chromatogram obtained with the membrane surrounded by air, and the lower trace represents the chromatogram obtained with the membrane purged with helium.

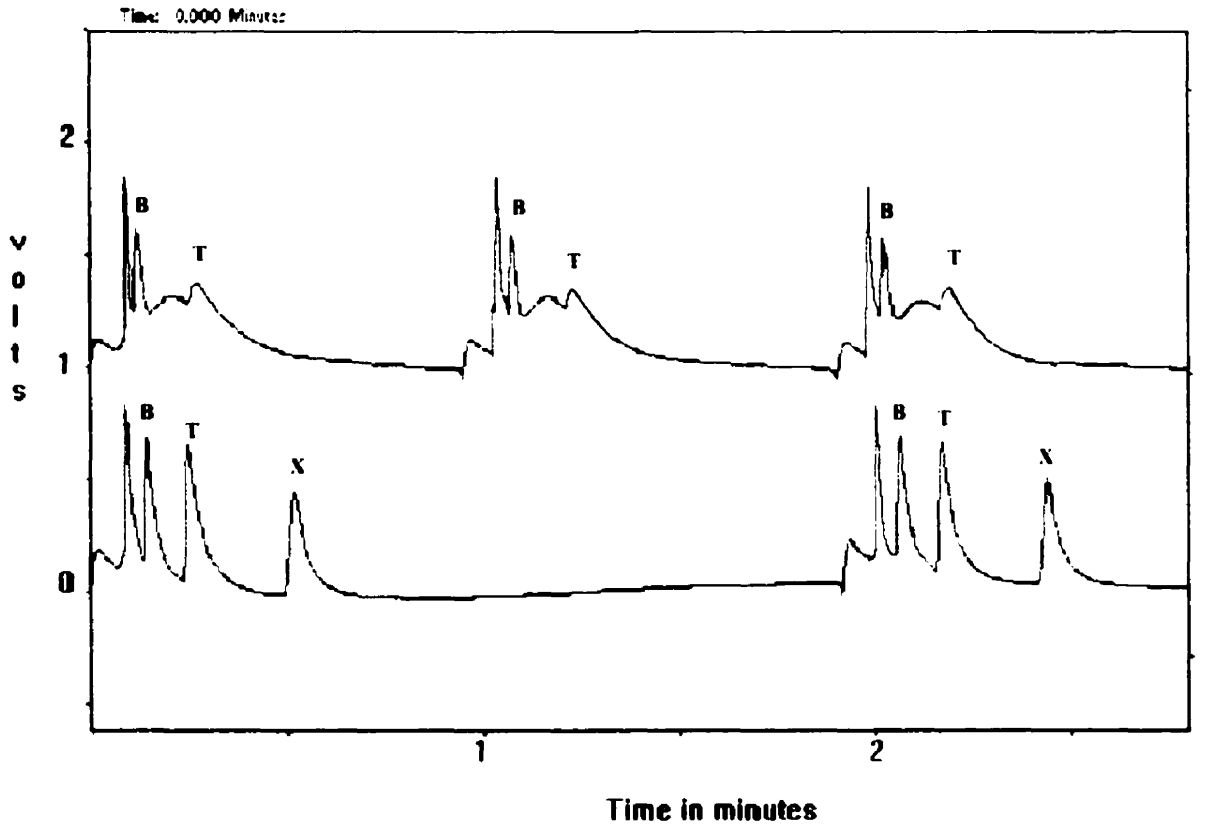

Figure 4- 27. Comparison of chromatograms obtained using the cap design. Upper trace = membrane in air atmosphere; lower trace = membrane purged with helium

Thus, by creating a helium atmosphere around the membrane, the WTP was eliminated. It can be seen in the chromatograms that the peaks in the lower trace appear well separated and no interference from the WTP can be seen.

The experiment proved that the cap design could be used to stop oxygen from entering the system. The fact that no additional gas cylinder was required was very important in maintaining the field portability feature of the system. At the same time, by surrounding the membrane with helium, the extraction efficiency was increased, due to the fact that the analytes diffuse faster through helium than through air. Overall, the use of the purged cap design was beneficial for the MESI-Micro GC system.

4.5 Summary

MESI has been shown with the work presented herein to be a viable analytical tool for the analysis of various environmental samples.

The coupling of MESI to a gas chromatograph equipped with a flame ionization detector proved to be easily achieved, resulting in a system that could be used for the analysis of volatile organic compounds in various matrices. The sensitivity of the detector proved to be very good, providing complex chromatograms for the analyzed samples. Thus, the only inconvenience of the system is the fact that the peaks can not be identified with FIDs.

Even with the high requirements of mass spectrometers, MESI could be coupled to a quadrupole MS. The detection was poor in terms of sensitivity, due to the age of the MS used. However, the detection limits achieved with new MS detectors are much better than the ones obtained when using FID. Thus, the very important issue of identifying the peaks present in a sample mixture was resolved by coupling MESI to a GC-MS.

The portability problem was solved by coupling MESI to a Micro GC equipped with a micro TCD. Even though the detection limits achieved with regular TCD are not very low, by providing preconcentration of the sample, detection a limit of 60 ppt was achieved. However, due to the fact that the TCD responds to water and air, a water trap had to be introduced in the system to eliminate the interference from the moisture permeating the membrane. The problems resulting from the degradation of the sorbent due to the presence of oxygen in the carrier gas was solved by using the cap membrane design and by purging it with helium.

4.6 References

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CHAPTER 5

MESI FOR THE ANALYSIS OF HUMAN BREATH

5.1 Introduction

5.1.1 The use of breath analysis for clinical chemistry

The "odor of decaying apples" in the breath of patients with severe diabetes was first recorded in 1798 by John Rollo in his classic monography "Cases of the Diabetes Mellitus".[1] In 1857 Petters identified the odor producing substance as acetone[2], and in the Bradshawe lecture to the Royal College of Physicians of London in 1886 Dreschfeld stated that the odor of acetone in the breath was a characteristic feature of diabetic coma.[3,4]

Proved to be a useful technique for clinical chemistry, breath analysis became more and more popular, and a lot of research has been performed in this field. Significant methodological advances were made by Scott-Wilson[5], Marriott and by Folin and Denis,[6] and in 1920 Hubbard[7] and Widmark[8] successfully measured the concentration of acetone in the breath of normal humans. These methods were insensitive and required tedious procedures for concentrating the sample prior to analysis.

The lung is the most efficient boundary organ. The adult lung has $3*10^8$ alveoli, which form a total surface of about 70 m^2 that are in contact with capillaries. The pulmonary blood flow consists of a total cardiac output that averages about 6 liters/min, and the mean daily respiratory volume is of about 10^4 liters.[9] It follows that the breath should faithfully reflect

the concentration in the pulmonary capillary circulation of those substances that are capable of ready transfer across the alveolar-capillary membrane. Volatile organic compounds produced by metabolic processes partition from the blood stream via alveolar pulmonary membrane into the alveolar air. The presence of some of these compounds in the expired air was correlated to some diseases or metabolic disorder.

Breath can be seen as the headspace of the blood present in the lungs, and breath analysis, as a headspace extraction of the VOCs present in the blood stream. The concentration of analytes in the breath is directly related to their concentration in the blood form the capillary bed which lies in the walls of the alveoli, but not necessary related to their concentration in the venous blood. For example, if a subject has only one hand exposed to a chemical, venous blood samples taken from the two hands will give different concentrations of the chemical to which one of the hand was exposed. This is due to the fact that the venous blood collects substances from the tissues, and if analyzed, it would indicate the concentration of analytes in the part of body the blood sample was taken from. However, the venous blood is mixed before it reaches the heart, and so breath, as well as the blood form the capillary bed which lies in the walls of the alveoli gives a mean concentration of substances for the entire body.

Breath tests open a unique window into the composition of the blood. Normal human alveolar breath contains a large number of volatile organic compounds derived from the blood by passive diffusion across the pulmonary alveolar membrane.[10,11] Approximately 200 compounds have been detected in the breath of normal human subjects.[12] The compounds present in human breath include hydrocarbons, alcohols, ketones, and aldehydes. The major VOCs in the breath of healthy individuals are isoprene, acetone, ethanol, methanol and other alcohols.[13]

Breath testing for VOCs is intrinsically safe and noninvasive, and might offer a new approach to the early diagnosis or evaluation of several common disorder including lung cancer,[14] heart disease,[15] exposure to environmental toxins,[16] schizophrenia,[17] malnutrition,[18] rheumatoid arthritis,[19] and inflammatory bowel disease.[20]

Some of the diseases for which VOCs from breath were used for assessment are:

Diabetes

The most extensively studied use of breath analysis, excepting toxicology, is the concentration of breath acetone in relation to diabetes. The presence of acetone in an increased concentration can indicate a relative deficiency of carbohydrates in muscle and adipose tissue, leading to an increased catabolism of fat.

Weight Reduction

For obese subjects, acetone has been used to monitor their commitment to the diet program. The breath acetone doubles by the second day of a low-carbohydrate diet, and by the third or fourth day, increases approximately five fold. When the breath acetone concentration of a subject has stabilized, it is very easy to establish whether a high carbohydrate meal was taken.

Renal Disease

Dimethyl- and trimethylamine have been found in high concentrations in the breath of uremic patients. Both these amines return to normal values after renal dialysis. Because their concentration is also reduced after antibiotics administration, it is believed that intestinal flora are involved in their production.

Increased concentration of isoprene has been found in the patients' breath following haemodialysis.[21]

Hepatic Disease

Increased concentrations of dimethyl sulfide, mercaptans, and fatty acids have been found in the breath of cirrhotic patients.

Circulatory System Disease

The level of isoprene in the breath has been correlated to the level of cholesterol in the blood.[22,23]

Periodontal Disease

Methanethiol, produced in periodontal pockets and on the surface of the tongue, is the major cause of halitosis.[24]

Increased concentration of volatile sulfur compounds and nitrogen-containing aromatic compounds as well as hydrogen sulfide, pyridine, and butadienes were found in the saliva and breath of patients with periodontal disease.[25]

Drugs

Many drugs have been found to be excreted via the lungs. Disulfiram (Antabuse), a drug used to render alcoholics averse to alcohol, is converted to CS_2, which has been found in the breath, but not in the blood of patients taking the drug.[26]

Delta-9-tetrahydrocannabinol (THC), detected in breath, shows a rapid decay below the limit of detection after 10 to 15 minutes. This disappearance is not due to its conversion to 11-hydroxy-THC, because THC remains unchanged in the blood for considerably longer periods after smoking, at high concentrations. Therefore, the THC in the breath is probably derived from the mouth or tongue surface.[27]

Anesthetics: 11 to 20 days after anesthesia, halothane ($CF_3CHClBr$) can be detected in the breath of patients, and up to 64 hours in the breath of anesthesiologists, after occupational exposure.[28]

Methoxyflurane ($CHCl_2CF_2OCH_3$) was detectable in the breath of patients for up to 18 days after anesthesia and in the breath of anesthesiologists for up to 30 hours after occupational exposure. Nitrous oxide was detectable in the breath of patients after anesthesia, but not in the blood.[29]

Toxicological use of breath analysis

The greatest use of breath analysis has been in monitoring inhalation exposure to gaseous and volatile compounds. Some of the compounds monitored by breath analysis are: acetone, toluene, styrene, ethanol, trichloroethylene, and dichloromethane.

The evaluation of work related exposure to VOC is one of the areas in which breath analysis has been applied. Researchers have applied breath analysis for the monitoring of low-level solvent and fuel exposure of aircraft maintenance personnel.[30]

5.1.2 Advantages and limitations of breath analysis

Breath analysis is a non-invasive technique, and with recent developments, potentially rapid in application. Other procedures for studying digestion, adsorption, or utilization of dietary nutrients are often difficult for both, patient and physician. Those procedures can be painful or uncomfortable, for example, intestinal perfusion studies, which require prolonged intubation, or classic tolerance tests, which necessitate multiple blood samples. They are also time-consuming, and subject to errors, such as in the 24-hour collection of urine or 3-day fecal balance. All those inconveniences often discourage the patient or the doctor from pursuing a given line of clinical evaluation.

The advantages of breath analysis are:

- Breath samples closely reflect the blood concentration of biological substances and may obviate the collection of blood samples.
- Samples are easily obtained: the sampling process is simple and non-invasive.
- Breath is a much less complicated mixture than serum or urine, and requires less complicated separation steps.
- Breath analysis provides direct information on respiratory function that is not obtainable by other means.
- Dynamic real-time monitoring of the decay of volatile toxic substances in the body is straightforward.
- Breath is suitable for immediate "field" analysis, or samples can be collected and analysed in the laboratory.
- However, breath testing is technically difficult because most breath VOCs are excreted in nanomolar or picomolar concentrations. Since these levels are too low for detection by most instrumentation, breath VOCs must be concentrated prior to assay. This, in turn, requires special apparatus for the collection and concentration of breath.

<u>The limitations of breath analysis are:</u>

- Only volatile compounds can be detected in the breath.
- Standardisation for breath is more difficult than for serum.
- Instrumentation for breath analysis is expensive.
- The analytes in the breath are usually present in very low concentrations, and therefore, are more difficult to detect.

5.2 Breath sampling

5.2.1 Currently used breath sampling methods

Only volatile organic compounds can penetrate the alveolar membrane to reach the alveolar air. Due to their very low concentration in breath, the VOCs have to be preconcentrated prior to analysis. However, because of the high moisture level in the breath, water traps have to be used when the VOCs are preconcentrated.

Separation of the compounds present in the exhaled air is required prior to detection, because of the complexity of the samples. Gas chromatography is the most suitable technique that can be applied for the separation of the breath samples.

Thus, there are three main requirements for breath analysis: elimination of the water; preconcentration of the sample; and sample separation. All these requirements are met by MESI, which due to the presence of the membrane, prevents most of the moisture from entering the system. It also provides sample preconcentration, and the samples are separated by gas chromatography prior to detection. Thus, MESI seems to be suitable for breath analysis.

Proper breath sampling is fundamental to all applications of breath analysis, because an exhaled breath sample is not homogeneous.

The breath profile for solvents may be considered as consisting of three phases. At the beginning of the expiration, phase I, the solvent concentration appears as a horizontal line which represents the fact that the airway dead space contains little or no solvent vapor. Rapid rise in solvent concentration follows in phase II, and slows down as the alveolar air is reached, in phase III.[31] The variation of the concentration of the CO_2 in the breath during one expiration is presented in Figure 5- 1. In the alveolar air, the analytes have a relatively

constant concentration, directly related to the concentration in the blood. Sampling from the end-tidal air (alveolar air) has been proven to be the most satisfactory method for quantitative breath analysis.[32]

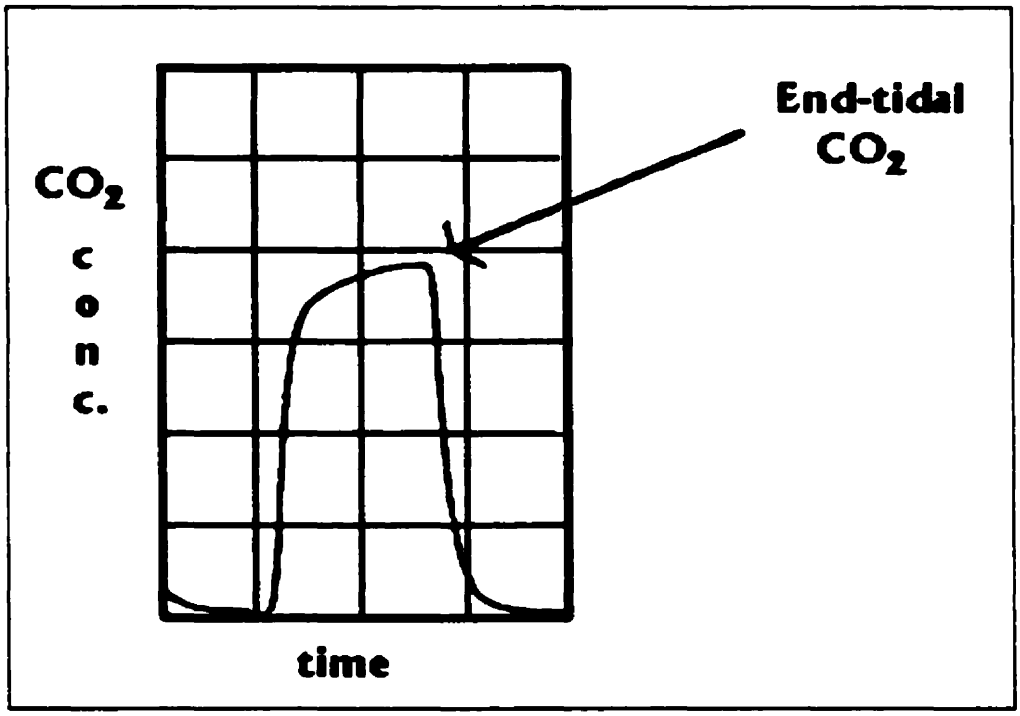


Figure 5- 1. CO_2 concentration profile in the breath

Many sampling techniques have been developed for the collection of the breath. Some of the most used ones are:

Haldane-Priesley tube. Consists of a rubber tube (120 cm) with a sampling syringe inserted through the wall, close to the mouth piece. At the end of the expiration, the tongue is placed over the tube, and a sample of air is collected from the stationary column of air by drawing it into the syringe. The patient is asked to expire into the tube only the last third part of the breath.

Glass pipettes. Is probably the field technique that has gained the most acceptance in occupational health monitoring. The pipettes are made out of glass, about 25 cm long, having a volume of 50 mL, and are fitted with screw caps containing saran liners. The patients are asked to flush the pipettes three times and then, after holding the fourth breath for 30 seconds, they are to exhale through the pipette and collect the end portion of breath by replacing the screw caps. The sample is usually removed from the pipette by a gas syringe.[33]

Aluminum tube. Consists of a cylindrical sampler (34 mL) constructed of aluminum, with a valve system at each end. The subject is asked to breathe into the sampler three times, followed by a period of apnea, and then exhale through the sampler. When the operator decides to trap the air, activation of the locking device traps the alveolar air.[34]

Syringe. Breath samples collected in pipettes or bags usually have to be transferred to a gas chromatograph by a syringe, with unavoidable losses and poor precision. To avoid those problems, attempts have been made to collect the alveolar sample directly into the syringe. A 10 mL syringe with an open side vent at the 9-mL mark, was used. The patient exhales through the barrel of the syringe with the plunger at the 10-mL mark. The plunger is pushed back beyond the outlet, trapping the alveolar air in the syringe. The mouthpiece is exchanged for a needle, and 3 mL of the breath are injected into a vacuum tube. The sample is analyzed by gas chromatography with a thermal conductivity detector.[35]

Recoil bag. Comprises a mouthpiece with sample port, a flutter valve, and a spring-loaded nylon recoil bag of about 100 mL. The subject blows through the mouthpiece, and the bag unwinds. As the velocity through the bag decreases, it recoils, trapping the alveolar sample, which is removed through the sample port into a syringe, for analysis.[36]

Partially evacuated containers. A metal container is partially evacuated at a pressure of 68528 Pa at 25°C. The subject exhales through a tube attached to the container, and the

tidal air vent through a slit. At the moment the breath sample is required, depression of the button activator breaks the vacuum, and a predetermined volume of breath is drawn into the can. A sample of air is withdrawn through a septum in the valve cap into a syringe and is analyzed.[37]

Bag sample. The system consists of two bags connected by an injector "T" mouthpiece. The subject blows into the discard bag, and the high velocity through the jet produces a negative pressure that prevents air entering the collecting bag. When the discard bag is full, the pressure in the system rises, and air enters the collecting bag.[38]

Glass vial. This consists of a sealed, heated glass vial with two hyperdermic needles penetrating the septum. After normal inhalation, the patient exhales through a mouthpiece connected to one of the needles, and the operator removes the vial from the needle assembly after complete exhalation.[39]

Face mask. The sampler is constructed from a pediatric face mask, two one-way flutter valves which control inspiration and expiration, and a three-way stopcock. The mask is fitted over the nose and mouth, and 5 mL sample is aspired through the stopcock into a syringe.[40]

5.2.2 Breath sampling by MESI

All sampling devices noted above use various containers to collect the breath, and a portion of the sample is then analyzed. Some techniques use large volumes of the sample for preconcentration. The sample is pumped in such cases either through a sorbent or a cold trap. However, the analysis is fractionated this way, and part of the sample could be lost during these steps.

A sampling device was designed in order to enable the analysis of human breath by MESI. The device was meant to enable on-line, semicontinuous monitoring of the exhaled air, and a picture of it is presented in

Figure 5- 2.

The device was made out of a piece of plastic tubing of about 12 cm long, and having a diameter of 5 cm. The total volume of the sampling device was of about 250 mL. Two one way flutter valves closed the ends of the tubing. One end of the tubing was equipped with a mouthpiece made out of rubber, and the other end had a plastic piece that prevented the air from escaping, and it could be closed with a plastic cap. The membrane module was placed inside the device, and thus, the sample could be collected online. A small fan was also placed inside the sampling module, to facilitate the circulation of the air inside the module. The fan was oriented in such a way that it pulled the air along the membrane module and it pushed it out against the wall of the plastic tubing. This prevented the opening of the flutter valve from the far end of the device during extraction.

The volume of the sampling devices was chosen to be small, in order to enable only the trapping of the alveolar air. The subject was asked to inhale deeply, hold the breath for a few seconds, and then exhale as much air as possible. During exhalation, the pressure created by the air opened both valves, allowing the breath to be vented out of the sampling device. When the exhalation stopped, the last 250 mL of the breath remained trapped in the sampling device.

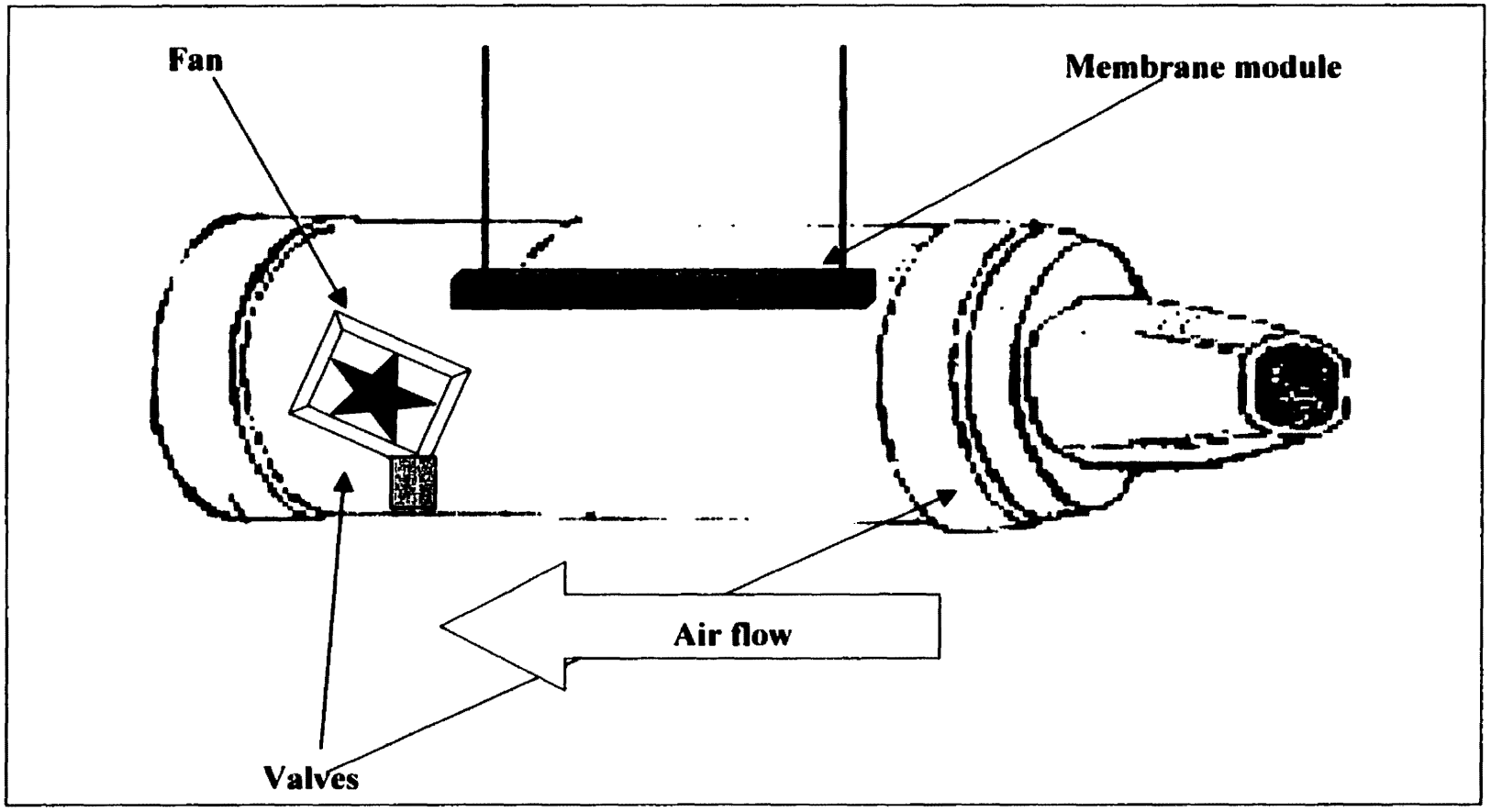


Figure 5- 2. Breath sampling device

The membrane extracted continuously the analytes present in the chamber. The exhalation was repeated every few minutes. Depending on the compound of interest and its concentration in the breath, the sampling time was extended up to 25 minutes, with samples collected every 4-5 minutes.

The collection of only one expiration was enough for some applications. Using this sampling device and MESI as sampling technique, the analysis of the exhaled air is performed online. At the same time, only the concentrated portion of the sample is analyzed. This portion not only contains higher amount of analytes, but it also reflects the concentration of the analytes in the breath.

5.3 Breath profile obtained with a MESI-GC-FID system

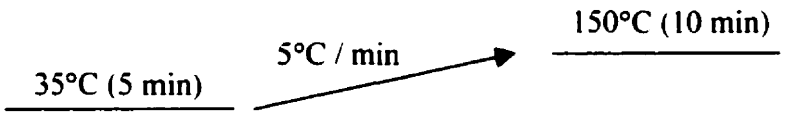
The sampling device described in the previous section was used to collect breath samples that were further separated by gas chromatography and detected using a flame ionization detector. The functionality of the sampling device was tested this way.

Experimental setup

The breath samples were collected using the device described in the previous section. The Chrompack CP 9002 gas chromatograph equipped with a flame ionization detector was used. The system was described in the section 4.2 of the previous chapter. For separation, a SPB-5 column (Supelco, Oakville, ON, Canada) of 30 m length, having an i.d. of 0.25 mm, and a film thickness of 0.25 μm was used. XAD-2 resin (Polystyrene divinyl benzene) purchased from Supelco (Oakville, ON, Canada) was used as packing material for the sorbent

trap. The length of the sorbent bed was 1.3 cm. The trap was cooled using the same type of Peltier cooler as in the experiments described in the previous chapters. A silicone membrane (SSP-M100), of 50 μm thickness, purchased from Membrane Components (Ballston Spa, NY, USA) was used for analysis. The membrane was placed in the membrane module, which was then placed in the sampling device. The previously described capacitive discharge power supply was used for analyte desorption. Helium was used as carrier gas, at a flow rate of 1.5 mL/min. All gases required for the FID (air, nitrogen and hydrogen) and for the chromatographic column were purchased from Praxair (Kitchener, ON).

The temperature program used for the column was:



The sampling period was of 25 minutes. The breath profile of a healthy, nonsmoker subject was recorded. The subject exhaled in the sampling device every 5 minutes.

The breath of a smoker was also analyzed in order to verify the difference between the breath profile recorded before and after smoking.

Results and discussions

The breath profile obtained from the healthy, nonsmoker subject is presented in Figure 5- 3. Even though the identity of the peaks was not known, the first major peak in the chromatogram is presumed to be acetone, which is one of the most abundant compounds in the breath. Approximately 70 peaks were obtained in the chromatogram. Definitely, if only some of the compounds from the breath are of interest, the selectivity has to be increased.

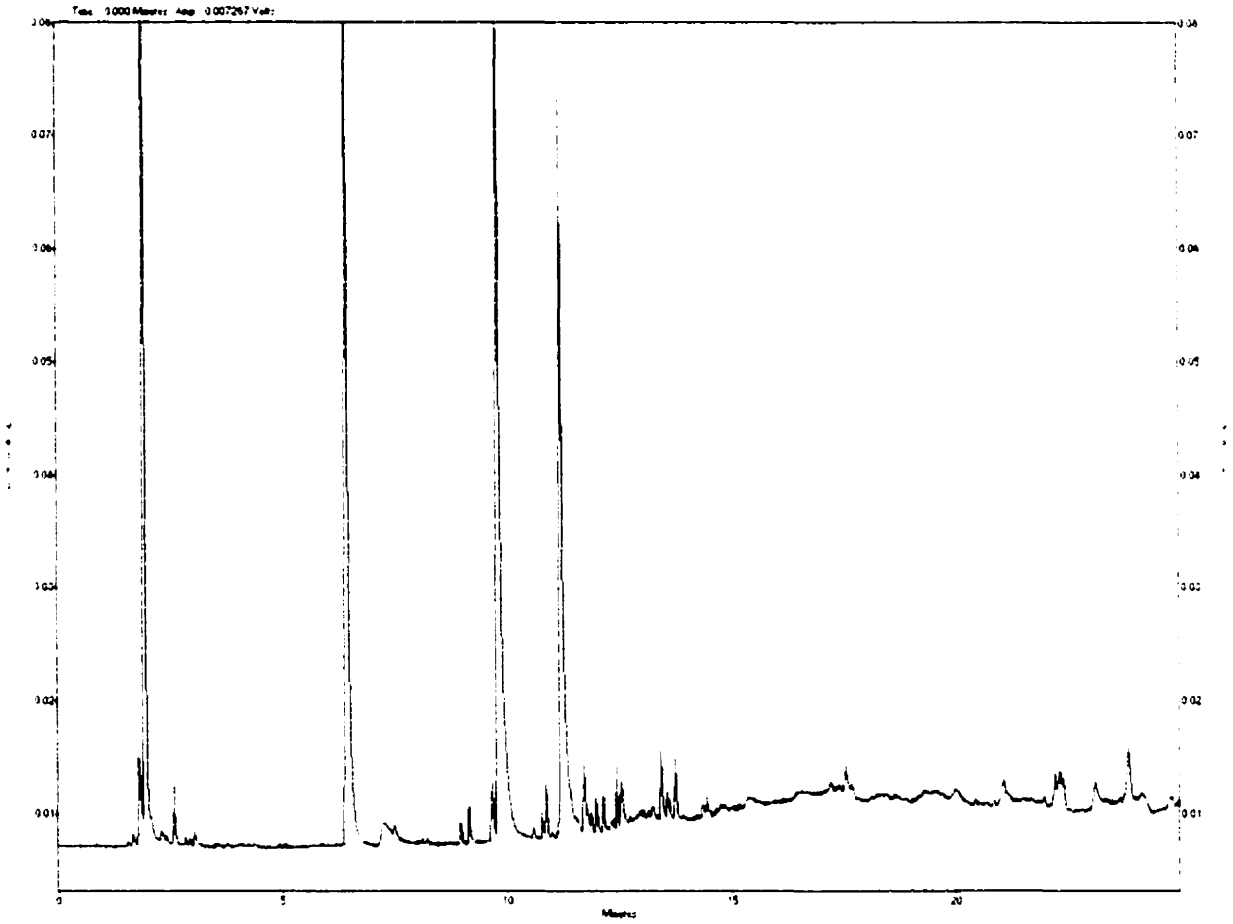


Figure 5- 3. Breath profile obtained from a healthy individual

The same sampling technique was used to analyze the breath of a smoker. The subject abstained from smoking for about 10 hours before sampling the "blank".

The chromatogram obtained before smoking is presented in Figure 5- 4.

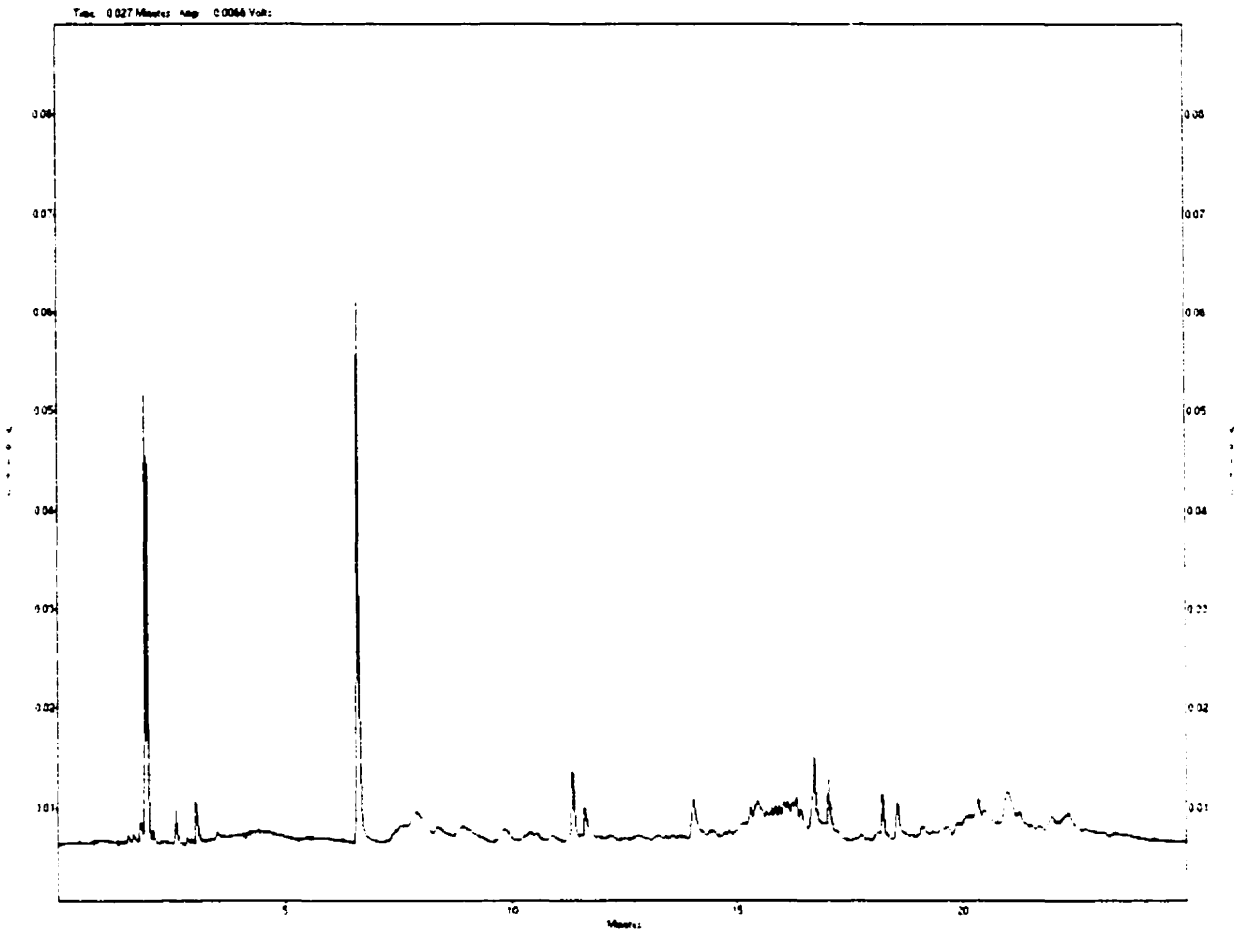


Figure 5- 4. Breath profile obtained from a smoker, as a blank before smoking.

Two major peaks were present in the chromatogram obtained as a blank. The subject's breath was again analyzed about 30 minutes after finishing smoking a cigarette. The chromatogram obtained after smoking is presented in Figure 5- 5.

If the chromatogram in Figure 5- 5 is compared with the one obtained as blank. it can be seen that more major peaks are presented in the "after smoking" sample. Due to the fact that the sampling device collects only the alveolar air. while the air from the mouth and the superior respiratory tract get in contact with the membrane for only a few seconds. it is assumed that the extra peaks present in the chromatogram are an effect of smoking. A good

indication that the sample process was consistent during the two analysis is the height of the first two major peaks from the chromatograms, which is the same for both samples.

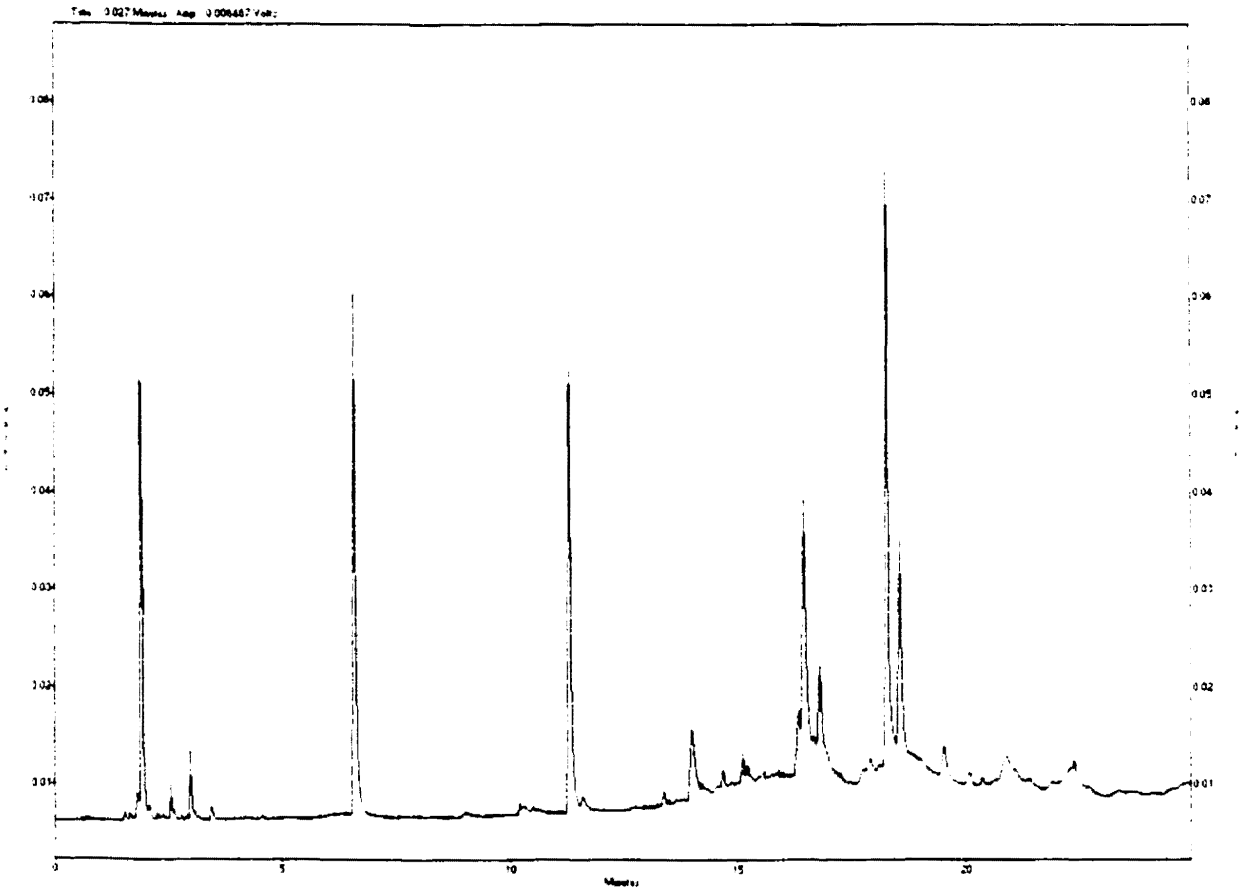


Figure 5- 5. Breath sample profile obtained after smoking.

The concentration of the compounds in the exhaled air, that is measured this way, can be strongly affected by the way the subject inhales and exhales. If the subject holds the breath for a longer time before exhalation, more VOCs get to cross the alveolar barrier, and thus the tidal air becomes more concentrated in VOCs. At the same time, the exhalation is very important since the collection of a sample in which the alveolar air is diluted by a portion of the air from the dead respiratory volume would result in the detection of lower amounts of VOCs.

If the chromatogram obtained from the nonsmoker (Figure 5- 3) is compared with the one obtained from the smoker subject before smoking (Figure 5- 5), it can be seen that the number and the height of the peaks from the first chromatogram is much higher than the one from the second chromatogram. A possible explanation for this is that the lung of a smoker is not able to exchange compounds in the same way the lung of a nonsmoker is. Previous clinical studies have indicated the degradation of the respiratory functions in smokers. Chronic respiratory symptoms and spirometric evidence of airflow limitation have been associated with smoking history.[41,42] Due to the fact that part of the alveoli of smokers' lungs are blocked, the exchange of VOCs between blood and breath is performed less efficiently than in nonsmokers' lungs. At the same time, a smoker can not exhale as efficiently as a nonsmoker[43], thus the sample might be diluted by dead volume air. However, further clinical studies would have to confirm these assumptions.

Even though these things seem to be very inconvenient for the quantification of the compounds in the breath, this phenomenon is encountered also for the analysis of other biological samples, like for example urine. In the case of urine, the concentration of analytes in the sample depends on the amount of liquid that the subject consumed. For this reason, most of the results obtained for the analysis of the concentration of various compounds in the urine are rapported to the concentration of creatinine (which is used as a reference). In the same way, carbon dioxide could be used for the normalization of the concentration of the compounds present in the breath. Sensors for CO_2 measurements can be connected online with the breath sampling device, in order to get an estimation of the concentration of a breath sample.

The above presented experiments proved that the sampling device worked properly, and the breath sample could be analyzed using MESI.

5.3.1 The influence of humidity on the MESI analysis

Since the high level of water present in the breath is one of the main inconveniences when performing breath analysis, the influence of the humidity on the MESI extraction was evaluated.

Experimental setup

The experimental setup was very similar to the one described in the previous section. The MESI-GC-FID system was the same. The temperature of the column was maintained at 30 degrees to enable the separation of acetone and ethanol. The standard gas mixture was delivered by the same standard gas generator that was previously described. However, due to the fact that the influence of humidity was examined, a glass container containing water was introduced in the carrier gas line. The container was filled half way with water, and the diluting carrier gas (compressed air) was allowed to pass through the headspace of the water. Three different humidity levels were examined. For the dry air experiment, the water container was not placed in the carrier gas line. In order to obtain a humidity level of 30%, the container was connected to the gas line, and the water was maintained at 22°C by insulating the container with glass wool. In order to increase the moisture level to 90%, the glass container that supplied the water vapors was heated using heating tape.

A mixture of benzene, toluene, acetone and ethanol was used for the experiment. The mixture contained 1.1654 g acetone, 1.5486 g ethanol, 4.6791 g benzene, and 4.2721 g toluene. The diluting flow rate was of 500 mL/min, and the standard mixture was supplied at a rate of 312 nL/min.

A Sunbeam thermometer (Canadian Tire) equipped with a hygrometer was placed in the sampling chamber of the standard gas generator and was used to monitor the temperature and the humidity in the chamber.

Results and discussions

The mixture was analyzed at three different levels of humidity. The first measurements were conducted with dry gas (air). The hygrometer indicated zero moisture level (unmeasurable) in the dry air. After the data were collected. the glass container that was supposed to increase the humidity in the system was connected in the standard generator's gas line. When the water was maintained at 22°C the moisture level was about 30%. When the temperature was increased at about 60°C the moisture level increased to 90%.

The results obtained for the extraction of the four compounds from the mixture is presented in Figure 5- 6.

From the figure it can be seen that an unexpected extraction profile was obtained for the four compounds. For acetone. benzene. and toluene the amount extracted increased significantly at higher humidity. while the amount of ethanol extracted maintained relatively constant for all three values of humidity. Thus. the humidity does not affect the analysis in a negative way. but further research is required to explain this unexpected behavior.

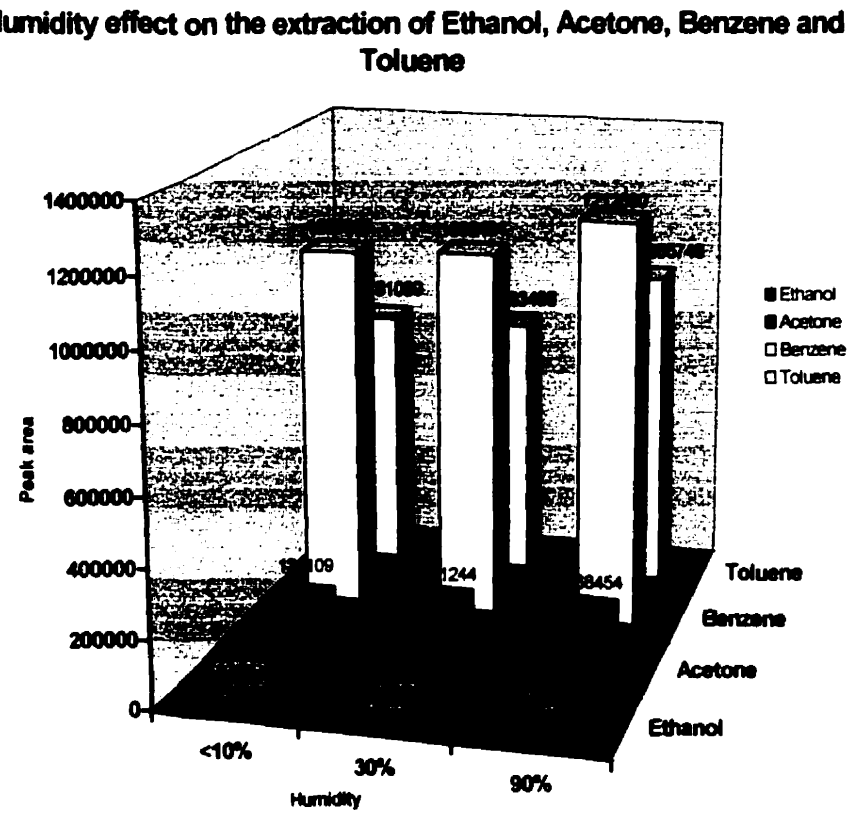


Figure 5- 6. Influence of the humidity on the extraction of acetone, ethanol, benzene and toluene

5.4 Analysis of selected compounds present in breath by MESI, using an ion mobility detector

A Barringer IonScan instrument was used for the identification of selected compounds in the breath. The IonScan detector is based on a technology called ion mobility spectrometry (IMS). The commercial instrument is a powerful analytical tool that can detect and accurately identify trace residues of a wide variety of chemicals. It has been optimized for the detection

of illicit drugs and explosives. Almost all the US airports are equipped with one of these instruments, in an attempt to prevent the smuggling of drugs and explosives.

The instrument is relatively small and it can be easily transported in the field. Air pumped from the atmosphere is purified and used as carrier gas. Thus, no additional gas cylinders are required. As currently designed, the instrument allows for the analysis of solid sample only. By coupling the instrument with MESI, the analysis of VOCs in various matrices would be enabled. However, the most interesting feature of the system would be its applicability for field analysis, since both, the IonScan and MESI are specially designed as portable tools.

The heart of the IonScan is the IMS detector. The identification of many substances by ion mobility spectrometry is made possible by a few basic principles. Many chemicals give off vapors or particles that are adsorbed by, or cling to the surface of materials they come into contact with (clothing, skin, containers, paper, etc.). These traces can be collected by either vacuum or by wiping the surface. Even microscopic traces of such chemicals can be desorbed by application of heat. The vaporized substances can then be ionized, and the ions are allowed to "drift" in a controlled electric field. In this field, the ions move at different speeds, depending on their molecular size and structure. The characteristic speed at which an ion moves (the ion mobility) is a distinct "thumbprint" that enables the identification of the original substance.

In an ion mobility detector the sample of interest is heated to vaporize the collected compounds, which are then introduced into the reaction region of the instrument by a carrier flow of clean, dry air. The reaction region has a weak radioactive ionizing source, a Nickel 63 Beta emitter. Both positive and negative ions are formed by collision of the Beta particles with the gases from the sample stream. The IMS has an electric field applied along its length.

The polarity of this field is either positive or negative, depending on the type of substances that are to be analyzed. The ions of the correct polarity are allowed to move from the reaction region into the drift region by the momentary opening of a gating grid. The various ions travel at different, but characteristic speeds and arrive at the collector electrode at unique drift times, enabling their identification.

A Barringer IonScan instrument was used as detector for the analysis of various compounds present in breath. The instrument was coupled to a gas chromatograph by inserting the chromatographic column directly into the IMS. The end of the column was placed at about 1 cm in front of the gating grid. The column was taken out of the GC through the oven insulation. The part of the column that was outside the oven (about 30 cm) was heated at a temperature higher than the oven temperature in order to prevent compound condensation at this level.

5.4.1 IMS parameters optimization for the analysis of benzene, toluene, acetone and ethanol

The temperature of the drift tube, as well as the air flow inside the drift tube can be optimized in order to obtain the best sensitivity for a certain chemical. Four compounds were selected for the optimization experiment: acetone, ethanol, benzene, and toluene. Acetone was chosen because it is one of the main constituents in the breath and the results were to be used for the analysis of breath samples. Ethanol is very easily obtained in the breath, thus it can be used for the optimization of the sampling technique, and comparison with other techniques. Benzene and toluene where selected in order to compare the results obtained using the ion mobility detector with the results obtained using other detectors.

Experimental setup

A CP-9002 GC (Chrompack, Middelburg, The Netherlands) equipped with a SPB-5 column (Supelco, Oakville, ON, Canada) of 30 m length, having an i.d. of 0.25 mm, and a film thickness of 0.25 μm. XAD-2 resin (Divinylbenzene) purchased from Supelco (Oakville, ON, Canada) was used as packing material for the sorbent trap. The length of the sorbent bed was 1.3 cm. The trap was cooled using the same type of Peltier cooler as in the experiments described in the previous chapters. Silicone membrane (SSP-M100), of 50 μm thickness, purchased from Membrane Components (Ballston Spa, NY, USA) was used for analysis. The detector was a IonScan M400B IMS (Barringer, Mississauga, ON). The water present in the air (that flows inside the drift tube) was used to induce the ionization of the compounds.

A mixture of benzene, toluene, ethanol, and acetone was used. A standard solution was prepared by mixing 2.7610 g acetone, 2.8799 g ethanol, 3.2257 g benzene, and 3.0613 g toluene. The standard gas generator described in Chapter 2 was used to prepare a mixture of the four compounds in air. Air was used as diluting gas at a flow of 500 mL/min. The mixture was used for the determination of the optimum drift tube temperature and drift flow for the detection of each compound. The syringe pump supplied 31.2 nL of mixture/minute.

In the first experiment the drift flow was maintained at 300 cc/min, while the temperature of the drift tube was varied. The response of the detector was recorded for 190, 200, 210, 220, and 230°C. A trapping time of 7 min. was used. In order to achieve separation between acetone and ethanol, the column temperature was maintained at 30°C.

In the second experiment the temperature of the drift tube was maintained at 200°C, and the drift flow was varied. The response of the detector was recorded for flows of 260, 280 and 300 cc/min.

The calibration curve was constructed for acetone and ethanol, and the estimated limit of detection was calculated. For this experiment, a mixture of 0.8095 g acetone, 1.1142 g ethanol, 4.7180 g benzene, and 4.8515 g of toluene was used. However, only limits of detection for acetone and ethanol were determined due to a very poor response of the detector for the other two compounds present in the mixture. The other experimental conditions were the same as for the previously described experiment.

Results and discussions

Acetone and ethanol are easily ionized in the IMS, and thus they produced a much better response then benzene and toluene.

In the first experiment the response of the detector was examined for different temperatures of the drift tube. The results obtained for the four compounds present in the mixture are presented in Figure 5- 7. As it can be seen in the figure, for acetone the peak height increases with an increase in the temperature of the drift tube. For ethanol, the peak height increases until 210°C, after which it decreases. However, the difference in the peak height is not as significant for ethanol as it is for acetone.

In the case of benzene and toluene, the response of the detector is so poor that a significant change in the height of the peak can not be noticed from the graphic. Though, it should be mentioned that the detection of toluene improves at lower temperatures of the drift tube, while in the case of benzene the opposite was noticed.

Thus, it can be seen that a high drift tube temperature favors the detection of acetone and benzene. However, the response of the detector for benzene and toluene is very poor due to ionization difficulties for these two compounds.

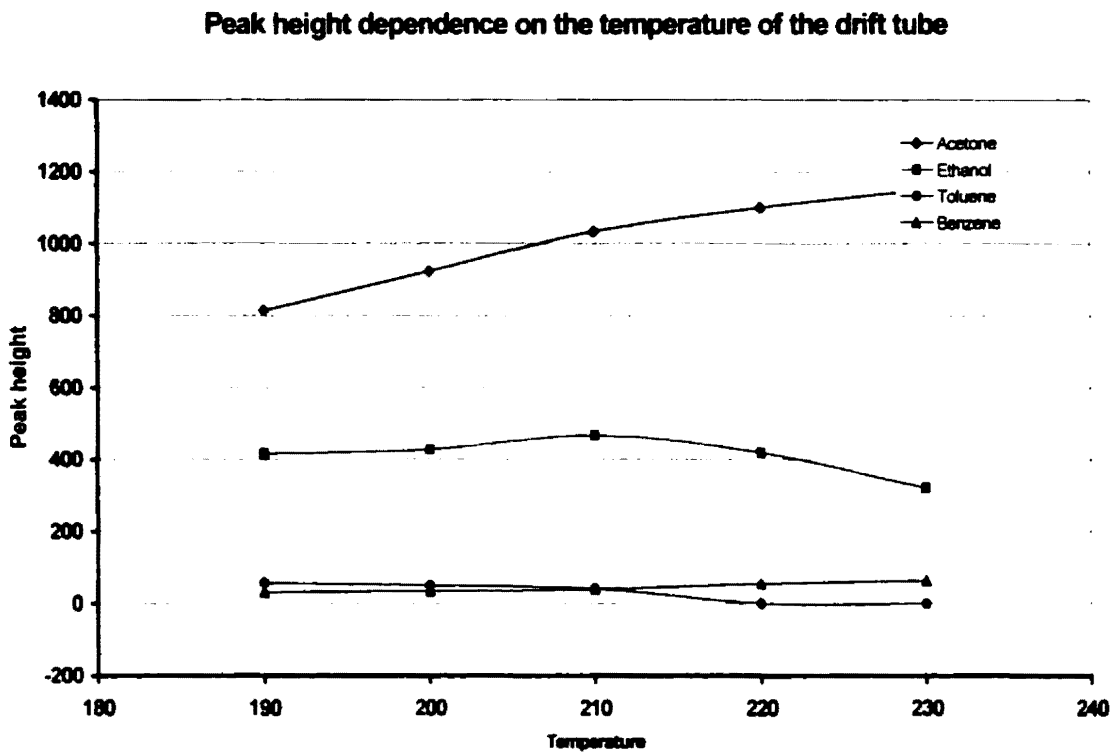


Figure 5- 7. Peak height dependence on the drift tube temperature

A second experiment tried to evaluate the response of the detector, as a function of the of the drift flow. The temperature of the drift tube was maintained at 200°C, while the drift flow was varied from 260 to 280 and 300 cc/min. For a better view, the results are presented in two different figures.

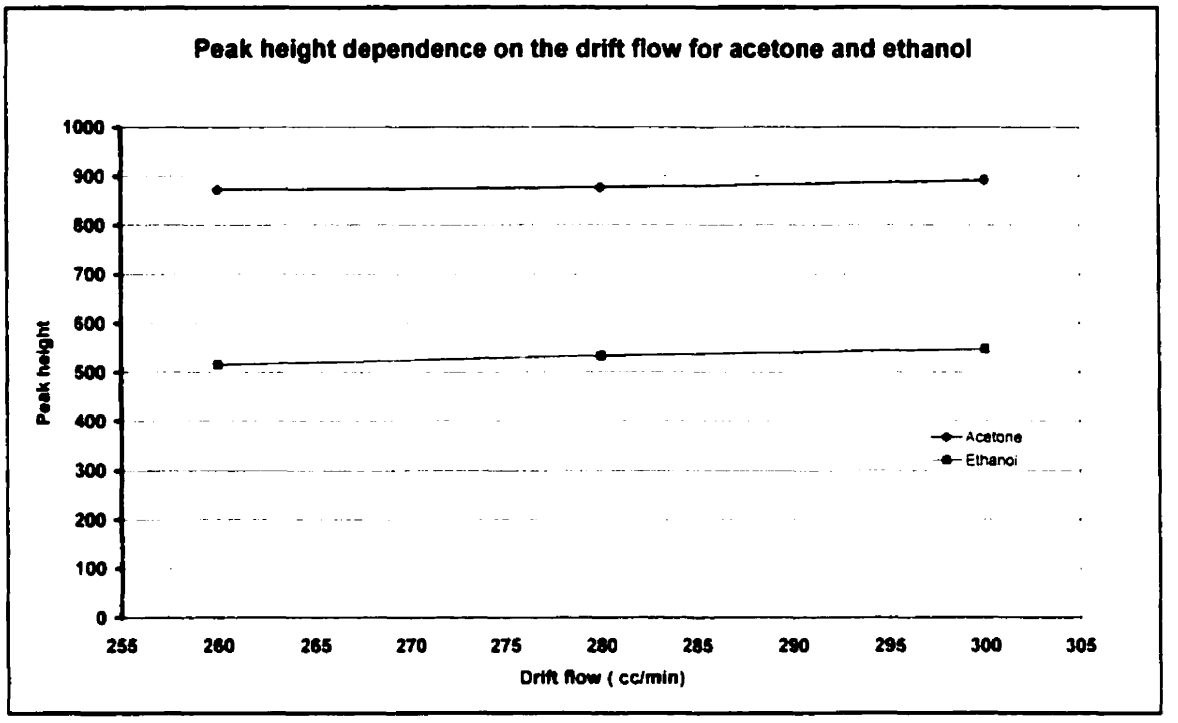


Figure 5- 8. Acetone and ethanol peak height dependence on the drift flow

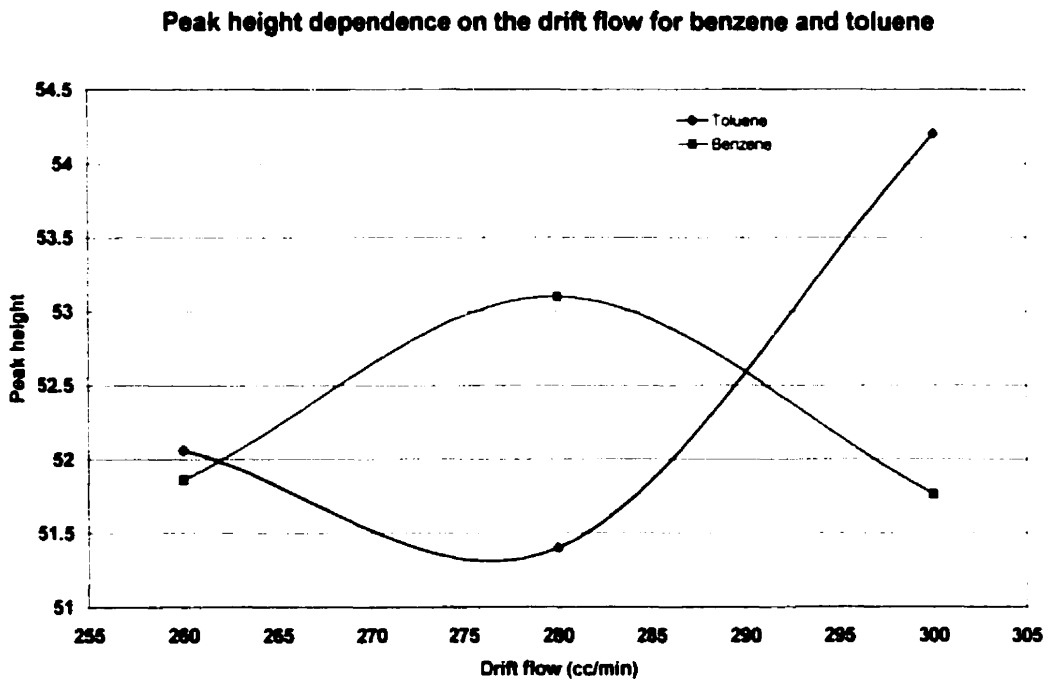


Figure 5- 9. Benzene and toluene peak height dependence on the drift flow

The results obtained for acetone and ethanol are presented in Figure 5- 8, and the results obtained for benzene and toluene are presented in Figure 5- 9.

For acetone and ethanol, the height of the peak increases at higher flow rates. However, the difference can not be considered significant. On the other hand, the results obtained for benzene and toluene show that benzene is better detected at a flow of 280 cc/min, while the detection efficiency decreases at higher or lower flow rated. The exact opposite happens to toluene, which is best detected at 300 cc/min. The detection becomes less efficient at a flow rate of 280 cc/min.

The results obtained in these experiments can be used in future experiments in order to be able to set the optimum experimental conditions for the detection of the target analytes.

Due to the poor sensitivity of the detector for benzene and toluene, the detection limit was not calculated. IMS is definitely not the detector of choice for the analysis of these two compounds.

For acetone, the standard gas generator supplied a constant concentration of 3.6, 1.8, and 0.9 μg/L of air, at flow rate of 0.5, 1 and 2 L/min respectively for the diluting gas. For ethanol, the concentrations supplied by the carrier gas were of 6.6, 3.3, and 1.65 μg/L for the above mentioned carrier gas flows. The calibration curves obtained for the two compounds are presented in Figure 5- 10. The calibration curve obtained for acetone has a relatively good linearity. However, the calibration curve obtained for ethanol does not present a very good linearity. This is partially due to the fact that the ethanol peak is not very well separated from the acetone peak by the chromatographic column. The drift times of the two compounds is also very similar, thus making the detection a bit more difficult.

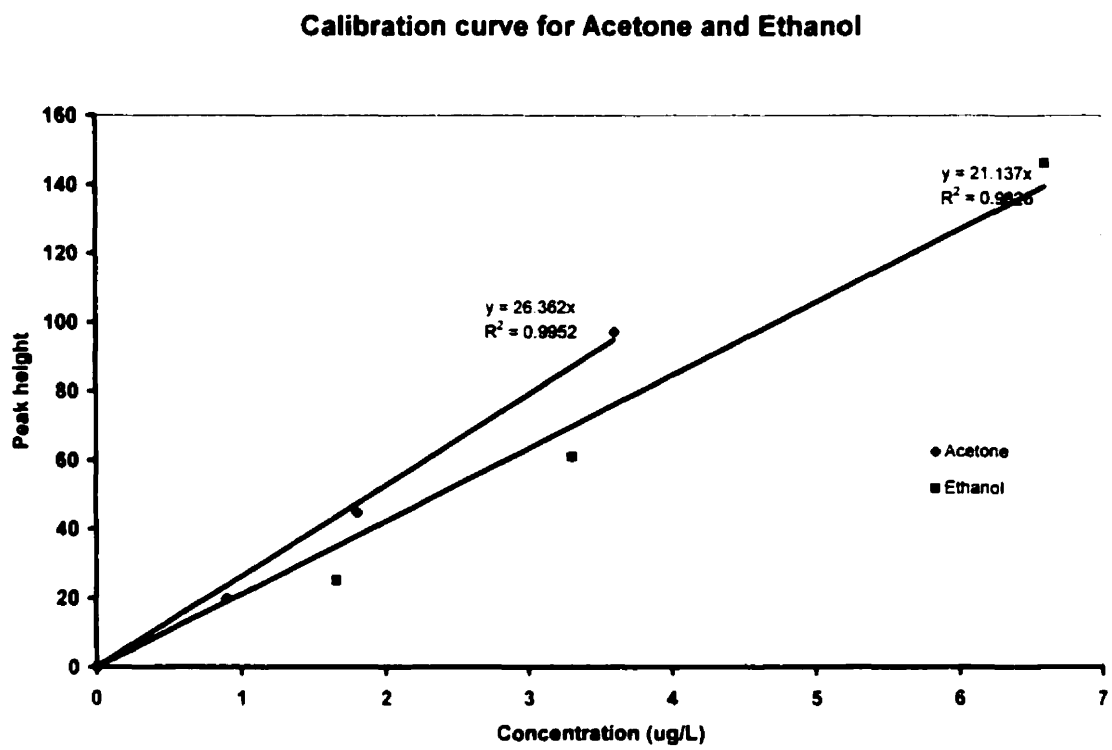


Figure 5- 10. Calibration curves obtained for acetone and ethanol, with MESI-GC-IMS

The detection limits were calculated for the two compounds. The noise level was about 2.25, with a standard deviation of 0.465. Thus, the estimated limits of detection for acetone and ethanol were 0.4 μg/L and 0.5 μg/L, respectively.

The IMS proved to be more sensitive for acetone and ethanol then other detectors are, but its sensitivity for benzene and toluene is poor.

5.4.2 Applications

Acetone, ethanol, and chloroform were analyzed in breath. The level of acetone in breath changes during the day, depending on the amount of carbohydrates received by the body. Due to this, acetone is used for the control of weight reduction and as a marker in the breath of diabetic patients.

Ethanol was used to prove that the volatile organic compounds entering the body through the skin are rapidly distributed in the body through the blood circulation, and they can be found in the breath. Ethanol was used to simulate exposure to toxic compounds.

Chloroform was analyzed in the breath of a subject after the subject spent about 30 minutes swimming in a pool. The water from the swimming pool was treated with chlorine-based disinfectants.

Experimental setup

The MESI-GC-IMS setup was the same as the one described in the previous section. The sampling device used for breath collection was described in section 5.2.2.

For the experiment in which the permeation of ethanol through the skin was evaluated, Absolute Vodka was used as a source of ethanol. The permeation of the ethanol through the skin was facilitated by the application of a small amount of Lanolin on the skin, prior to the application of Vodka. Different amounts of Vodka were rubbed on the hands, and the concentration of ethanol was measured in breath. In order to eliminate the possibility of inhaling ethanol while rubbing it on the skin, the application of the Vodka on the hands was performed in a fume hood.

A constant concentration of ethanol was obtained in the breath by constantly applying Vodka on the skin. This enabled the optimization of the breath sampling. The trapping time used for the extraction of ethanol from the breath was 4 minutes. During this time the subject was asked to exhale once, twice or three times and the results were compared.

For the experiment in which acetone was measured in breath, samples were collected over a 4.5 hour period, and the variation of the concentration of acetone in the breath was correlated with the amount of food ingested. Each measurement used a trapping time of 20 minutes, with breath being exhaled in the sampling device every 4 minutes.

For the detection of chloroform in the breath the sample from a swimmer was collected 40 minutes after swimming for half an hour in the pool. The sample was collected in the same way as for the previous experiment.

Results and discussions

Ethanol in breath

1 mL of vodka was applied on the skin after the application of a small amount of Lanolin, and the collection of the breath started 3 minutes after the application. One breath sample was analyzed each time, using a preconcentration time of 4 minutes. Two other samples were collected at 10 minutes time interval. The result obtained are presented in Figure 5- 11.

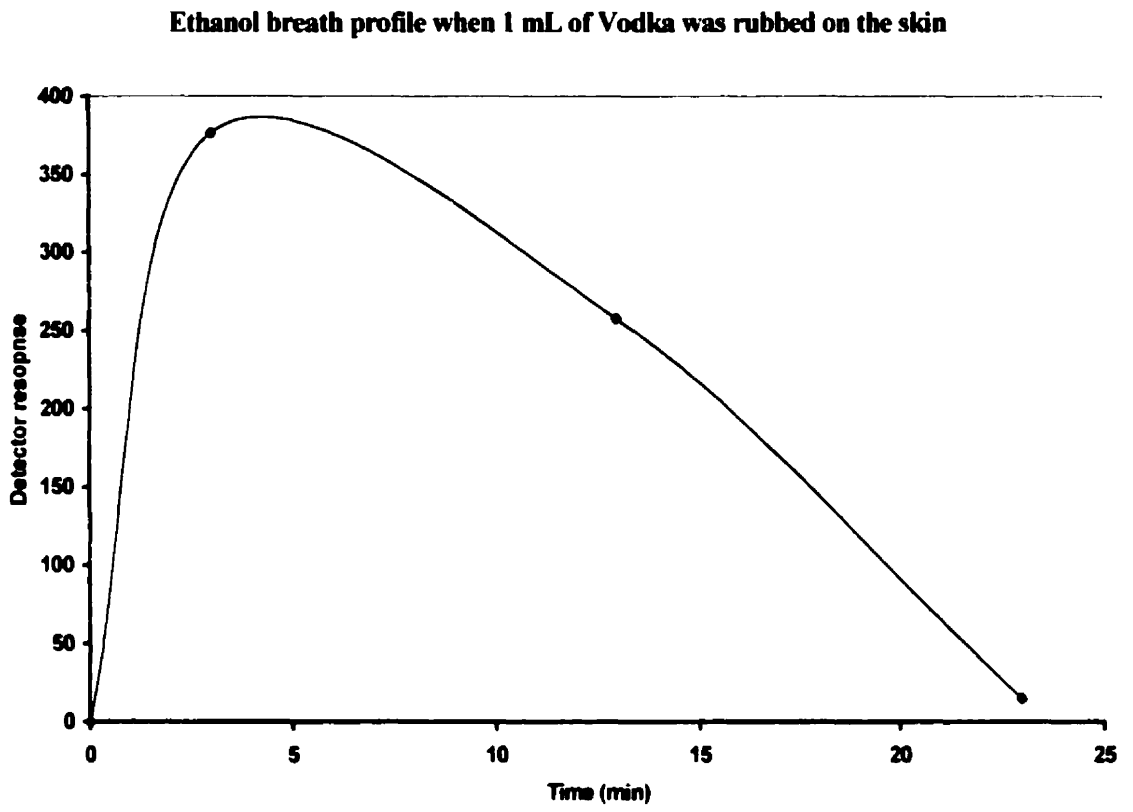


Figure 5- 11. Breath ethanol profile after the application of 1 mL of Vodka on the skin

It can be seen in the figure that the ethanol traveled rapidly through the body to reach the breath. The profile is very sharp, with the concentration dropping rapidly after the first sampling.

The permeation through the skin was used in order to optimize the sampling procedure. It is very difficult to find a marker compound in the breath that would maintain at a very constant concentration over a long period of time. Thus, the permeation of ethanol through the skin was used to obtain the desired marker. Different time intervals were tested

for the Vodka application, and the concentration of ethanol was measured in the breath aiming to reach a concentration plateau. It has been found that by applying 1 mL of Vodka every 5 minutes, the concentration of ethanol maintains constant over a long period of time. Samples were recorded every 15 minutes, using 4 minute trapping times and one breath per sample. The results are presented in Figure 5- 12.

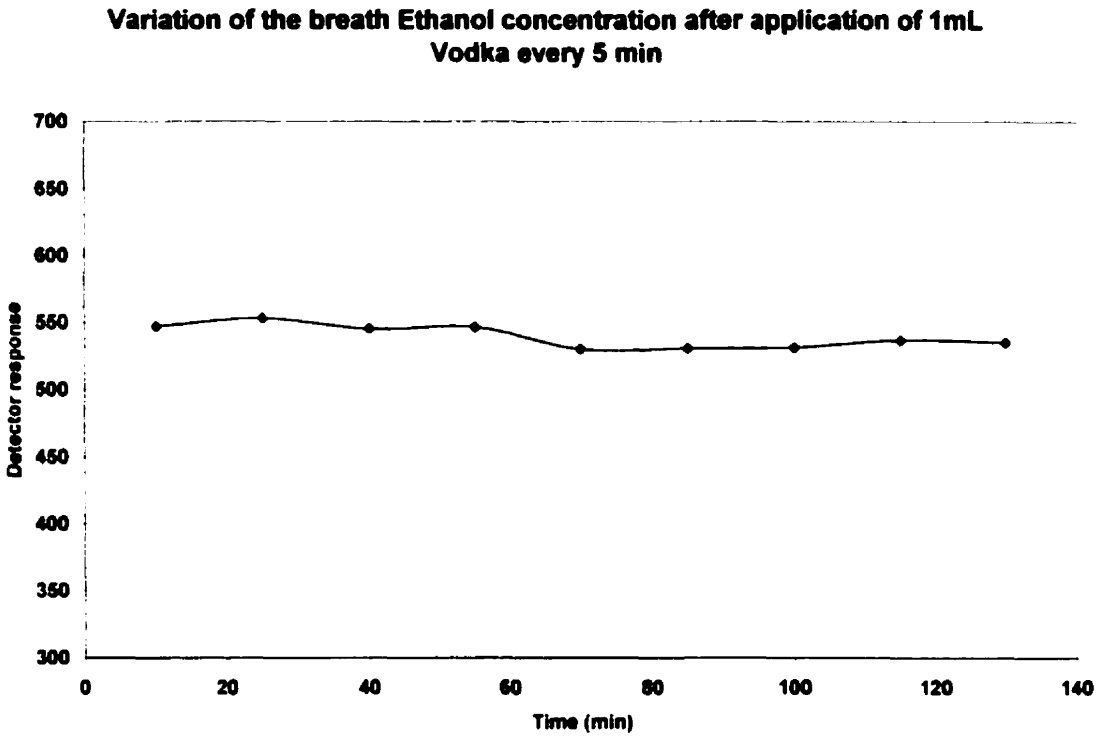


Figure 5- 12. Breath ethanol profile after application on the skin of 1 mL of Vodka, every 5 minutes

Once a constant concentration of ethanol was obtained in the breath, the sampling technique could be evaluated. Different numbers of breath samples were taken per analysis and the results were compared. The trapping time was maintained at 4 minutes, and during the trapping time, the subject was asked to exhale once, twice, or four times. The goal was to

find out whether it was better to breathe more often in the sampling device, or if one sample would suffice. The results are presented in Figure 5- 13.

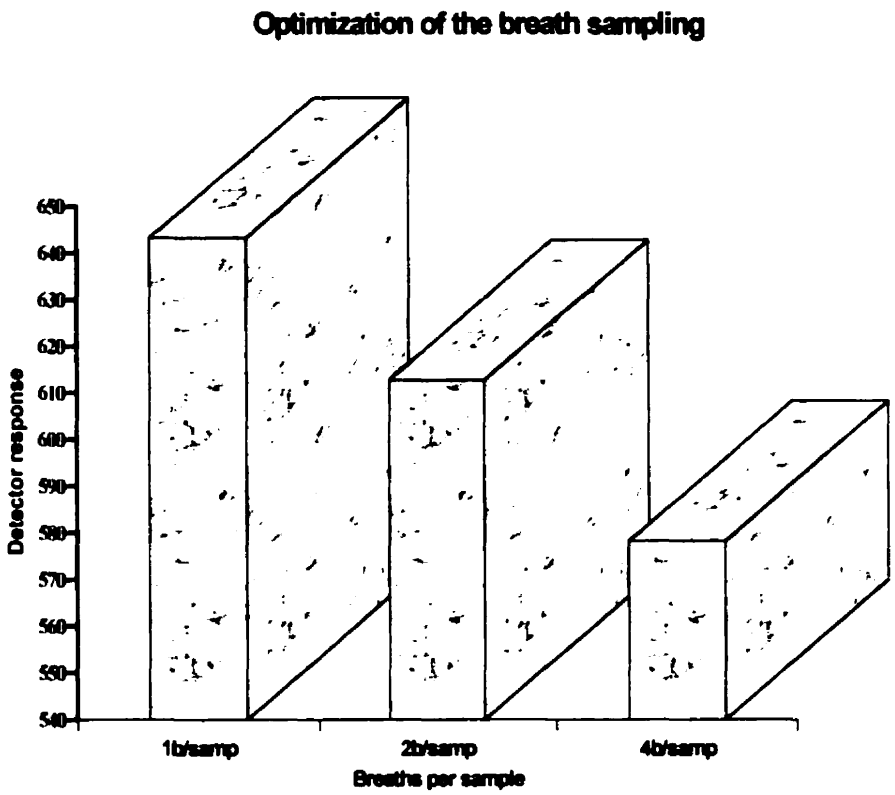


Figure 5- 13. Optimization of the breath sampling

From the results it can be seen that it is better to sample only once rather then two or four times. During each sampling a few seconds are wasted, which might be the cause of the decrease in the extraction efficiency. However, if longer trapping times have to be used due to the low concentration of the target compounds in the breath, one sampling would not be enough because of the sample depletion that occurs after a certain period of time.

Acetone in the breath

Acetone was measured in the breath over a 4.5 hour period. During this time, the subject ate various amounts of food. The correlation between the level of the acetone in the breath and the amount of food ingested is presented in Figure 5- 14.

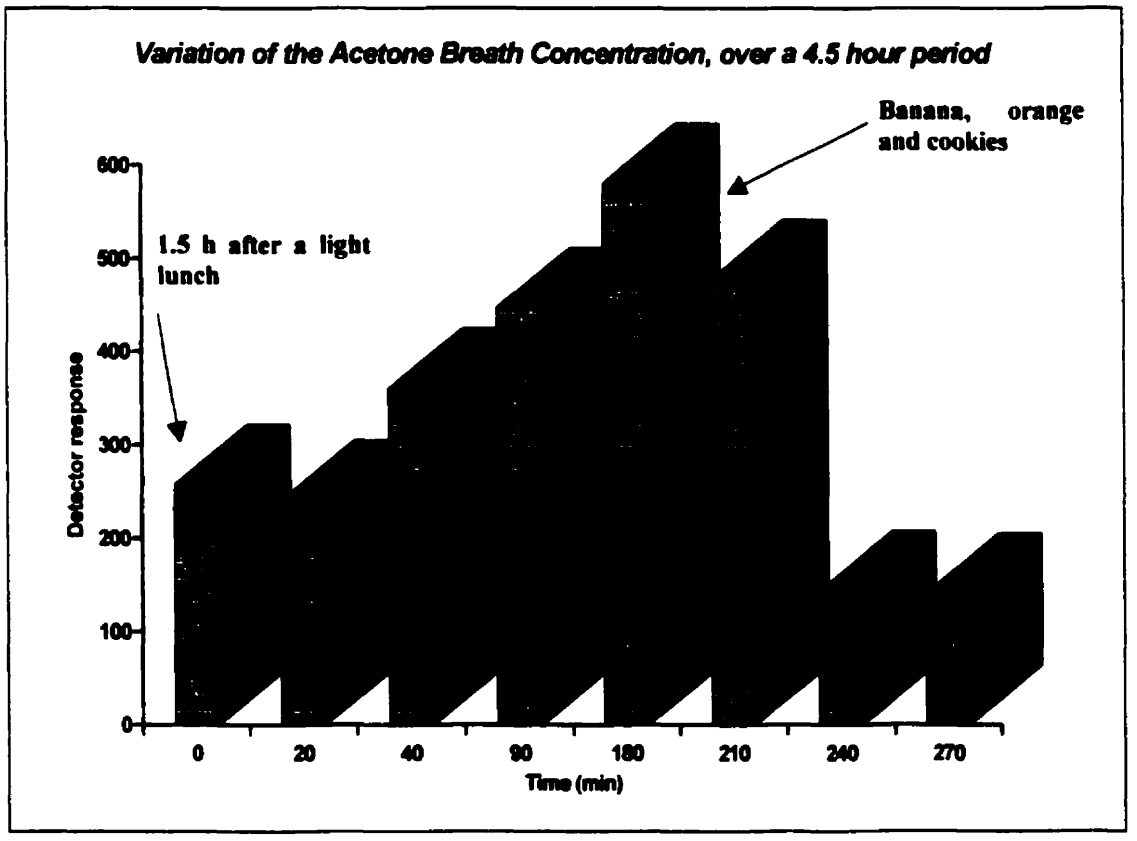


Figure 5- 14. Breath acetone profile recorded over a 4.5 hour period

After lunch, the concentration of acetone in the breath drops, and it starts increasing again after more than two hours after the lunch time. The concentration started to drop after the subject ate a banana, an orange and some cookies. It can be seen that the concentration of the acetone in the breath is in direct relation with the food that is consumed by the subject.

Chloroform in breath

MESI was used to measure the chloroform in the breath of a swimmer. A longer trapping time (20 min) was used in order to preconcentrate the chloroform which was assumed to be present in low concentrations in the breath. The subject was asked to replace the breath sample from the sampling device, every 4 minutes. The plasmagram that is presented in Figure 5- 15 presents the segment in which the chloroform peak reached a maximum. The same peak is presented as a whole peak in Figure 5- 16. Since the IMS can be set to look for certain compounds, in this particular case only the chloroform was measured. The software of the IMS offers the possibility of seeing the peak (or peaks) as in a normal chromatogram in which only the desired peaks are observed (Figure 5- 16).

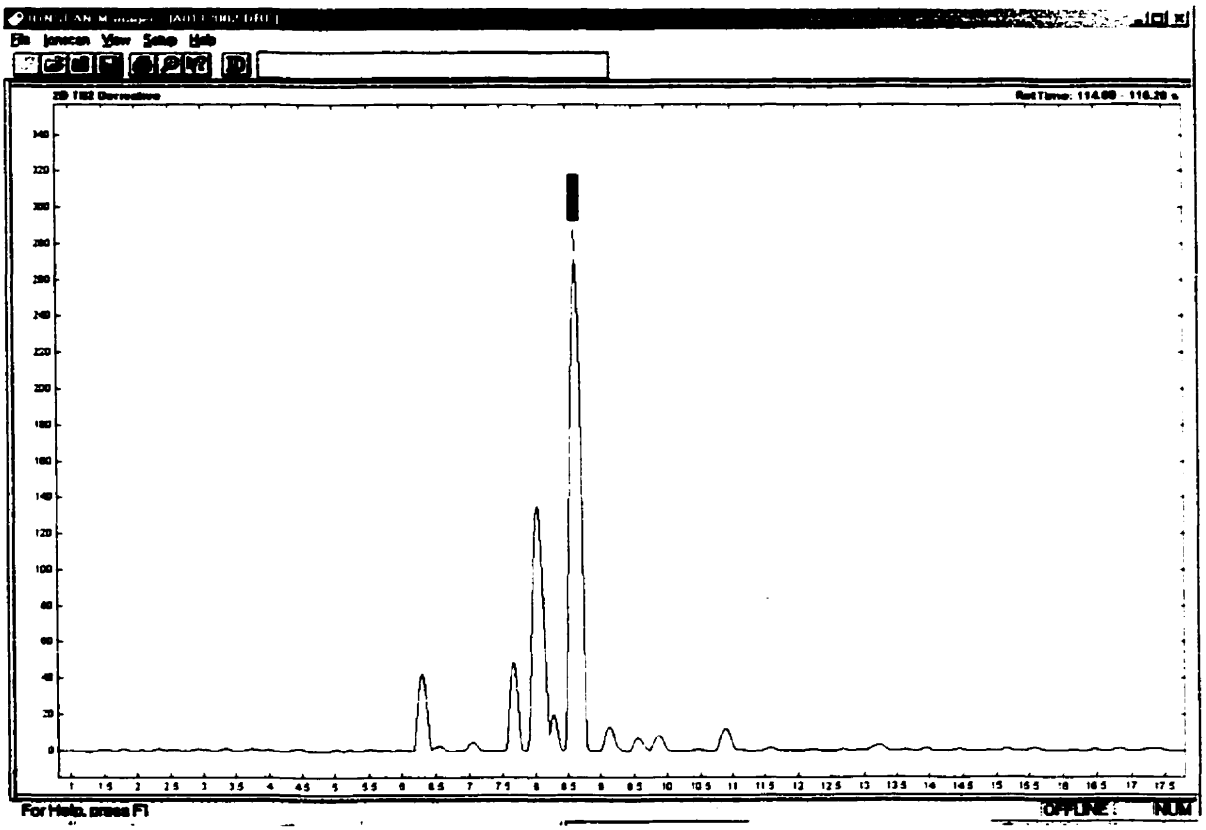


Figure 5- 15. The peak of chloroform in one of the segments of the plasmagram

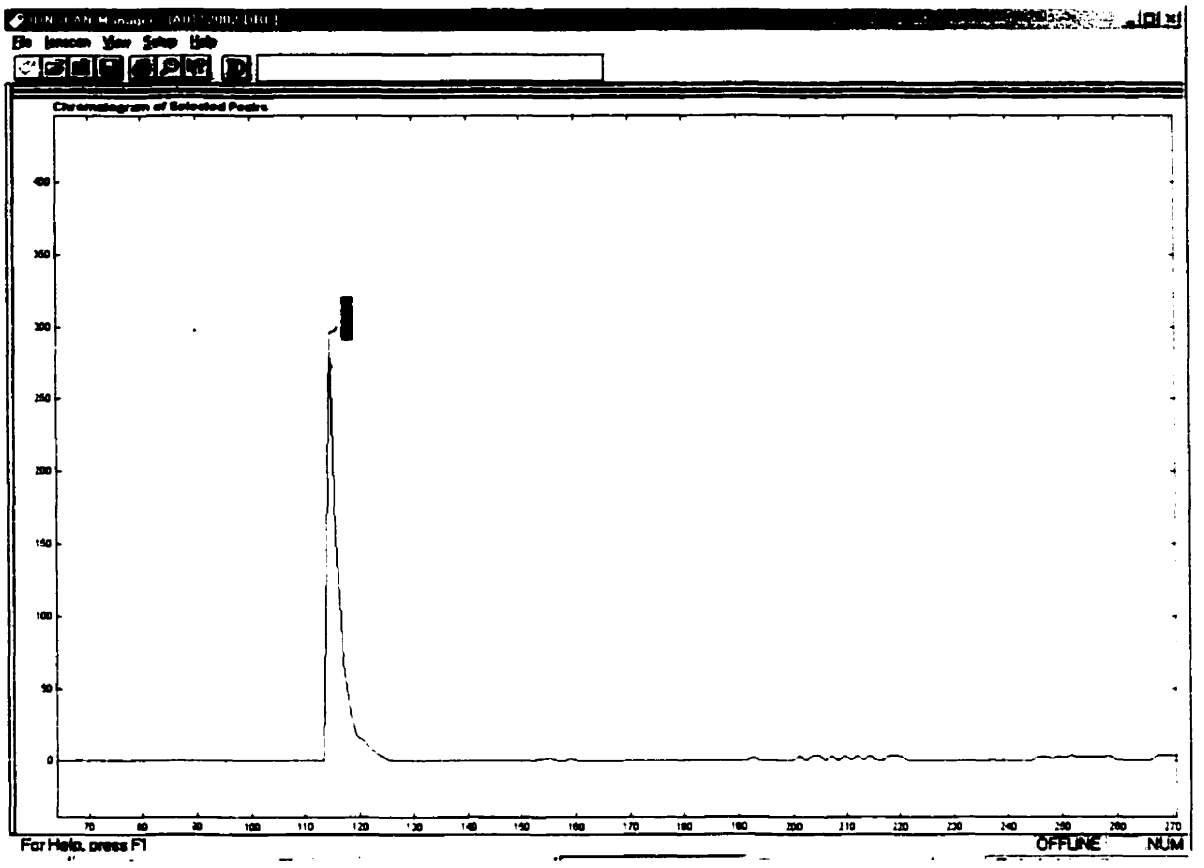


Figure 5- 16. The chloroform peak obtained by analyzing the breath of a swimmer

It can be seen that the chloroform peak was very well defined and the response of the detector was very good. Thus, if further developed, MESI could be used to assess the exposure of swimmers to the chloroform present in the water. The results could be used for the implementation of new regulations.

5.5 Summary

Breath is a noninvasive technique that can be used in clinical chemistry. However, the analysis of the breath is difficult. Powerful analytical techniques are required for the analysis of the low levels of VOCs in the breath. The samples have to be preconcentrated prior to analysis.

The experiments proved that MESI could be used for the analysis of the human breath. Different areas in which the technique could be applied are of great interest (e.g. work related exposure to toxicants).

In cases in which only one compound is to be analyzed, the ion mobility detector represents a good choice, if the chemical can be properly ionized. By using ionization agents other than water, the sensitivity can be improved.

At the same time, the IonScan represents a great detector for applications in breath analysis since it can be easily transported in the field and it is capable of identifying marker compounds in breath. The fact that the instrument can inform about the presence of certain target compounds in the breath is extremely important, since only a few compounds are usually to be measured in the relatively complex breath matrix. The instrument can be preset to alarm only at target compounds, and minimum training is required for its use.

The analysis of human breath by MESI could become a very powerful technique for clinical chemistry and toxicology. In the recent years breath analysis has gained the interest of the scientists around the world. Further research should improve the technique and provide quantitative information about the compounds present in the breath.

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CHAPTER 6

SUMMARY

6.1 Development of MESI, a one-step sample preparation technique

There was a great need for changes in the sample preparation field. This need has determined the development of solvent free sample preparation techniques, like solid phase microextraction and membrane extraction with a sorbent interface.

Membrane extraction with a sorbent interface is a solvent free, one-step sample preparation technique that was developed to enable continuous monitoring of volatile organic compounds in various matrices. MESI introduces selectivity in the system and provides high sensitivity. It is simple, cost effective, and it can be applied for the analysis of VOCs in gaseous, aqueous, or solid samples. It can be designed either for laboratory use, or for field analysis.

6.1.1 The membrane extraction module

The membrane module represents the sampling part of the MESI system. For a field portable system, the response time and memory effect are very important. Due to the fact that diffusion through the membrane is a slow process, the thickness of the membrane is an important factor determining the rapidity by which the analytes cross the membrane barrier.

This affects the rapidity by which the system can respond to variations in the concentration of the target compounds, as well as the sensitivity of the measurement.

Hollow fiber membranes are self supported and very easy to connect to the carrier gas line. However, they have thick walls. The flat sheet membranes are thinner and there is a bigger variety of flat sheet membranes available on the market. However, the latter need to be placed in special holders in order to enable their connection to the carrier gas line.

The design of the membrane module is very important. The flow of the carrier gas on the inner side of the membrane has to be linear and even along the whole surface of the membrane. An improper design of the membrane holder could reduce the advantages of the flat sheet membranes. Long and narrow designs provide an efficient flushing of the membrane, providing a carrier gas flow profile similar to the flow from inside the hollow fiber membrane.

6.1.2 The sorbent interface

Preconcentration of the sample prior to detection is very often required. The use of a small amount of sorbent capable of increasing the concentration of the analytes present in the matrix by a few hundred times, is very advantageous.

The sorbent trap from MESI systems provides sample preconcentration, thus increasing the sensitivity of the method. The use of a packed sorbent trap enhances the trapping efficiency, and makes the system easily transportable in the field.

The amount of sorbent present in the trap, as well as the size of the sorbent particles is very important. The more sorbent that is packed in the trap, the longer trapping times can be used without reaching the breakthrough volume. The size of the sorbent particles has to be

small enough to prevent the analytes from "channeling through" the trap without being retained, and large enough to create enough space for the carrier gas to pass without creating a big pressure drop.

The efficiency of the trap can be increased by using a cooler. The analytes are better retained in a cold trap, and longer trapping times can be used.

The sorbent interface has to be designed in a way in which desorption of the analytes is produced very rapidly in a sharp injection band, since the trap replaces the injectors from regular gas chromatographs. Proper heating pulses have to be applied, and the heating pulses have to maintain the highest temperature long enough to allow the entire sorbent bed to reach the proper desorption temperature. At the same time, the analytes must be prevented from getting flushed back during desorption. A restriction can be placed at the front part of the trap, to prevent the carrier gas from expanding backwards during desorption.

6.2 Application of MESI for the analysis of VOCs in environmental and clinical samples

MESI has been proven with the work presented herein to be capable of analyzing volatile organic compounds in various samples.

The environmental applicability has been proven by the analysis of tap water samples, fume hood air, cigarette smoke, parking lot snow and eucalyptus leaves. The system provided very high sensitivity, and by coupling it to various detectors complex chromatograms have been obtained (FID); the compounds present in the samples have been identified (quadrupole MS); and a portable system has been obtained (Micro GC-TCD).

Human breath has been analyzed by MESI. When FID was used as detector, complex chromatograms have been obtained. However, additional selectivity can be achieved. The ion mobility mass spectrometer has been proven to be a good detector for the analysis of acetone and ethanol, which were determined in breath. Due to low ionization efficiency, the IMS proved to be inappropriate for the detection of benzene and toluene. However, the IonScan was specially designed as portable instrument, and in combination with MESI it could be applied for the analysis of target VOCs in various complex matrices. The MESI-IMS system could be conveniently used for on-line continuous monitoring of industrial processes or for on-site clinical applications. Owing it to the fact that the IonScan can be set to alarm to certain compounds present in samples, it does not have to be operated by chemists.

The use of MESI for the analysis of human breath proved to be a very useful technique especially for toxicological applications.

6.3 Conclusions

MESI is a very efficient, simple and cost-effective sample introduction technique. The system can be easily coupled to any gas chromatograph. The membrane module can be immersed into liquid samples or exposed to their headspace to collect the volatile organic compounds. The membrane acts as a selective barrier, preventing unwanted compounds or dirt from entering the system. The method offers good sensitivity, due to the preconcentration step. The sorbent interface is placed online, and thus, continuous monitoring of VOCs can be achieved. The technique can be applied for the evaluation of industrial processes or analysis of natural waters.

It can be concluded that MESI combines the advantages of other sample introduction technique, like purge-and-trap and SPME: it is an on-line sample preparation method, like purge-and-trap, but offers the selectivity and sensitivity of SPME. At the same time, little handling is required for sampling, making MESI easily to automate.

GLOSSARY

A..... Unit area

AC/DC..... Alternating current / direct current

b..... Thickness of the membrane

C..... Concentration of the analyte in the sample

C_o..... Concentration of the analyte on the outer wall of the membrane

C_i..... Concentration of the analyte on the inner wall of the membrane

D..... Diffusion coefficient of the analyte in the membrane

DVB..... Divinylbenzene

δ_C/δ_x..... Concentration gradient of the analyte across the membrane wall

F..... Flux

FID..... Flame ionization detector

IMS..... Ion mobility spectrometer or ion mobility spectrometry

GC..... Gas chromatograph or gas chromatography

K..... Distribution constant of the analyte between membrane and sample

LC_{50}..... Concentration of analyte which kills 50% of the test organisms

LD_{50}..... Dose of analyte which kills 50% of the test organisms

LLE..... Liquid – liquid extraction

MESI..... Membrane extraction with a sorbent interface

MIMS..... Membrane introduction mass spectrometry

MS..... Mass spectrometry

MTBE..... Methyl tert-butyl ether

PDMS..... Poly (dimethysiloxane)

ppb..... Parts per billion

ppm..... Parts per million

pO_2..... Partial pressure of oxygen

ppt..... Parts per trillion

rpm..... Rotations per minute

SPE..... Solid phase extraction

SPME.....Solid phase microextraction

t..... Unit time

TCD..... Thermal conductivity detector

THC..... Delta-9-Tetrahydrocannabinol

THMs.....Trihalomethanes

USEPA..... U.S. Environmental Protection Agency

VOCs..... Volatile organic compounds

v/v..... Volume per volume

w/w..... Weight per weight