

Breath Analysis and Monitoring by Membrane Extraction with Sorbent Interface

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An analytical system consisting of a sampling chamber, membrane extraction module, sorbent trap and gas chromatograph with flame ionization or ion mobility detector was used for on-line monitoring of the composition of the last 250 mL portion of human expired breath. The sampling chamber consisted of a tube fitted with check valves on both ends to allow the air to pass through during expiration, but not to return or allow mixing with ambient air. The last portion of breath was held in the chamber at the end of breath expiration. The organic components in the trapped breath were transferred to the carrier gas by permeation through the membrane in the extraction module and were concentrated in the sorbent trap before introduction as a sharp plug on the front of chromatographic column. Moisture in the breath did not penetrate the membrane to a substantial degree. This system was used to investigate presence of acetone as a biologically important marker of human health as well as exposure to volatile compounds.

Breath analysis is an interesting opportunity and challenge to the analytical community. Breath is essentially the headspace of blood and can provide much information about the state of human health as well as about exposure to volatile environmental organics. It is a relatively noninvasive technique and so is generally well-accepted in both clinical and work-place settings. Breath analysis is regarded as a simple, noninvasive, easily repeated and useful alternative to blood and urine analysis.^{1–4} Many different organic compounds that can penetrate through the alveolar membrane have been identified in breath. Although many of these are nonpolar, some polar compounds present in the blood are also present in the breath, such as acetone produced during normal metabolic processes.

In the lungs, only a thin barrier separates the air in the alveoli from the blood in the capillaries. This barrier is called the pulmonary alveolar membrane. The basic assumption underlying breath analysis is that there is a relatively fast gaseous equilibrium between alveolar air and pulmonary blood, based on partitioning into the membrane and passive diffusion across it.^{2,5} Therefore,

analysis of volatile substances or solvents in breath should provide a good indicator of the levels of those in blood.

Human breath contains numerous volatile substances derived from both endogenous metabolism and external exposure to vapors and gases or their metabolites. More than 200 different VOCs (volatile organic compounds) have been observed in most breath samples, and more than 3000 different VOCs have been observed at least once.⁶ Normal humans differ widely from one another in the composition of their breath VOCs, both qualitatively and quantitatively. Despite the large total number of different VOCs observed, there is a comparatively small “common core” of breath VOCs that are present in all subjects. Some of the major VOCs in the breath of healthy individuals are isoprene (12–580 ppb), acetone (1.2–1880 ppb), ethanol (13–1000 ppb), and methanol (160–2000 ppb), which are present as the result of normal metabolic processes.⁷ However, the source and physiological significance of most of the other VOCs are still unknown. Should a correlation between a breath VOC and a particular disease be consistently observed, this may present a unique opportunity for better understanding of the biochemical basis of that disease. In addition, breath testing might offer a new approach for diagnosis of diseases in their earlier and more treatable stages.

Breath analysis can be used for early diagnosis or evaluation of metabolic disorders and disease conditions, including lung cancer, heart disease,⁸ exposure to environmental toxins or pollutants, or for drug monitoring. For instance, acetone is produced from glucose metabolism through decarboxylation of acetoacetate, which derives from lipolysis or lipid peroxidation. Uncontrolled diabetes mellitus causes the blood glucose to rise to excessively high levels. As a result, the body generates large quantities of acetone as a major metabolite.^{2,9,10} Breath analysis has also been proposed and used for monitoring workers industrially exposed to solvents.^{1,11} Progress in each of these areas, however, has relied on the development of suitable sampling methods and measurement techniques.

MESI for Breath Analysis. Many different techniques have been applied for breath analysis. The most popular involves a bag

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into which the subject expels the air. The air is then processed by active trapping of the components on a sorbent trap followed by desorption into an analytical instrument. This is a time-consuming process consisting of a number of steps, which may lead to loss of compounds. Compounds may also adsorb onto the surface of the bag.¹ The objective of the current work is to investigate membrane extraction with a sorbent interface (MESI) as a simple and effective alternate means of performing breath analysis.

The silicone membrane used for MESI breath sampling is similar in nature to that of the nonpolar lipid bilayer cell membrane of the alveoli across which many compounds must travel to be expired in air, in that both are relatively nonpolar and preferentially transport nonpolar compounds. Most polar and nonvolatile compounds are excreted by the kidneys. As a result, compounds present in breath are relatively nonpolar and have good volatility. These are the compounds that are best analyzed by MESI, and so there should be little discrimination among breath components by the sampling system.

Membrane extraction with a sorbent interface (MESI), has been developed to allow rapid routine analysis and long-term continuous monitoring of VOCs in various environmental matrices.^{12,13} It minimizes the loss of analytes by interfacing the membrane extraction module directly to a capillary gas chromatograph. The standard GC injector is bypassed, because the MESI unit acts as an injector. The system includes a membrane module, including a supported flat sheet membrane to extract the analytes from the surrounding liquid or gaseous sample. Sample is exposed to one side of the membrane, and a gas flows along the other side and transports the extracted analyte molecules into a cooled sorbent trap. The analytes are desorbed from the sorbent trap by heating and are transferred to the GC for analysis. Several modifications to the system allow it to be used for breath analysis. These are described in the Experimental Section.

A healthy individual exhales 500 mL or more with each breath, and the breath profile for expired volatiles is composed of three phases. At the beginning of expiration, air originates from the airway dead space from the upper airways, where no gas exchange has occurred and little or no volatiles are expired. During the next phase there is a rapid rise in volatiles concentration followed by a plateau as the alveolar air concentration (plateau) is reached. Next follows a shallow positive gradient that can be attributed to the continued liberation of compound from the alveoli until the individual is unable to expire any further breath. This is referred to as the end-expired or alveolar air. Collection of this last part of expiration (about 200 mL) is generally considered to be the easiest sample to collect and probably the most reproducible.¹⁴ The sampler for the current analyses, as shown in Figure 1, is designed to trap the last 250 mL of breath.

Almost all conventional breath sampling devices capable of collecting alveolar air have two common disadvantages. First, adsorptive losses on the surface of the material may be significant, particularly when trace levels of analyte are being measured. The problem is exacerbated when a film of moisture forms on the surface of the container. Second, collected samples must be transferred to an analytical instrument, usually a GC, for analysis.¹

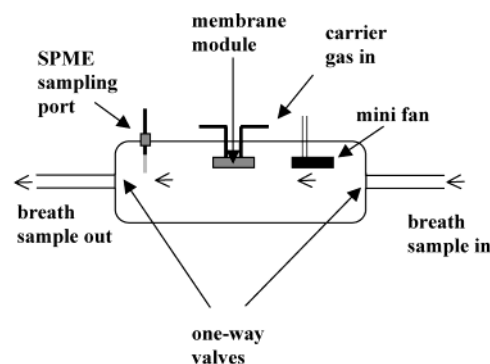


Figure 1. Schematic representation of breath sampling chamber for MESI analysis. The mini fan circulates the sample during trapping, which can enhance the extraction rate. The SPME sampling port is used to validate breath concentrations determined by MESI.

The MESI sampler addresses these difficulties by avoiding any storage of breath sample and by directly coupling sampling and analysis. In addition, moisture is eliminated early in the process, because it is essentially unable to pass the membrane module.

EXPERIMENTAL METHODS

The MESI system employed for the current work consisted of a helium supply (UHP grade, Praxair, Waterloo, ON), a membrane module with a silicone membrane, a sorbent trap, and a Chrompak gas chromatograph (model CP9002, Varian, Walnut Creek, CA) with a FID detector. In some studies, an ion mobility spectrometer was used for detection (Barringer Instruments, Toronto, ON). A SPB-5 column (Supelco, Bellefonte, PA), 30 m \times 0.25 mm i.d., 0.25- μ m phase thickness, was used for separation. Column programming was as follows: initial column temperature, 35 $^{\circ}$ C; hold 5 min; increase to 150 $^{\circ}$ C at 5 $^{\circ}$ C/min; hold 10 min. Ethanol (40%) for dosing studies (Absolut, The Absolut Company, Stockholm, Sweden) was obtained from a local liquor store. All other chemicals were of analytical reagent grade or better.

A standard gas generator was used to deliver the standard gas mixture that served as the sample for calibration experiments. A syringe pump (Razel Scientific Instruments, Inc., Stamford, CT) was used to deliver a solution of standards at a constant flow rate into a stream of purified gas. The gas stream was heated to a set temperature and was continually flushed through a sampling chamber. Although commercial standard gas generators are available, the one constructed in-house proved to have sufficient accuracy and flexibility for the work at hand and was constructed at a much lower cost. A 100- μ L gastight syringe (Hamilton) was used with the syringe pump. The pump was calibrated by measuring the mass of analyte mixture delivered over various time periods. A very fine metering valve (Swagelock, Solon, OH) was used to control the flow of the diluting gas. Copper tubing and copper and stainless steel connectors (Swagelock) were used to connect the parts of the generator. They were thoroughly cleaned with solvent, allowed to dry, and then flamed. 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, as was the transfer line that conducted the analyte gas mixture to the sampling chamber.

Construction of MESI System. The flat sheet membrane module was constructed as shown in Figure 2. A flat sheet silicone

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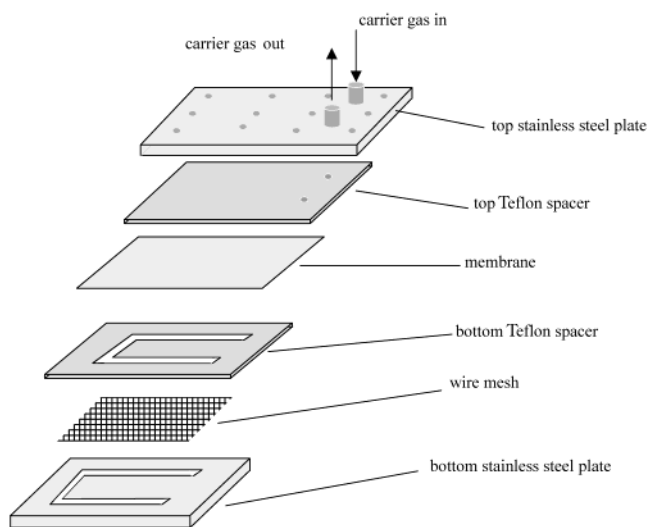


Figure 2. Schematic representation of the flat sheet membrane module used for MESI analysis.

membrane (50 μm , SSP-M100, dimethylsilicone, Membrane Components, Ballston Spa, NY) was mounted between the two Teflon spacers as shown. The upper Teflon spacer contains two holes that match the holes in the upper steel plate and permit passage of the carrier gas. The lower Teflon spacer is slightly thinner and has a u-shaped channel cut into it to match with the channel cut into the lower steel plate. During operation, the pressure of the carrier gas causes the membrane to balloon into the u-shaped channel in the lower Teflon spacer. Carrier gas travels along the channel so formed, and the dimensions of the channel in the lower Teflon spacer determine the overall dimensions of the channel on the receiving side of the membrane. Wire mesh was affixed to the lower steel plate to support the membrane and prevent it from ballooning out of the bottom of the module during sampling. The module was sealed to be gastight by evenly tightening the 12 machine screws that pass through the module and compress the two steel plates together. The entire assembled membrane module was obtained from Restek Corporation (part no. 551741, Bellefonte, PA.). The module can be ensured to be gastight by immersing it in water with carrier gas passing through, followed by thorough drying, before sampling.

The helium carrier/stripping gas flowing at 1.5 mL/min was passed over the receiving side of the membrane to strip analytes as they diffused through. The membrane module was mounted into an Aerochamber aerosol holding chamber equipped with a valve (Boehringer Ingelheim Canada Ltd., Burlington, ON Canada). A schematic representation of the assembled sampling chamber with the membrane module contained in the Aerochamber is shown in Figure 1. For sampling, the subject expired as much breath as possible into the device. The sampling chamber had valves mounted at both the inlet and outlet ends and had a volume of 250 mL. Thus, it was only the last 250 mL of breath that was sampled in the chamber. Because of this flow-through design, mixing is minimized, and no concentration or dilution occurs in the chamber. The sampling chamber also had a septum-plugged hole in it to allow parallel SPME sampling for validation.

The trap consisted of a 5-cm section of deactivated stainless steel tubing (MXT guard column, 1/16-in. o.d., 0.73- μm i.d., Restek Corp., Bellefonte, PA) with 1.3 cm of XAD-2 resin (Restek Corp.)

packed tightly in the center and held in place by two plugs of quartz wool. The assembled trap was obtained from Restek Corporation (part no. 551744). A schematic representation of the trap design is shown in Figure 3.

Analytes stripped at the membrane were transported by carrier gas to the trap, where they were retained until a sufficient amount had been trapped for thermal desorption and analysis. Trapping time was optimized so that the amount trapped was in the linear response range of the detector but did not result in breakthrough in the trap. Breakthrough time was determined as the time after which no additional analyte was trapped with increasing trapping time. During sampling, the trap was cooled with a three-stage peltier cooler (Melcor Corp., Trenton, NJ) to improve trap capacity and reduce breakthrough. The trap was desorbed at predetermined times by applying a capacitive discharge voltage from a custom-made power supply (Science Shops, University of Waterloo). After thermal desorption, analytes were carried in a narrow band to the top of the separation column mounted in the gas chromatograph.

Influence of Humidity on the MESI Analysis. The presence of saturating quantities of water in breath presents an additional problem. Condensation in the collecting apparatus may deplete the sample of polar volatile organic compounds that are soluble in water, resulting in falsely depressed concentrations of some of these analytes.¹⁵ Because the high level of water present in the breath is one of the main difficulties when performing conventional breath analysis, the influence of the humidity on the MESI extraction was evaluated.

In these experiments, the column was run isothermally at 30 $^{\circ}\text{C}$ to enable the separation of acetone and ethanol. A standard gas generator as described above was used to deliver the standard gas mixture that served as the sample for these experiments. Humidity was generated by introducing a glass container containing water into the carrier gas line. The container was filled halfway with water, and the diluting carrier gas (compressed air) was bubbled through the water prior to sampling. Three different humidity levels were examined. For the dry air experiment, the water container was not placed in the carrier gas line. To obtain a humidity level of 30%, the container was connected to the gas line, and the water was maintained at 22 $^{\circ}\text{C}$ by insulating the container with glass wool. To increase the moisture level to 90%, the container was heated to 60 $^{\circ}\text{C}$ with heating tape. A combination thermometer/hygrometer, purchased from a local hardware store, was placed in the sampling chamber of the standard gas generator and was used to monitor the temperature and the humidity in the chamber.

A mixture of benzene, toluene, acetone and ethanol was used to generate the standard gas for the experiment. The mixture contained 1.1654 g of acetone, 1.5486 g of ethanol, 4.6791 g of benzene, and 4.2721 g of toluene. The diluting flow rate (high purity air) was of 500 mL/min, and the standard mixture was supplied from the syringe pump at a rate of 312 nL/min.

System Calibration. The system response to ethanol and acetone was calibrated by use of standard gas with detection by ion mobility spectrometry. The standard gas generator as described above was used to prepare a mixture of the four compounds in air. The calibration curve was constructed for

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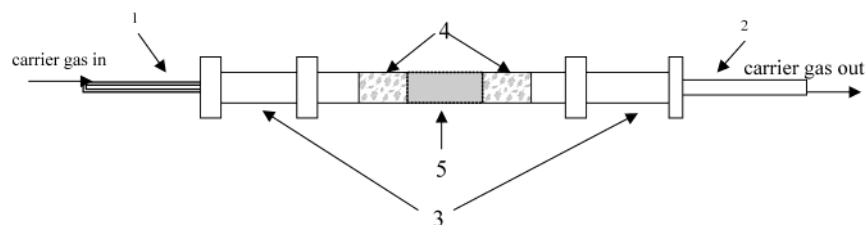


Figure 3. Schematic representation of the sorbent trap module used for MESI analysis: (1) upstream transfer line (inner tubing, 0.25-mm i.d. stainless steel tubing; outer tubing, 1/16-in. Teflon tubing); (2) downstream transfer line, 1/16-in. stainless steel tubing; (3) standard 1/16-in. HPLC fittings; (4) quartz wool; (5) polymer sorbent material.

acetone and ethanol with detection by ion mobility spectrometry (IMS), and the estimated limit of detection was calculated. For this experiment, a mixture of 0.8095 g of acetone, 1.1142 g of ethanol, 4.7180 g of benzene, and 4.8515 g of toluene was used. A syringe pump supplied 31.2 nL of mixture/minute. Air was used as the diluting gas at a flow of 500 mL/min. Limits of detection for only acetone and ethanol were determined because of a lower detector response for the other two compounds. The use of FID instead of the IMS would allow for more-sensitive but less-specific detection. The other experimental conditions were the same as for the previously described experiment.

Breath Analysis Applications. The utility of the device was investigated in several studies into both endogenous and exogenous components of breath. Breath samples were analyzed for acetone, ethanol, chloroform, and compounds arising from cigarette smoking. For acetone analysis, samples were collected over a 4.5 h 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 min with breath being exhaled in the sampling device every 4 min.

Breath ethanol was monitored to demonstrate that volatile organic compounds entering the body through the skin are rapidly distributed in the body through the blood circulation, and that they can be found in the breath. This was meant to simulate exposure to toxic compounds. Transdermal dosing (skin permeation) was employed instead of ingestion to avoid the effect of sample contamination from ethanol absorbed to the mouth tissues.

For skin permeation experiments, Absolute vodka was used as a source of ethanol. The permeation of the vodka through the skin was initially 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 skin of the forearm and the concentration of ethanol was measured in breath. To eliminate the possibility of inhaling ethanol while rubbing it on the skin, the application of the vodka on the skin was performed in a fume hood. Disposable nitrile gloves were used during applications to better control the amount of skin surface exposed to ethanol. In the first instance, 1 mL of vodka was applied on the skin after the application of a small amount of lanolin to aid absorption. The collection of the breath started 3 min after the application and a 7 min trapping time was used for each breath sample. In the second instance, 1 mL of vodka was applied to the skin after the application of a small amount of a mixture of lanolin and mineral oil.

For the detection of chloroform in breath, the sample was collected from a swimmer 40 min after swimming for half an hour in a chlorinated swimming pool. A longer trapping time (20 min)

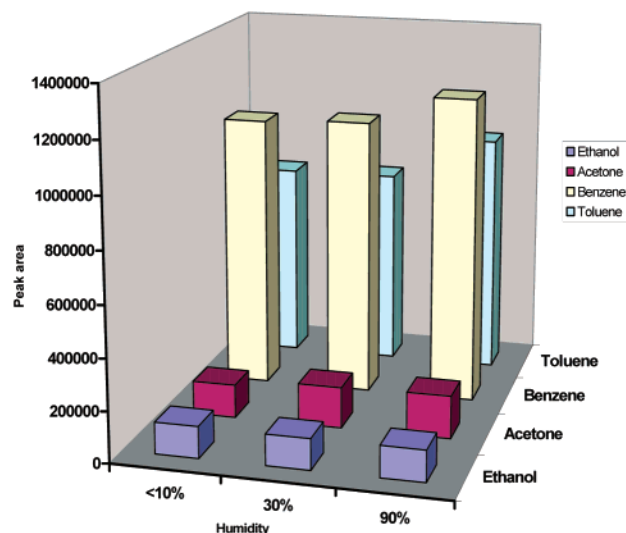


Figure 4. Influence of the humidity on the extraction of acetone, ethanol, benzene, and toluene.

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 in the sampling device every 4 min. To identify which components originated from cigarette smoking, a comparison was made of the volatiles profiles between breath samples acquired prior to smoking and 30 min after smoking. The subject replaced the breath sample every 5 min, and again, a longer trapping time (25 min) was used.

RESULTS AND DISCUSSION

Influence of Humidity on the MESI Analysis. The results obtained for the extraction of the four compounds from the mixture under varying levels of humidity are presented in Figure 4. For acetone, benzene, and toluene, the amount extracted increased slightly at higher humidity, although the increase was not significant. The amount of ethanol extracted was constant at all humidity levels. Thus, the humidity of the sample does not significantly affect the analysis in the range of humidities tested.

System Calibration. The calibration curves obtained had good linearity. Ethanol was linear to $7 \mu\text{g/L}$ ($r^2 = 0.983$), and acetone was linear to $3.5 \mu\text{g/L}$ ($r^2 = 0.995$). The slightly lower regression coefficient for ethanol likely results from the fact that the ethanol peak was not very well-separated from the acetone peak during the chromatography. The detection limits were calculated for the two compounds on the basis of $S/N = 3$. The noise level was ~ 2.25 , with a standard deviation of 0.465. The estimated limits of detection for acetone and ethanol were 0.4 and $0.5 \mu\text{g/L}$,

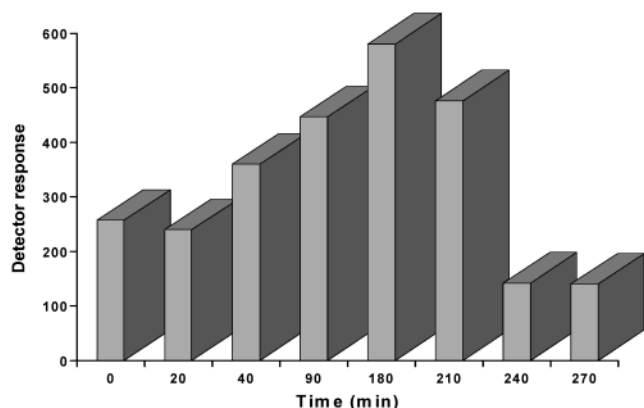


Figure 5. Breath acetone profile recorded over a 4.5-h period. Sampling was initiated 2 h after eating. Subject ate a light lunch just prior to the 210-min sample.

respectively. Linear ranges were limited by the linear response of the detector. Detection by FID would allow for a wider linear range of calibration and better correlation of system calibration with expected breath concentrations. When higher specificity is required, however, MESI trapping may be optimized to ensure extracted amounts are in the linear range of the detector simply by varying the trapping time.

Acetone Analysis. Uncontrolled diabetes mellitus causes the blood glucose to rise to excessively high levels. As a result, the body generates large quantities of acetone as a major metabolite.^{2,16,17} The concentration of acetone in breath changes during the day, depending on the amount of carbohydrates received by the body. Because of this, acetone measurement is used for the control of weight reduction and as a marker in the breath of diabetic patients.

The results of breath acetone analysis are shown in Figure 5. Acetone was originally present at a relatively low level. As the subject became progressively more hungry the acetone concentration in breath started to rise. After a light lunch of a banana and some crackers the acetone level is seen to drop off again to very low levels. The method is very simple, with a low level of requirement from the subject. It should provide a convenient means of monitoring carbohydrate metabolic status in either healthy or ill individuals.

Ethanol Analysis. It can be seen in Figure 6 that ethanol appeared in breath rapidly after initial application of the vodka with lanolin. This indicates that the ethanol traveled rapidly through the skin and blood vessels to reach the breath. After the initial high breath ethanol concentration, it dropped rapidly to nondetectable levels after ~25 min. When the vodka was applied with the mixture of lanolin and mineral oil, however, the appearance of ethanol in the breath was delayed and the total amount of ethanol in breath was reduced. It appears mineral oil acts as a barrier to the skin absorption of ethanol.

A marker compound present in the breath that remains at a constant concentration over a long period of time is beneficial for device design and optimization of the analytical method. Ethanol was selected as this marker compound. Different time intervals

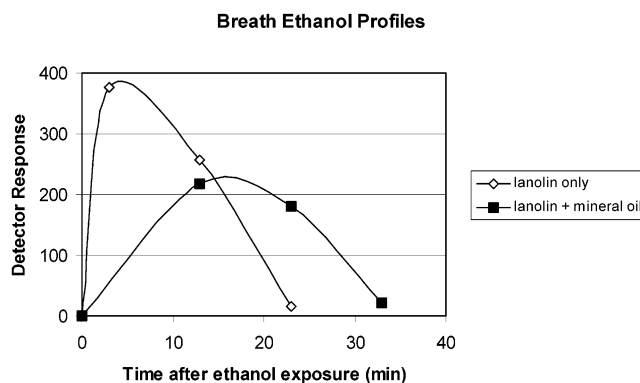


Figure 6. Progress of breath ethanol concentrations after transdermal exposure to ethanol. Ethanol was applied after application of either lanoline alone or lanoline mixed with mineral oil.

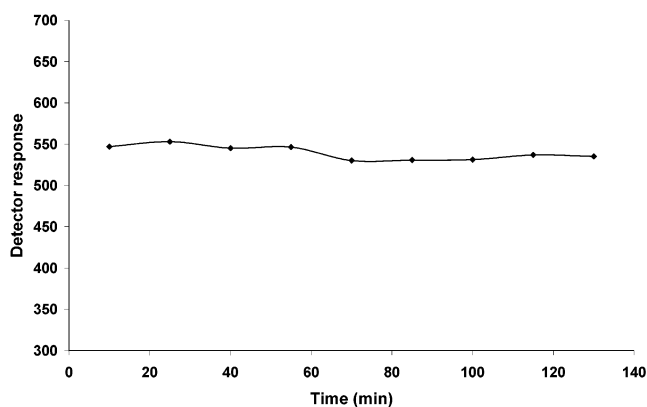


Figure 7. Demonstration of establishment of constant breath ethanol concentration through carefully timed transdermal exposures. Constant concentration was achieved by application of 1 mL of vodka every 5 min.

between applications were tested. It was found that by applying 1 mL of vodka every 5 min, the concentration of ethanol in breath remained constant over a long period of time. Samples were recorded every 15 min using 4-min trapping times and 1 breath/sample (Figure 7). The presence of a marker compound present at a constant blood level will also be beneficial for correlation between breath and blood concentration. This will allow correction for variability in transport efficiency across the alveolar membrane or bronchial tissues, which otherwise complicates correlation of breath concentrations to blood concentrations.

Recent studies on the physiology of gas exchange for highly water soluble polar volatile breath components have helped to explain why varied values of end-exhalation breath alcohol concentrations have been observed.^{18,19} The degree of the exchange is directly related to the solubility of the analyte in the airway mucosa and mucous lining and, hence, its polarity.^{20,21} The very high water solubility of alcohol implies a strong interaction with airway tissue. Because the interaction depends on temperature and airflow characteristics, variations in breath tidal volume

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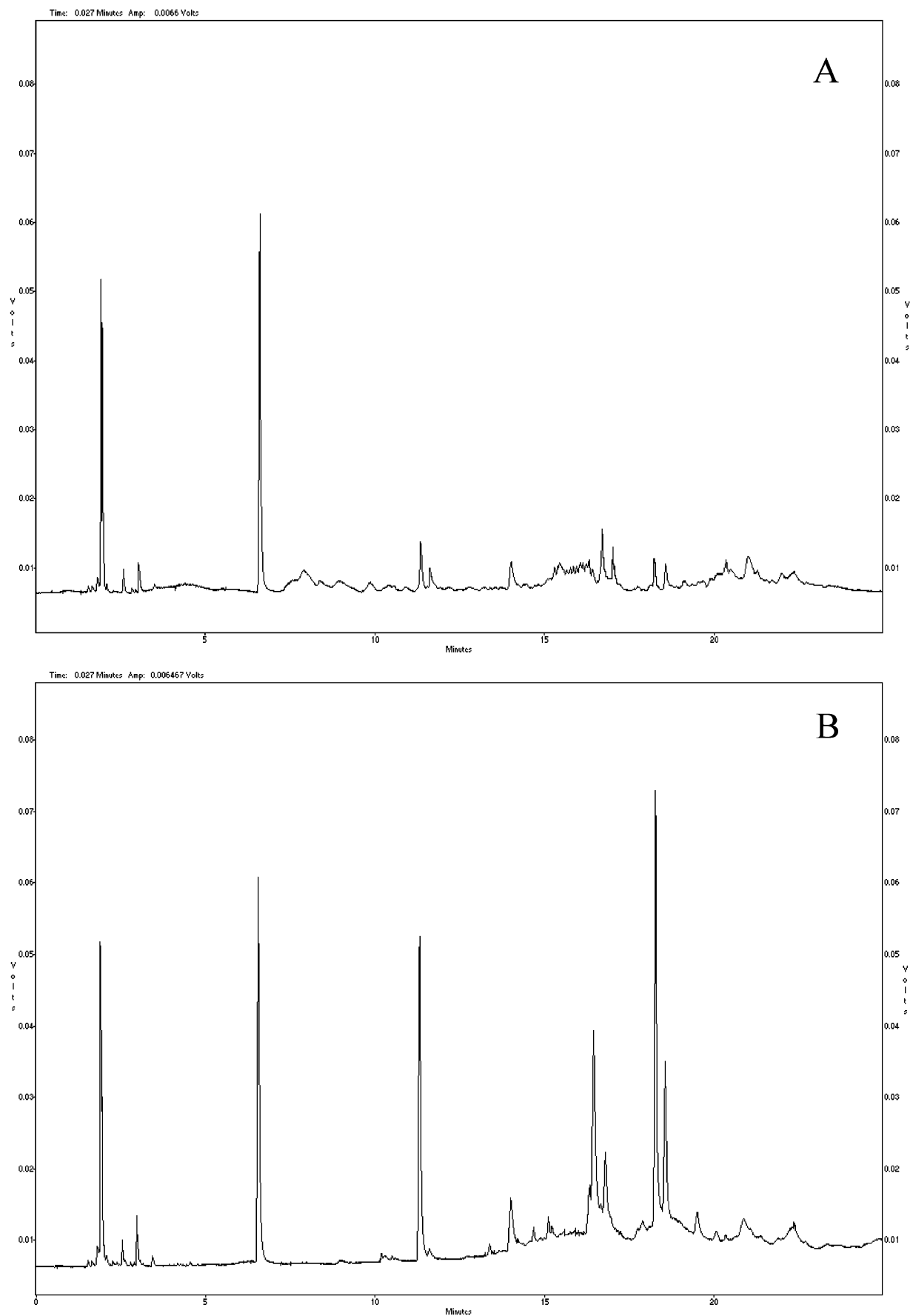


Figure 8. Chromatograms obtained from MESI analysis (and with detection by FID) of the breath of a smoker before and after smoking: (A) blank breath sample from smoker, no smoking in previous 10 h; (B) breath sample from smoker 30 min after smoking.

and frequency can have a significant effect on the alcohol concentration in a breath sample.^{22,23}

Thus, transport is known to be somewhat variable, depending on the analyte under study, and a means for correcting for the variation will be beneficial in developing methods for accurate quantification of blood concentrations based on breath analysis. In practice, the marker compound would ideally be structurally related to the compound of interest, so that its water solubility and transport characteristics are closely matched to the compound of interest. Standard blood analyses should be performed to ensure the blood concentration is constant. Analyte peaks from breath analysis can then be referenced to the marker compound peak to correct for transport variation.

Preconcentration of Breath Samples. Only the most abundant VOCs, such as ethanol, acetone, and isoprene, can be detected with assays of unconcentrated breath. More general breath testing is technically difficult, because most breath VOCs are present at levels too low to be directly detected by most instrumentation. These VOCs must be concentrated prior to assay. Preconcentration is usually done with traps to selectively capture the VOCs in the breath.²

The major advantage of breath analysis by concentration methods is that there is no theoretical lower limit to the sensitivity of the assay, since the volume of the collected sample is limited only by the patience of the donor, the capacity of the concentrating system, and the focusing power of GC. The use of concentration techniques makes it possible to detect several volatile compounds in the breath when their concentrations are too low to be detected in the blood. Common problems with breath analysis techniques employing trapping devices have been discomfort for human subjects donating a breath sample (e.g. expiring against resistance), complicated apparatus, poor trapping efficiency, and the need for specialized technical supervision.²⁴ MESI can address most of these.

Two experiments were conducted to evaluate the utility of MESI in preconcentrating analytes present at low concentration. For analysis of both components arising from smoking and breath chloroform, trapping times of 20–25 min were used.

Effect of Smoking. The results are shown in Figure 8. Although some peaks were common to both samples, several more appeared 30 min after smoking. Two major peaks were present in the chromatogram obtained as a blank. By comparison, after smoking, more significant peaks are present. Because of the fact that the sampling device collects only the end tidal air, whereas the air from the mouth and the superior respiratory tract are in contact with the membrane for only a few seconds, it is likely that the extra peaks present in the chromatogram arise from smoking. It can be verified that the sampling process was consistent between the two analyses in that the heights of the first two major peaks in the chromatograms are equivalent.

Chloroform Analysis. Breath chloroform was also monitored to investigate the use of the MESI system for preconcentrating and analyzing a trace analyte in breath. Trapping time in this case was 20 min. Chloroform was monitored in the breath of a swimmer

with detection by ion mobility spectrometry (IMS). Chloroform was not identified in the breath prior to swimming.

The chloroform peak was clearly visible in the IMS trace, which was programmed to monitor only chloroform.

CONCLUSIONS

Breath analysis is an attractive noninvasive procedure for medical diagnosis, drug monitoring and employee screening. It has been used in numerous laboratory-based studies and for field research. Despite its advantages for routine biological monitoring, it has not become widely accepted as a tool in medical diagnostics or occupational hygiene. Suitable sampling methods and measurement techniques are the bottleneck. MESI has several advantages, which can overcome the problems in breath analysis, such as low concentrations of components and high moisture in human breath. It is particularly amenable to the analysis of highly volatile compounds and has been shown to be effective for even the very difficult analyte methane.²⁵ Therefore, MESI is potentially a very useful technique in breath analysis.

The ion mobility spectrometer may be a suitable detector for breath analysis, because it is a highly selective detector capable of quantifying target compounds from a complicated mixture and is relatively portable and inexpensive. By using ionization agents other than water, the sensitivity can be further improved. For better sensitivity and linear range of detection, FID was observed to be a good choice.

The results show MESI to be a fast and quantifiable means of determining breath components originating from blood. When the device was calibrated using standard gases, it was shown to produce a linear response. This would allow for external calibration of breath concentrations of compounds. Calibration based on diffusion coefficient (D) and partition coefficient (K) is also possible.^{26,27} The potential has also been described for use of an internal standard for calibration of blood concentrations of compounds based on reference to a known steady concentration of a reference compound.

The MESI device is an example of the integration of sampling, sample preparation, and sample introduction, resulting in an integrated instrument that could potentially be used for point-of-care analysis for either medical diagnostics or exposure monitoring. Because the integration of many of the steps in the analytical process is fundamental to the design of the method and instrument, it is implicit that in device design, careful consideration be given to the sampling protocol. In conventional analyses, other experts (medical technicians in the case of breath sampling) assume responsibility for a correct sampling protocol. As the analytical process becomes integrated with the system under study, analytical chemists must assume responsibility for the requirements of the sampling protocol to ensure that results obtained are representative of the problem being investigated.

Further research into both device design and the physiology of breath analysis will improve the MESI technique. The device may also be beneficial in the study of breath physiology, because it is ideally suited to analysis of highly volatile compounds, which are problematic for analysis by other means. The device provides quantitative information about the concentrations of compounds

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present in the breath and ultimately should provide a means of correlating to blood concentrations.

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