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On-line monitoring of breath by membrane extraction with sorbent interface coupled with CO_2 sensor

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Abstract

In order to establish a simple and reproducible sampling device and method, which is crucial for a wide application of breath analysis, the original membrane extraction with sorbent interface (MESI) system was improved by coupling with a palm-size CO_2 sensor. Variations in analyte concentrations due to mass losses and different breathing patterns were normalized by simultaneously measuring the partial pressure of CO_2 and the concentrations of target analytes in the breath sampled. Analyte concentrations can then be expressed normalized to CO_2 as in the alveolar air. The MESI system was applied to study light hydrocarbons such as methane and ethane, which are difficult to analyse by other methods. A systematic study of breakthrough, which relates to the sorbent capacity and is characteristic of the analytical efficiency, was performed through the effects of analyte concentration, trap temperature, sample humidity and extraction time on breakthrough. Continuous on-line monitoring of breath methane and ethane was carried out under the optimum operation conditions based on the breakthrough study.

Keywords: Membrane extraction with sorbent interface (MESI); CO_2 sensor; Internal standard; Carboxen; Light hydrocarbon; Breath analysis; Breath methane; Breath ethane; Breakthrough; On-line monitoring

1. Introduction

The disadvantages of most of the existing methods [1–7] for breath analysis are briefly summarized here. The use of sampling bags, sampling loops, valves and vacuum pumps can cause contamination, leaks, errors and handling inconveniences. Off-line analysis and difficulty in automation due to the separated steps of sampling and sample preparation are time consuming, limit convenience and introduce handling errors. The need to eliminate CO_2 and water using silica gel, Drierite^{TM} or sodalime, etc. introduces high levels of hydrocarbon contaminant.

Membrane extraction with sorbent interface (MESI) coupled with gas chromatography is an analytical system that integrates sampling and sample preparation in one step. It is shown to have eliminated most of the disadvantages of the current methods. In our previous work [8], the system was described in detail and used to investigate the presence of acetone as a biologically important marker in human breath as well as exposure to volatile compounds such as ethanol and chloroform. In this study, the MESI system was improved by coupling with a palm-size CO_2 sensor and using the CO_2 in breath as an internal standard to correct for variations (from the alveolar air) or errors (from sampling or storage) by simultaneously measuring the partial pressure of CO_2 and the concentrations of target analytes.

One of the main reasons that breath analysis has not been used routinely as a diagnostic tool is the difficulty in the methodology (sampling and analysis) [6,9]. Breath cannot be considered as a homogeneous medium but as a mixture of air coming from different regions in the lungs. Physiological considerations have indicated that the analytical results could vary considerably, depending on the type of breath (including alveolar air, expired air, end tidal air and re-breathed air, etc.) or on the sampling technique used.

Although CO_2 levels vary from person to person according to several factors (such as metabolic rates and the amount

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of time the breath is held before providing a sample), the "true value" or the average of CO_2 concentrations in alveolar air should be steady for a single subject. Measuring deviations from the average established for each person permit correction of the measured analytes levels. In fact, CO_2 in alveolar air is about 5%, compared to only 0.035% in ambient air [10]. It is reasonable to assume that both the CO_2 and the analytes in an expired air will be affected in more or less the same way by hypoventilation, hyperventilation, dilution by dead-space air and the sampling method used. Therefore, the simultaneous determination of CO_2 in the breath sampled and its use as a normalization factor, in the same way creatinine is used for urine analysis, should improve the reliability of breath analysis [9,11]. Unfortunately, the importance of sampling correction in breath analysis has not been fully realized by many researchers and only a few have tried to calibrate the errors in their studies [12–14].

Methane (CH_4) and ethane (C_2H_6) are important VOCs in breath analysis. Because of their high volatility, at low levels they are difficult for analysis by conventional analytical means due to sampling and trapping problems. There are currently no widely accepted methods for collecting and analyzing highly volatile hydrocarbons in expired air. Most investigators have developed their own techniques. Some techniques are ineffective in trapping highly volatile hydrocarbons. There is often striking variability in the published data due to the different sampling and sample preparation methods applied by the different researchers [7]. The MESI technique is amenable to the studies of very volatile hydrocarbons such as methane and ethane, and their on-line monitoring. A two-stage Peltier cooler was used to produce a trap temperature as low as −25 °C for more efficient pre-concentration of breath analytes. There is no need to turn off the cooler when performing heating for desorption, thus simplifying the control circuitry. The required maximum temperature (for example, 140 °C for ethane) is reached at $t = 5$ s, i.e. 5 s after triggering the heater or at the end of the 5-s heating pulse. The temperature then quickly returns to the originally set cooling temperature value.

In the current study, the feasibility of using CO_2 in human breath as a natural internal standard in breath analysis with MESI is confirmed by demonstrating a linear relationship between acetone or isoprene concentration and CO_2 partial pressure in the collected breath samples. Acetone and isoprene rather than other breath components were selected for this demonstration because they are two of the most abundant volatile organic compounds (VOCs) in human breath and like CO_2, ethane or most other compounds in breath air, they originate from blood. Although methane is generated in humans through anaerobic bacterial metabolism in the colon and is excreted in flatus and expired breath, the errors due to sampling and storage can still be corrected with the internal standard in the same way. Hence, the experimental results with acetone and isoprene were expected to be representative, easier and more precise. The technique was then applied in the light hydrocarbon analysis.

2. Experimental

2.1. Materials and instruments

Helium (5.0 ultra high purity, 99.999%), nitrogen (5.0 ultra high purity, 99.999%), methane (3.7 ultra high purity, 99.97%), and ethane (2.0, 99.0%) were obtained from Praxair (Kitchener, Ont., Canada). Flat sheet silicone polycarbonate membranes SSP-M213 (0.001 in.) were purchased from Special Silicone Products Inc. (Ballston Spa, NY, USA). Tenax TA (for acetone and isoprene) and Carboxen (for methane and ethane) trap tubes, membrane modules, Rtx-VMS column (30 m × 0.25 mm i.d. × 1.40 μm d.f.) (for acetone and isoprene), aluminum oxide PLOT column (30 m × 0.32 mm i.d.) (for methane and ethane), Hydroguard MXT guard columns and transfer lines (0.28 mm i.d. and 0.53 mm i.d.), coiled Silcosteel tubing (0.53 mm i.d.) and gastight syringes (Hamilton, 25-μL and 1.0-mL) were obtained from Restek (Bellefonte, PA, USA). A two-stage Peltier cooler was purchased from Melcor (Trenton, NJ, USA). The gas chromatograph (GC, Chrompack CP9002) coupled with flame ionization detector (FID) was supplied by Varian (Walnut Creek, CA, USA). A dc power supply (hp Harrison 6427B) from Hewlett-Packard (Palo Alto, CA, USA), electronic thermometer (Fluke 53II) from Fluke Corporation (Everett, WA, USA) and electronic flow meter (ADM 2000 Intelligent flow meter) from J&W Scientific (Folsom, CA, USA) were also used. The power supply for the cooler (S&D −066), the temperature controller for the dc power supply (S&D −070), the heating timer (S&D −073) and the glass vial sampler (550 mL) were custom-made by the Science Technical Services Shops of the University of Waterloo (Waterloo, Ont., Canada). The model 8200 Capnocheck CO_2 sensor was purchased from Smiths Industries Medical Systems (WI, USA).

2.2. MESI system

The MESI system includes a membrane module (supporting a silicone flat sheet membrane) to extract the analytes from the surrounding liquid or gaseous sample. A stripping gas (usually helium) flows inside the membrane and transports the extracted analyte molecules into a cooled sorbent trap, where they are enriched and subsequently desorbed and transferred to GC/FID for separation and quantification (Fig. 1). The detailed structure of the MESI system has been described previously [8].

2.3. Standard gas generating systems

(1) Dynamic standard gas generator. A dynamic standard gas generator was constructed and used to continuously supply the standard gas mixtures of known concentrations (v/v) for calibration and other experiments.
(2) Static standard gas generator. A static standard gas generator was also used to produce standard gases by spiking quantified pure hydrocarbons into pure nitrogen or helium in the sealed glass vial sampler.

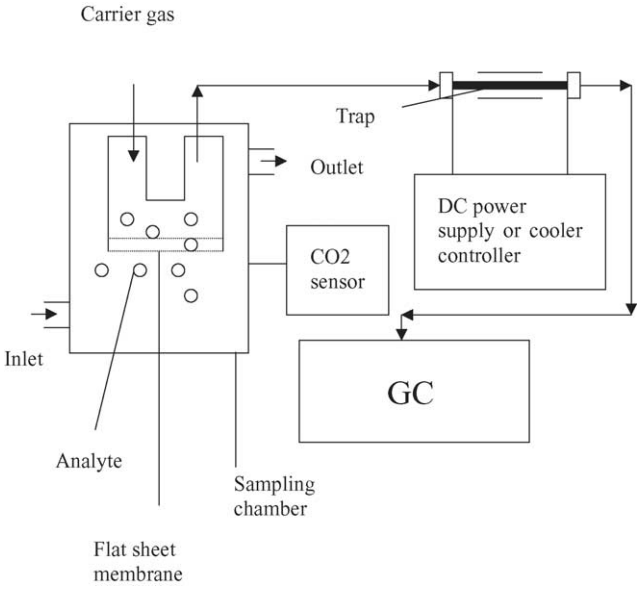

Fig. 1. Schematic diagram of MESI–GC system.

The measured concentrations of the standard gases produced with the dynamic system were verified with the static system. The values determined by the two methods agreed with each other well (e.g. R.S.D. within 1.1% for methane). Therefore, both the standard gas generating methods were applied in this study.

2.4. Experimental conditions

Unless otherwise indicated, the GC was operated at an isothermal oven temperature of 25 °C and a helium flow rate of 2.3 mL/min. Trap cooling temperatures ranged from −22 to +20.5 °C. Desorption heating parameters were 100–140 °C for 5 s for acetone and isoprene, 100–120 °C for 5 s for methane, and 120–140 °C for 5 s for ethane. The GC column (aluminum oxide PLOT) was regularly conditioned at 200 °C. The Tenax TA trap was conditioned on-line at up to 180 °C by several 5-s desorptions and the Carboxen trap at up to 250 °C by several 10-s desorptions in pure nitrogen or helium gas until a sufficiently low contaminant background was achieved.

3. Results and discussion

3.1. Quantitative correlation between analytes and CO_2 in the expired air

The model 8200 Capnocheck CO_2 sensor is a medical device that measures CO_2 partial pressure in expired breath. The commonest type of CO_2 sensor detects CO_2 levels on the basis of changes in the infrared light transmission properties in gas mixtures containing CO_2. A typical CO_2 sensor contains the infrared transducer, a small pump to draw the gases through a narrow tube connected to the breathing circuit, a water trap, controls and a display unit.

A breath sample was collected by asking a subject to randomly inhale and then blow breath air into the sample chamber through the inlet nozzle, so that samples other than alveolar air were collected. For each measurement of CO_2 partial pressure, part of the breath sample is drawn out of the sampler by a micro-pump in the CO_2 sensor. The lost sample volume in the sampler is replaced by the ambient air, resulting in a dilution and contamination of the sample. Dilution can also result from mass loss due to analyte diffusion through or leakage from the sampler. When CO_2 is to be considered as an internal standard in breath analysis, it should be confirmed that the ratio (or proportion) of analyte concentration to CO_2 partial pressure is not affected by the dilution due to the above mentioned causes and is independent of breathing patterns or sampling styles. Theoretically, for this purpose, the samples should have been collected from the same subject at the same moment, as the analytes concentrations in the alveolar air might fluctuate from time to time. However, in practice, it is impossible to do so. Alternatively, the representative samples could be collected within a period as short as possible and it could be reasonably assumed that the analytes concentrations in the alveolar air from the same subject should not have changed significantly during the period. During the experiment, the breath samples in the sampler were continuously refreshed in a 10-min cycle and a 5-min extraction time was applied. Therefore, two tests could be performed for each sample. For the current study, however, the analytical result from the first test was neglected because the sample refill was performed within the first 5-min extraction period of the test. In order to get samples with different CO_2 partial pressures, the samplings were performed randomly or with varied breathing patterns. Ten breath samples from the same subject were continuously obtained and monitored over 100 min. For each sample, the peak areas of isoprene and acetone and the partial pressure of CO_2 were recorded. The experimental result is indicated in Fig. 2. The linear correlation between acetone or isoprene and CO_2 is demonstrated. The main contribution to the deviation from the linear relationship could be the slight fluctuation of the analytes concentrations from the subject during the experimental period. After the

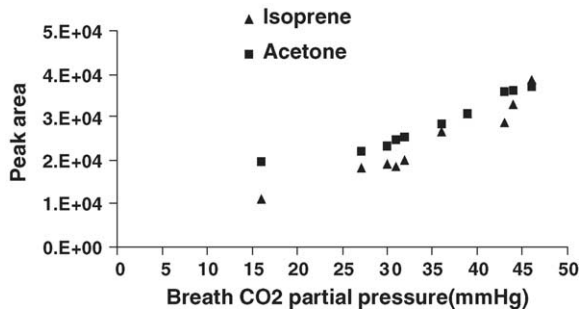

Fig. 2. Quantitative correlation between various analytes and CO_2 in breath sampled. Trap temperature: −22 °C, desorption: 140 °C for 5 s.

use of CO_2 normalization had been validated with the higher concentration analytes (isoprene and acetone) the technique was applied in the analysis of ethane and methane described below.

3.2. The characteristics of the MESI system for light hydrocarbon analysis

Breakthrough is a phenomenon that occurs when sampled analyte molecules exit the trap (sorbent) prior to desorption, either because of saturation within the sorbent bed or displacement by other molecules. t_1 is defined as the time when the outlet stream concentration increases by 5%. t_2 is the time when a total or complete breakthrough occurs. After the complete breakthrough of an analyte (when $t > t_2$), the sorbent trap cannot retain any more analyte.

In the MESI system the membrane module is a sampling probe, which is exposed in the sample matrix. There is a continuous flux of analyte molecules from the sample matrix through the membrane, which is carried away by the stripping gas and accumulated on the sorbent trap. Since the stripping gas (helium) also works as the GC carrier gas, the GC baseline level reflects the real-time analyte concentrations in the stripping gas exiting the trap.

The breakthrough discussed here is caused by a capacity overload (or limitation) when all the trap adsorption sites are occupied by analytes, and thus is called "capacity breakthrough" [9,11]. If the sorbent quantity, the analyte concentration and the flow rate remain fixed, the larger the sorbent capacity, the longer the breakthrough time t_1, or t_2.

Factors, such as analyte concentration, trap temperature and humidity in sample matrix will affect the sorbent capacity, causing the changes of t_1 and t_2. A clear and complete understanding of these effects is essential to find the optimum experimental conditions for the analytes of interest.

Carboxen is one of the commercially available carbon molecular sieves. It is made by the pyrolysis of polysulfonated polymers. Carboxen was selected for this study as it is relatively hydrophobic and is ideal for trapping small organic analytes (C_2–C_5 VOCs) [15,16].

3.2.1. Effect of analyte concentration on sorbent capacity

Carboxen sorbent extracts analytes via adsorption. Since the pores in Carboxen are small enough to cause capillary condensation, equilibrium on Carboxen sorbent can hardly be reached within a reasonable extraction time and the Langmuir isotherm model, which is applicable to other sorbent, is not exactly applicable to Carboxen sorbent [17]. No theory has been developed yet for the Carboxen sorbent. However, a quasi-Langmuir isotherm model can still be approximately used to qualitatively explain the adsorption process on carboxen sorbent. As expected, ethane (in standard gases) breakthrough occurred faster at higher concentrations (Fig. 3). The faster breakthrough at a higher concentration can easily be understood as the result of a faster saturation process on the sorbent. However, the faster breakthrough does not mean smaller sorbent capacity as usual; on the contrary, it result in larger sorbent capacity as indicated in Fig. 3, in which a higher plateau is obtained with the extraction time profile of higher concentration. However, the two plateau heights are not proportional to their corresponding concentrations. The ratio of the former is only about 2, but that of the latter is 65. This can be approximately explained with the quasi-Langmuir adsorption isotherm. At low concentrations, the dependencies between amount of analyte adsorbed and analyte concentrations in the stripping gas are approximately linear. At higher concentrations, the dependencies level off [17]. For ethane, at the level (ppbv) in real human breath, the breakthrough time is much larger than the actual trapping time under our operation conditions, therefore, the concentration effect can be ignored. For methane, since its breakthrough time is very short (about 2.5 min), effect of its concentration on sorbent capacity is not apparent.

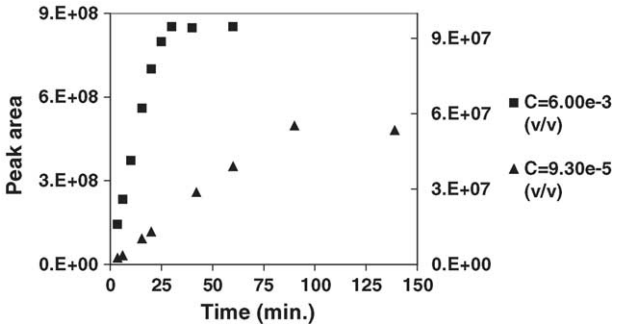

Fig. 3. Ethane extraction profiles at different concentrations. Trap temperature: −22 °C.

3.2.2. Effect of trap temperature on sorbent capacity

The sorbent capacity depends on the distribution constant (K) of the analyte between the sorbent material and the stripping gas. The larger the K, the higher is the sorbent capacity. K is related to the temperature T by $K = Ae^{-\Delta H/RT}$ or $\ln K = -\Delta H/RT + \ln A$. The adsorption of analytes on the sorbent is an exothermic process, i.e. $\Delta H < 0$. Therefore, K decreases with increasing T. This means that an increase in the trap temperature will result in a decrease in the sorbent capacity or breakthrough time.

The corresponding extraction time profiles of ethane at −22 and +20 °C trapping temperatures are presented in Fig. 4. It can be noted that before the breakthrough started ($t < t_1$), the extraction rates (m_t/t, the amount (m_t) of the trapped analyte retained in a certain trapping time (t)) are independent of the trap temperatures. The lower trap temperature enhanced the total sorbent capacity and caused a later breakthrough.

The above results are very useful in practice. In this example, if an extraction time of $t = 5$ min provides sufficient sensitivity, maximal trapping efficiency is obtained at ambient trap temperature. No advantage is gained by cooling the trap and no cooler will be necessary. Generally, for a given extraction time and analyte concentration, a maximal trap

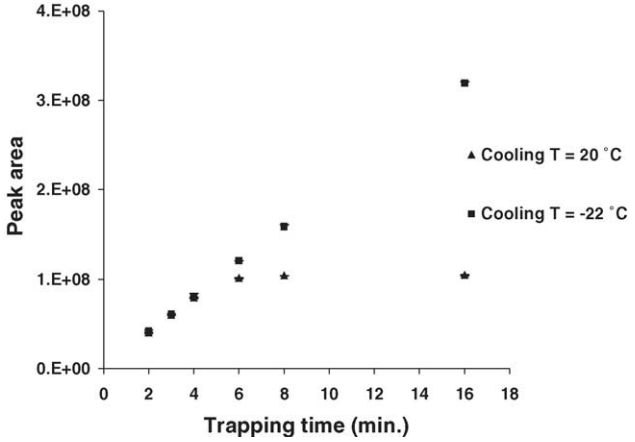

Fig. 4. A comparison of ethane extraction time profiles at different trap temperatures. Ethane concentration: 3.36×10^{-3} (v/v).

temperature can be determined according to the corresponding breakthrough parameter, to avoid saturation of the trap capacity.

3.2.3. *Influence of sample humidity on sorbent capacity*

Compared with absorption, which is a non-competitive process, adsorption is a competitive process and a molecule with higher affinity for the sorbent surface site can replace another molecule with lower affinity. Therefore, sample matrix composition can affect the amount of analyte extracted. The effect of the sample humidity on the breakthrough of ethane is shown here: $t_1 = 11.9$, 11.8 and 60.6 min versus different matrices of real breath air, 100% RH water vapor and dry helium, respectively (ethane concentration: 1.8 ppmv, trap temperature: −22 °C). It can be seen that moisture greatly reduces the breakthrough times (or sorbent capacity) due to water molecules occupying adsorptive sites.

Fig. 5 shows that for methane or ethane, the sorbent capacity is affected by both the sample matrices ("dry" or "wet" matrices; nitrogen or helium was randomly chosen as a dry matrix and no distinction was expected between them) and trapping temperature. Fig. 5a and b represent the situation after the breakthrough occurs, while Fig. 5c before the breakthrough. Whether the humidity will really affect the trapping efficiency also depends on other conditions. When the applied trapping (sampling) time $t \leq t_1$ (before breakthrough starts), high humidity in the sample should have no significant influence on the trapping efficiency as in Fig. 5c. Under our optimum operation conditions (trapping temperature = −22 °C, trapping time = 5 min for methane and 15 min for ethane), the MESI system can reach detection limits of less than 500 ppbv for methane and 20 ppbv for ethane.

3.3. *Breath methane/ethane on-line monitoring by MESI*

Contamination by ambient hydrocarbons is a significant technical and practical problem for implementation of breath monitoring. Two approaches have been used to deal with the

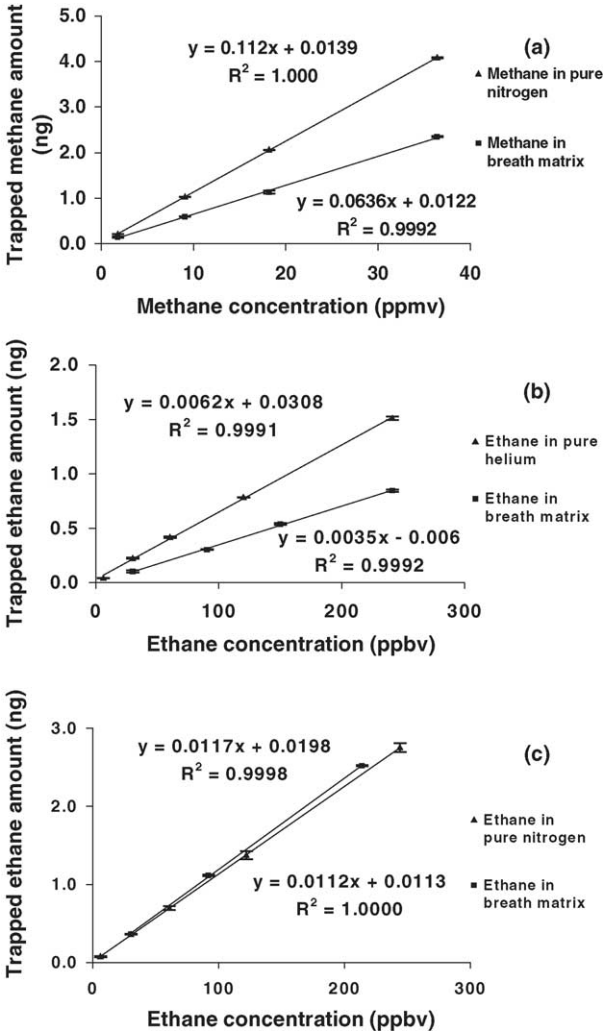

Fig. 5. The effects of matrix, trap temperature and trapping time on calibration curves. (a) Trap temperature: −22 °C, trapping time: 5 min; (b) trap temperature: +20 °C, trapping time: 15 min; (c) trap temperature: −22 °C, trapping time: 15 min.

problem of ambient air: one is the employment of washout periods to clear the lungs with hydrocarbon free air (HCFA) before expired air sampling; the other is the correction for the actual background concentrations [1,3] by subtracting them from the exhaled breath concentrations. Washout with HCFA might be a more appropriate alternative or logical approach, but it is less convenient achieve in practice [18]. It requires an additional source of purified air, submits the subjects or patients to an additional maneuver and takes more time and effort. We have followed the background subtraction method in this study.

A breath sample was taken by asking a subject to deeply inhale and then blow breath air into the sample chamber three times through the inlet nozzle at normal exhaling speed, so that an end-expired air (or alveolar air) sample without the "dead-space" air from the airway was taken. The inlet and outlet nozzles were sealed as soon as a sample was obtained. It took about 20 s to collect a proper breath sample. The suit-

Table 1
A typical quantification of breath methane and ethane (trap temperature: −22 °C)

	Methane (ppmv)	%R.S.D. ($n = 3$)	Ethane (ppbv)	%R.S.D. ($n = 3$)
Lab air	2.2	5.8	26.1	2.3
Breath air (before background correction)	2.3	1.8	31.7	6.0
Breath air (after background correction)	n/d[a]	–	5.6	6.4

[a] A *t*-test at the 95% confidence level indicated no difference of methane level in the breath from that in the ambient air background.

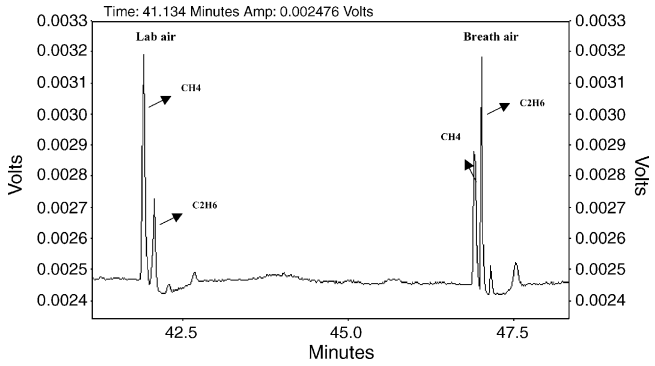

Fig. 6. A typical chromatogram of simultaneous lab air and breath air monitoring. Trap temperature: −22 °C.

ability of this sampling procedure was confirmed by monitoring the normal CO_2 partial pressure levels in breath samples. To collect another breath sample, another subject was asked to blow into the vial using the same method. A disposable inlet nozzle cone was used to ensure a clean surface for each subject. Therefore, an on-line continuous monitoring is available. According to this procedure, no significant deviation of analyte levels has been noted between the repeated breath samples from the same subject.

A typical chromatogram of breath methane/ethane in comparison with lab air (as the background) is presented in Fig. 6. The first pair of peaks was obtained when the membrane module was exposed to the lab air and the second while the membrane module was in a breath sample. The breath sample was collected immediately after the membrane module was quickly transferred from the lab air into the sampling chamber. The quantitative results based on the peak areas are shown in Table 1. Due to different properties ("dry" or "wet" as discussed previously) of the matrices, the concentrations of methane and ethane in lab air were calculated with the corresponding "dry" calibration curves and those in breath air with the "wet" calibration curves in Fig. 5. From Table 1, it is seen that the methane levels from the two samples are almost the same. In Fig. 6, it is noted that after the sample change, the second peak (ethane) significantly increased, but interestingly, the first peak (methane) decreased. This is because the extraction time used here was 5 min (>methane breakthrough time t_1 which is only about 2.5 min due to the high volatility of methane.) and as discussed previously, the humidity in the breath air lowered the methane extraction efficiency in this after-breakthrough case. The longer extraction time had to be applied in this study only because the methane breakthrough time is too short to be practical for general breath (including other analyte like ethane) pre-concentration. The breath sample in Fig. 6 happened to be from a non-methanogenic individual and nearly all the methane in this breath was from the ambient air, but the equivalent level of methane appeared lower in the wet matrix. Some of our volunteers were methanogenic individuals (breath methane at least 1 ppmv higher than ambient air methane) and their breath methane was up to 12.8 ppmv after ambient background correction. From Table 1, it is seen that the breath ethane is only about 20% of the ambient ethane. This percentage agrees with literature data from the lung washout technique [1].

4. Conclusion

In breath analysis, the errors in sampling and storage for the period between sample collection and sample analysis can be significant. It is essential to correct for the errors before using the analytical results; otherwise, the results cannot be compared inter-individually or even intra-individually. Carbon dioxide in human breath was shown to be a good and convenient natural internal standard for correction. The analyte concentration measured in a randomly collected breath sample can be normalized to its corresponding value under a normal CO_2 level with the actually measured CO_2 level and the constant ratio of the analyte concentration to CO_2 partial pressure.

The application of a hydrophobic silicone membrane prevents excessive amounts of moisture from entering the analytical system. Since the content of the moisture in expired breath is high, the advantage of the hydrophobic silicone membranes makes the MESI technique be well suited for breath analysis. No extra drying device, which has been regularly applied by other researchers and frequently contributing to errors, is necessary in the MESI system. Since the stripping gas also works as the GC carrier gas in this system, one of the apparent advantages of the system is that it eliminates any switching valve and allows rapid routine analysis and long-term continuous on-line monitoring of VOCs in breath air and various environmental matrices. The latter includes plant emissions (ethylene, isoprene and α-pinene, etc.) and atmospheric greenhouse gas monitoring.

The aluminum oxide PLOT column used demonstrated retention time changes which caused lower resolution of methane and ethane over time because of the moisture in breath samples. This effect can be eliminated by using a polydivinylbenzene PLOT column which is not moisture sensitive and is ideal for the applications where moisture is of ma-

jor concern. Since the detection limit for ethane is currently 20 ppbv, the breath ethane concentration level of 5.6 ppbv, which was obtained by background correction in Table 1, can hardly be detected directly as in the case of HCFA washout method. However, the analytical sensitivity for ethane can be significantly enhanced by increasing the sorbent quantity filled in the trap. Since only about 7.5 mg of sorbent is available in the current commercialized traps, there is great potential in this aspect. These will be investigated in future work.

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