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Sampling and Monitoring of Biogenic Emissions by Eucalyptus Leaves Using Membrane Extraction with Sorbent Interface (MESI)

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Membrane extraction with sorbent interface (MESI) has been applied to monitor plant fragrance volatiles emitted into indoor air. The main components of the MESI system are a membrane module and a trap, which can be connected directly to a GC or GC-MS for simultaneous multicomponent extraction and monitoring. A polydimethylsiloxane (PDMS) membrane and two different traps, PDMS and Tenax, as well as a DC current supply for trap desorption have been applied in this research. After the membrane module is placed in contact with the plant, the MESI/GC-MS provides semicontinuous characterization of volatile compounds emitted. The MESI device has been applied to monitor the biogenic volatile organic compounds released during the first 8 h after a branch was cut from a *Eucalyptus dunnii* tree. The study demonstrates that the MESI system is a simple and useful tool for monitoring changes in emission processes as a function of time.

KEYWORDS: Membrane extraction; sorbent interface; gas chromatography; mass spectrometry; fragrance volatiles; eucalyptus

INTRODUCTION

The availability of analytical air-monitoring methods of high sensitivity and reliability is very important for qualitative and quantitative determination of organic substances present in the atmosphere in low concentrations. In particular, biogenic volatile organic compounds (BVOCs), which are emitted from vegetation, are of great importance for atmospheric chemistry processes (*1*). Analysis of BVOCs may be applied to macerated or cut parts of a plant (*2*). Determinations of BVOCs released from living plants are usually performed by enclosing the whole plant or some of its parts in glass or plastic chambers, followed by collection of the emitted organics in sorbent traps and chromatographic analysis of the sorbates (*3*). A solvent-free sample preparation technique, membrane extraction with sorbent interface (MESI), is a new sample preparation method for trace organic analysis (*4*). The main features of MESI include its solvent-free nature, a rugged and simple design for long-term reliable performance, the fact that it is an integrated and uninterrupted process (eliminating the possibility of analyte loss), and its ease in automation and feasibility for on-site operation (*5*).

The MESI technique has some similarities to purge-and-trap analysis but also has several advantages over it. First, it simplifies the whole analysis system. With the membrane added to the system, no off-line operation mode is required. The membrane introduces complex sample analyte selectivity into the system. Second, because of the hydrophobic nature of the PDMS membrane, water vapor is prevented from entering the system. Finally, the solubility/diffusion mechanism provides concentration of the analytes as they penetrate through the membrane.

Membrane extraction consists of two simultaneous steps: extraction of analytes from the sample matrix through the membrane and stripping of analytes from the other side of the membrane into a flowing carrier gas. Transport through the nonporous membrane occurs by a solution/diffusion mechanism, and selectivity is achieved either by differences in membrane/sample material partition coefficient or diffusivity (*6*). This implies that analytes with a large partition coefficient (membrane/sample) and a large diffusion coefficient (in the membrane) are preferentially extracted (*7*). The analytes that have permeated through the membrane and into the stripping gas are carried to the trap, where they are concentrated. After having been extracted and concentrated, the analytes can be introduced into the GC or GC-MS for analysis by thermal desorption of the trap.

Membrane extraction in air, aqueous phase, and headspace has been investigated, and the corresponding mathematical models have been used for defining the extraction process (*8*−*10*). In this paper, for monitoring of real samples, a branch of a *Eucalyptus dunnii* tree was removed and put into a glass

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chamber with air flowing through the chamber to mimic a real environment. To optimize the experimental conditions, different desorption methods, trap and membrane temperatures, and stripping gas flow rates have been studied. Because most compounds emitted from *Eucalyptus* leaves are semivolatiles, heating of the transfer line and membrane module was employed. A group of organic compounds including eucalyptol, α-pinene, γ-terpinene, and caryophyllene (a C_{15} compound) have been selected as target compounds.

The mass of analyte extracted by MESI can be estimated by eq 1

$$M/t = DAKC/b \tag{1}$$

where A is the membrane surface area, M is the extracted amount, t is the extraction time, D is the diffusion coefficient of analyte in the membrane, K is the partition coefficient between the membrane and gas phase, C is the concentration of the analyte in the sample, and b is the thickness of the membrane (*11*). Equation 1 assumes that the mass transfer is controlled by diffusion in the membrane, which is valid for volatile analytes under natural convection conditions and high linear carrier gas flow. As long as there is no breakthrough in the trap, this equation can be applied to estimate the extracted amount. Permeation of the stripping gas (helium) through the membrane does not affect analyses significantly. The partition coefficient of helium to PDMS is very low, and so the mass transfer of helium through the membrane can be ignored. Equation 1 shows the extracted amount is proportional to the concentration of analyte and extraction time. At a low concentration of analyte, the extracted amount can be increased by prolonging the extraction time, resulting in improvement in the sensitivity of MESI. In addition, this paper demonstrates that MESI can be used to automatically monitor the changes of biogenic emissions from plants.

EXPERIMENTAL PROCEDURES

Materials. An *E. dunnii* tree was maintained in the Department of Biology greenhouse at the University of Waterloo. Both the leaves and a branch portion of the tree were sampled. A branch was brought into the laboratory for monitoring of BVOCs.

Monoterpene standards were purchased from Sigma-Aldrich (Mississauga, ON, Canada).

Instrumentation. An Agilent 6890A GC coupled with an Agilent 5973 MS detector was used for analysis and was fitted with an Rtx-5MS column, 30 m × 0.25 mm, df 0.25 μm (Restek Corp., Bellefonte, PA). Helium was used as the carrier gas/stripping gas at 1 mL/min. Column temperature programming was as follows: initial temperature, 50 °C; hold for 3 min; ramp at 10 °C/min to 250 °C; hold for 2 min. Temperatures of the ion source (EI, 70 eV, 39.6 μA), quadrupole, and transfer line were 230, 150, and 280 °C, respectively. For analysis the quadrupole was scanned in the range of 50−300 m/z at a scan frequency of 2.94 scans/sec. Identification of eluants was performed using a NIST 98 spectra database.

MESI System. A flat sheet of PDMS membrane (dimethylsilicone membrane, SSP-M100C.001, 25 μm thickness) was purchased from Membrane Components (Ballston Spa, NY). The membrane module was obtained from Restek Corp. (Bellefonte, PA, catalog no. 551741) and is shown in **Figure 1**. A piece of the membrane sheet was inserted between the two Teflon spacers in the membrane module. A stainless steel housing compressed the Teflon spacers using screws. With this construction, the membrane module was leak-proof. A PDMS trap (catalog no. 551743) and a Tenax trap (catalog no. 551745), both from Restek Corp., were selected for this study. These traps were prepared by packing the polymer material into a 5 cm × 0.75 mm i.d. piece of deactivated stainless steel tubing. The bed length was 1.7 cm, and the sorbent was 60/80 mesh. Quartz wool was placed at the ends of the sorbent bed to retain the packing.

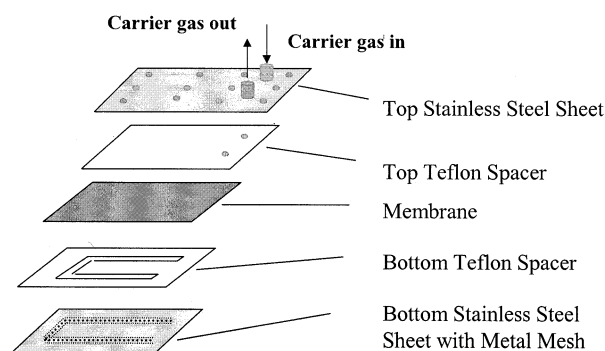

Figure 1. Schematic diagram of a commercial membrane module.

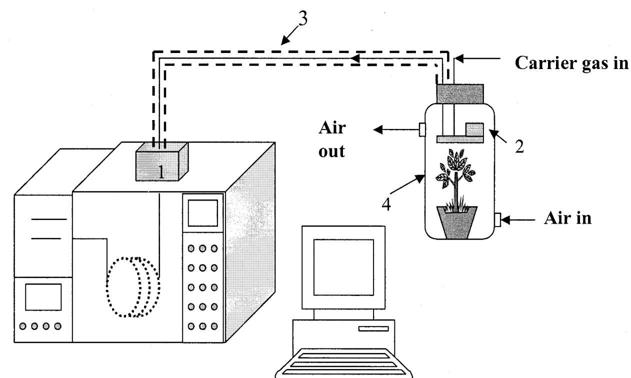

Figure 2. Schematic diagram of MESI online system: 1, MESI control box with trap inside; 2, membrane module with heater; 3, heating tape; 4, extraction chamber.

To facilitate the extraction of the BVOCs emitted by *E. dunnii*, a glass chamber with a brass lid was constructed. A solid phase microextraction (SPME) sampling port was designed on the lid to facilitate simultaneous SPME and MESI extraction. Different flow rates of air through the chamber were obtained by adjusting the flow rate from an air cylinder connected to the sampling chamber. **Figure 2** shows the schematic diagram of the MESI on-line system.

A heater connected to a controller was mounted on the top of the membrane module. With this construction, different membrane module temperatures were obtained.

A MESI enclosure was designed to facilitate temperature control of traps. **Figure 3** shows the construction of the MESI control box and a commercially available trap. During the period of trapping, a one-stage thermocouple cooler (catalog no. CP1.0-07-08L, Melcor Corp., Trenton, NJ) included in the enclosure lowered the temperature of the trap to around −10 °C and increased the partition coefficients of the compounds between stripping gas phase and the polymer material of the trap. A DC current power supply (catalog no. AB-8, ABRA, Ontario, Canada) was used with this cooler. The whole cooling unit was constructed at the University of Waterloo. After a specified trapping period, a separate DC current was applied to the trap to thermally desorb all analytes into the carrier stream for GC analysis. For these experiments, the DC current was controlled manually by applying 8.5 A until the trap temperature reached 210 °C for the Tenax trap and 240 °C for the PDMS trap. The temperature was maintained for 0.7−1 min by adjusting the current. A thermocouple thermometer monitored the changes of temperature during desorption. The thermal desorption device was prepared using a 20 V, 10 A DC power supply (catalog no. AB-10AV, ABRA) to supply heating current to the trap for desorption. A Fluke 53II thermocouple thermometer (Fluka Corp.) was used to monitor the change of temperature while applying heating current to the trap.

The connection between the trap and the membrane module was made with $^{1}/_{16}$ in. i.d. Teflon tubing with 0.25 mm i.d. deactivated stainless steel tubing inserted inside it. The arrangement of the tubing

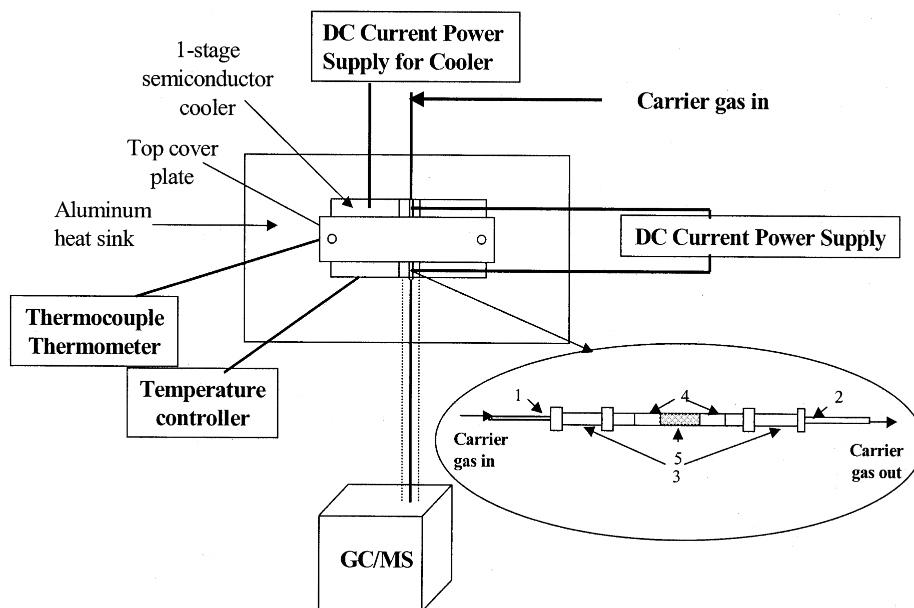

Figure 3. Schematic details of MESI control box and commercial trap: 1, transfer line (inner tubing, 0.25 mm stainless steel tubing; outer tubing, $^{1}/_{16}$ in. Teflon tubing); 2, transfer line, $^{1}/_{16}$ in. stainless steel tubing; 3, fittings; 4, quartz wool; 5, polymer material.

in this way prevented heat loss and back-flushing during desorption. Transfer lines used to connect the other components were $^{1}/_{16}$ in. deactivated stainless steel tubing. All of the transfer lines were maintained at 170 °C to eliminate condensation of target compounds.

Standard Gas Generation System. A "liquid injection" standard gas generation system was used for method development for its flexibility in generating standard gases of widely varying concentration and composition. The standard gas concentration is described by eq 2 (*12*)

$$C_{g(T)} = \left[\frac{(m/t)_{\text{mix}}}{F_g} \times \left(\frac{\%\ \text{analyte}}{\text{total}}\right)\right] \quad (2)$$

where $C_{g(T)}$ is the gas concentration of analyte in mass/volume at a given temperature, m/t is the mass delivery rate of the liquid mixture, F is the dilution gas flow rate, and % analyte/total is the mass ratio of an individual analyte to the total.

Methods. Standard gas mixtures were used to optimize the experimental conditions. Daily blank runs were carried out to check for the presence of artifacts and contamination from previous analyses. The reproducibility of the MESI system was verified by on-line monitoring a standard gas mixture. During trapping, the temperatures of the PDMS membrane and the PDMS-packed bed of the trap were 25 and −10 °C, respectively. The flow rate of the carrier gas, which also served as a stripping gas, was 1.8 mL/min.

A branch of an *E. dunnii* tree was put into a bottle filled with fresh water and sealed inside the glass-sampling chamber. The chamber air circulation rate was 4 mL/s, and the carrier gas flow rate was 1.0 mL/min. The desorption temperature of the PDMS trap was 240 °C, held for 1 min. To monitor the changes of BVOCs emissions, a continuous extraction cycle was performed for 7 h. This consisted of 1 h of extraction time, followed by a 1 min desorption. After 7 h, both MESI and SPME methods were performed simultaneously to extract analytes.

A 2.5 g sample of *E. dunnii* leaf pieces (~1 cm^2) was used to study the impact of freshness on green leaf volatiles. One extraction was performed immediately after the leaves had been removed from the tree. The samples were then stored in a refrigerator and 24 h later, following re-equilibration to room temperature, the extraction was repeated and the results were compared. The membrane used was PDMS, and the trap material was Tenax. The desorption temperature of the trap was 210 °C, held for 1 min. System blanks were performed between the two experiments to eliminate the possibility of carry-over in the system.

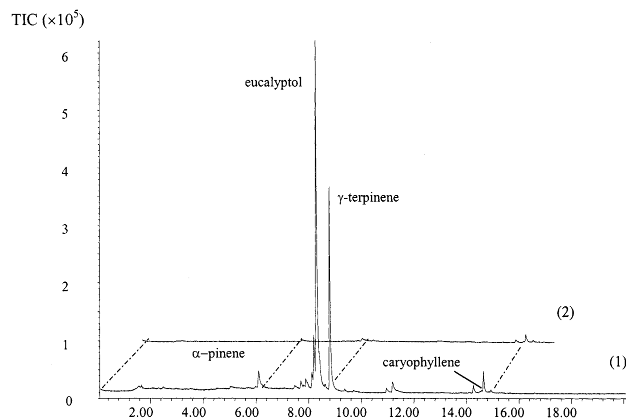

Figure 4. Chromatograms of desorptions of a Tenax trap after trapping leaves for 20 min: 1, desorption immediately after trapping; 2, carry-over of 1. Tentative identifications of the peaks are shown, based on comparison to standards.

RESULTS AND DISCUSSION

A DC current power supply was employed to desorb the extracted analytes from the traps. Adjustment of the current and its applied duration allowed optimization for different temperatures. The optimized desorption temperatures for PDMS and Tenax were 240 and 210 °C, respectively. **Figure 4** shows the chromatogram obtained from a sample of 3 g of *E. dunnii* leaves by using a DC current power supply to desorb the Tenax trap. The column temperature was initially set at 50 °C, held for 3 min, ramped at 5 °C/min to 80 °C, and ramped at 20 °C/min to 240 °C with a final hold time of 2 min. Compounds in group I (monoterpenes) such as eucalyptol and α-pinene can be completely desorbed; however, compounds in group II, such as caryophyllene, were not released completely. Caryophyllene is a 15-carbon molecule, and so it requires a longer time for complete desorption because its affinity for the trap material is very high. As shown in **Figure 4(2)**, it is difficult to eliminate carry-over for this compound.

Many compounds emitted by *Eucalyptus* leaves have large molecular weights and low diffusion coefficients. The boiling points of these compounds are high (most are >150 °C).

Techniques to increase diffusion and to prevent condensation would improve the method. Heating is the most convenient and efficient way to increase the diffusion of semivolatile compounds and prevent condensation. The temperature of the membrane influences the partition coefficient between membrane and sample (K) and the diffusion coefficients of compounds within the membrane (D). In addition, an increase in sample temperature affects the Henry's constant and the concentrations of the compounds in the headspace above the leaves. The overall change depends on which factor is most affected by the temperature change (*13*). We observed that the temperature of the membrane did not affect the extraction efficiency significantly. After the MESI system had been simplified and the effect of membrane temperature had been considered, room temperature (25 °C) was chosen as the extraction temperature for the membrane.

The PDMS sorbent is a polymeric material, and temperature can significantly affect its capacity as well. The higher the temperature, the lower the capacity of the sorbent. Different trapping temperatures (−5 and −11 °C) of the trap were studied. The trapping capacity at −11 °C is higher than that at −5 °C. Because the sorbent material was PDMS, nonpolar VOCs have a high affinity for the polymer. When the stripping gas flows through, the analytes are trapped by the sorbent. The absorption is an equilibrium process, which means the sorbent simultaneously absorbs and desorbs analytes. At low temperatures the analyte partition coefficients between the sorbent and the gas are very large, generally several orders of magnitude higher than at room temperature (*14*). Analytes are essentially completely trapped in the sorbent under these conditions.

Trapping time is affected by flow rate, sample amount, trap capacity, and ambient temperature. The optimal trapping time is the longest time possible before breakthrough is reached. By optimizing experimental parameters, the extraction time can be decreased; hence, extraction efficiency can be increased. Some modifications were made, such as adjusting the temperature of the membrane. Another approach was applying lower temperature to the trap during the extraction step. Applying a high stripping gas flow rate could also increase extraction efficiency. The flow rate of the stripping gas can affect the extraction significantly. A low linear stripping gas flow results in slow transfer of analytes from the membrane to the sorbent. In MESI the trapping time is limited by trap breakthrough, namely, the elution of the analytes from the sorbent by the stripping gas (*15*). Similarly to optimization of separation and extraction in gas chromatography, which are interrelated, a ramp of flow rate can be applied to the stripping gas (carrier gas) to provide an increase in extraction efficiency of the membrane.

The reproducibility of the MESI system was tested by online monitoring of a standard gas mixture. The measurements were based on three replicate injections. The makeup of the standard gas mixture used was α-pinene (5.8 ppb), eucalyptol (3.4 ppb), and γ-terpinene (3.9 ppb). The relative standard deviations for the extracted amounts of α-pinene, eucalyptol, and γ-terpinene were 5, 7, and 9%, respectively. The linearity of the calibration curves was acceptable (correlation coefficients for α-pinene, eucalyptol, and γ-terpinene were 0.9925, 0.9662, and 0.9920, respectively). The linear range can be increased by adjusting the experimental conditions, such as the size of the membrane and sorbent, the trapping and desorption parameters, and the flow rate of stripping gas.

Because the linearity and reproducibility of the MESI system were found to be acceptable, this technique was applied to monitor the change of biogenic emission of *Eucalyptus* leaves.

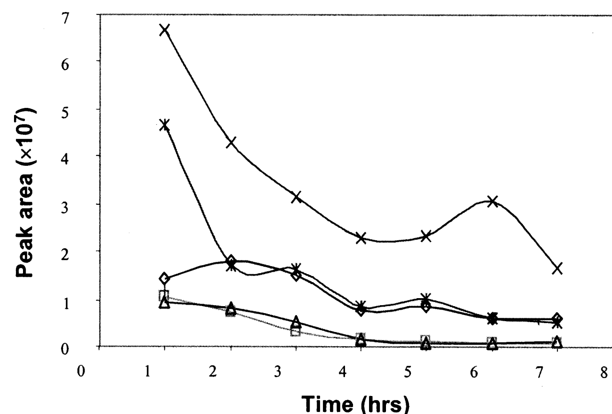

Figure 5. Dependence of biogenic emission of *Eucalyptus* branch inside the sampling chamber with time: (◇) α-pinene; (□) *m*-cymene; (△) limonene; (×) eucalyptol; (∗) γ-terpinene. Tentative compound identities are shown.

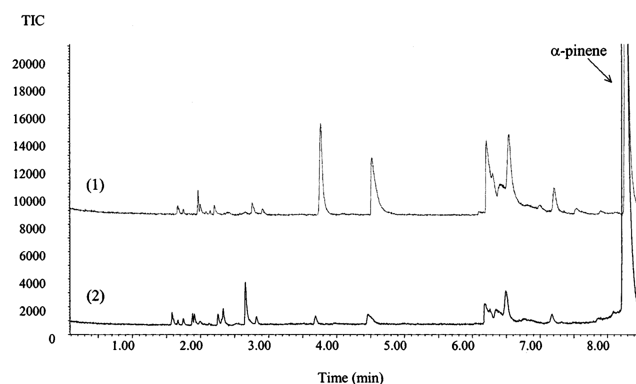

Figure 6. Effect of freshness on green leaf volatile emissions (early peaks): 1, 1-day-old leaves; 2, fresh leaves. α-Pinene is tentatively identified, based on comparison to standards.

To obtain good resolution, 1.0 mL/min was used as the carrier gas flow rate during monitoring of the *E. dunnii* branches. **Figure 5** shows the changes of the biogenic emission of eucalyptus leaves contained in the chamber during the first 8 h after being cut from the tree. Unlike the behavior of semivolatile compounds, the concentrations of which decreased with time, the concentrations of the green leaf volatile compounds emitted by the pieces of *E. dunnii* leaves increased after 24 h. **Figure 6** shows the chromatograms obtained by headspace extractions of 2.5 g of leaves. Appropriate blanks were run between the two analyses to verify that the results shown are not due to carry-over. The biochemistry of these phenomena is very complicated and is not the focus of this paper. However, these figures demonstrate that the MESI system can be applied to monitor plant biogenic emission changes, which can be used to analyze metabolic processes. SPME can also monitor BVOCs change (*16*), so it is important to identity the difference between these two sampling methods. Retention time differences seen in **Figures 4**, **6**, and **7** are due to variations in carrier gas flow rates and column temperature programming between the experiments and differences in the timing of the manual trap desorptions. **Figure 7** illustrates the difference in sensitivities between SPME and MESI. Seven hours after being picked, the biogenic emission of the branch was very weak. At low concentrations, MESI was much more sensitive for extracting volatile compounds. However, when low-volatility compounds such as caryophyllene were extracted, MESI was not as effective as SPME. An increase in membrane temperature would assist

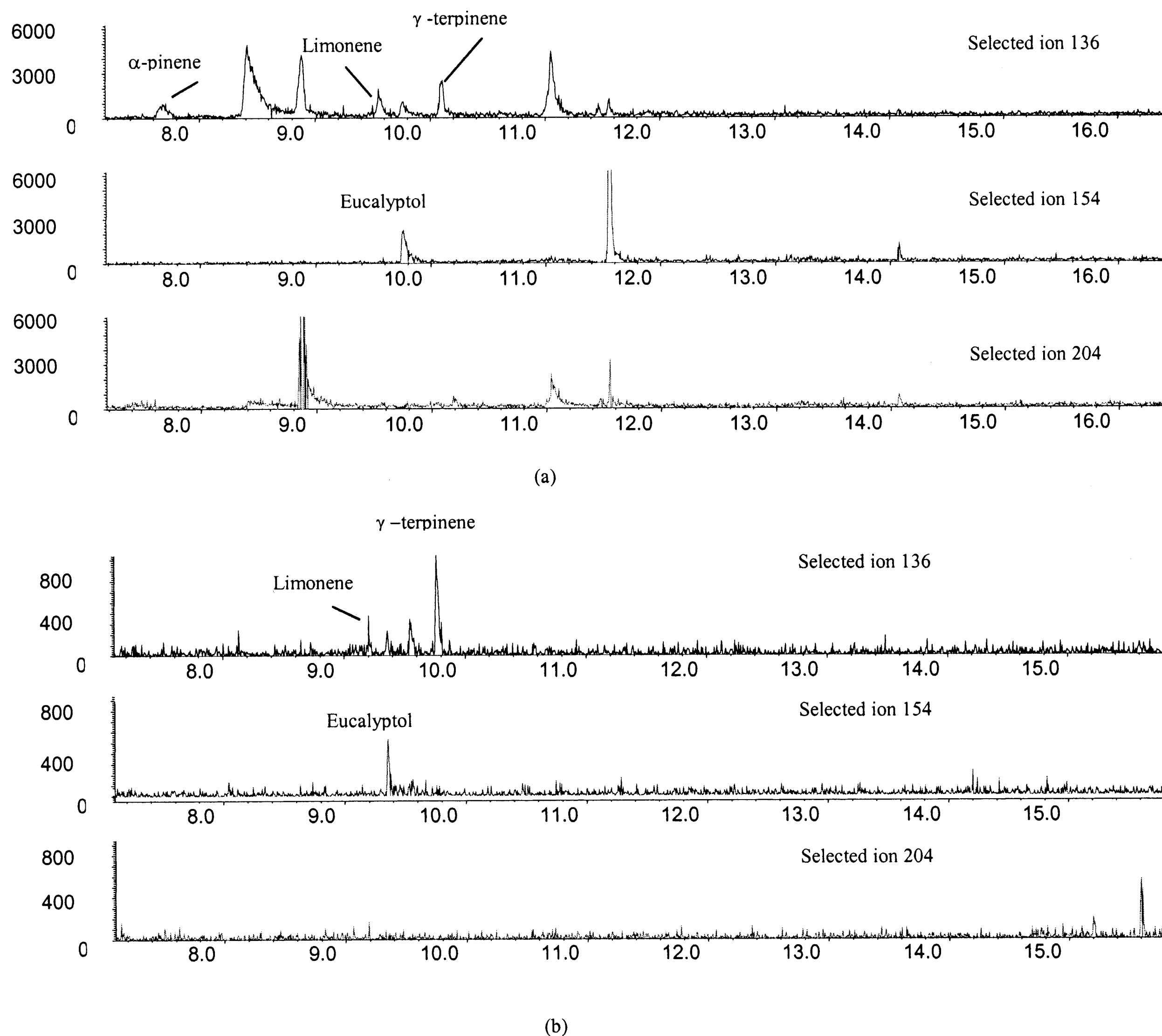

Figure 7. Selected ion chromatograms from the application of MESI (a) and SPME (b) for analyte extraction at low concentrations. Several peaks are tentatively identified. Selected *m*/*z* values were 136, 154, and 204, respectively.

in more efficient permeation and stripping of semivolatile compounds from the membrane, and the extraction efficiency may be improved. It should be emphasized that unlike SPME, which requires human intervention, MESI can be operated automatically.

CONCLUSION

The results reported above indicate that MESI is a very useful tool for field monitoring and analysis. MESI integrates sampling, sample preparation, and introduction to an analytical instrument resulting in a continuous process. It can be used to monitor biogenic emission without loss of volatile analytes. Because headspace extraction has been used, no contamination (irreversible fouling by nonvolatile sample components) or mechanical damage of membrane occurs, which prevents loss of membrane performance with time. No mechanical operation is needed when the MESI system is applied. During extraction, the membrane unit can be exposed directly to the studied plant. This method can be easily automated. The MESI system does not disrupt the analysis system, and the analysis itself does not affect emission. It can monitor changes in emissions as a function of time and can be automated to run without human intervention. This method can be extended to study volatile emissions by animals, foods, etc. and demonstrates that the MESI system combined with GC is a good practical approach for field monitoring of biogenic emission because of its general simplicity and convenience of deployment. The application of MESI can be extended to the analysis and monitoring of volatile fragrance compounds in foods and other matrices.

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