

Field Application of Membrane Extraction Sorbent Interface-IMS for Analysis of Pheromones

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Abstract

Pheromones are chemical compounds involved in insect communication. They have been used in effective integrated pest management program in the agricultural and forestry markets. Growers have a real desire, and need to get away from the traditional pesticides approach in their pest management practices. Pheromones are natural substances and are considered to have less adverse environmental impact than the use of pesticides. Pheromones are formulated into lures and used in traps to capture specific species or a number of related species. They are also used to disrupt insect mating or manipulate insect behavior at the hive level as well as improve crop pollination.

Monitoring pheromones, released at low levels is an important process for the successful application of this approach. This demands sensitivity and specific method for preconcentration and analysis of these substances at the lowest possible concentrations.

A promising technique developed at Waterloo University by Pawliszyn et al is membrane extraction with sorbent interface (MESI). It has been used in this study to concentrate pheromones at low levels over period of minutes to hours. MESI is a single step sample preparation technique which uses a membrane module to exclude water and allowing organic compounds to permeate the membrane and concentrate on a selective adsorbent material, like Tenax GC, Carbopack or thick films GC liquid phases, packed in small Stelcosteel tubing. The trap is resistively heated to release the adsorbed materials into a capillary gas chromatograph interfaced to an ion mobility spectrometer model GC-IonScan 400B.

MESI enables semi-continuous monitoring of pheromones and other gaseous compounds owing to the selectivity of the membrane material and trapping properties of the sorbent used. The sensitivity of the method allows concentration in sub-parts per billion levels of pheromones to be detected.

1.0 Introduction

Insect communication is a fascinating and rewarding area of entomology with much applied potential. Pheromones are communication chemicals, which can be used against pest insect species to help prevent pesticide misuse, spraying the wrong type or too much or spraying in the wrong place or at the wrong time. During the past 40 years, pheromones of hundreds of insect species have been chemically elucidated (1-3). Generally, they are classified as hydrocarbons, epoxides, acetates, aldehydes, ketones, carboxylic acids and they vaguely resemble fatty acids, from which they are biogenetically derived. A novel extraction method was developed at Waterloo University (4-6) consisting of membrane extraction with sorbent interface (MESI). A permeable silicone membrane is coupled with an adsorbent trap for the purpose of sampling, concentration of organic compounds. This method affords some advantages over existing technologies in that it has no moving parts, and is easily transported to the field. The MESI technique was explored for the collection and concentration of pheromones and was compared to the solid phase microextraction fibers (SPME).

Monitoring pheromones, released at low levels is an important process for the successful application of this method to improve crop pollination and control of insect population.

This demands sensitivity and specific method for preconcentration and analysis of these substances at the lowest possible concentrations. The MESI technique has been used in this study to concentrate pheromones at low levels over periods of minutes to hours. Instrumental analysis involved release of the adsorbed materials into a capillary gas chromatograph interfaced to an ion mobility spectrometer model GC-IonScan 400B. MESI enables semi-continuous monitoring of pheromones and other gaseous compounds owing to the method selectivity of the membrane material and trapping properties of the sorbent used. The sensitivity of the method was substantially enhanced by the addition of the MESI system allowing sub-parts per billion levels of pheromones to be detected.

2.0 Experimental

The SPME and MESI methods were deployed for the concentration and sample introduction into the GC-IMS. The SPME fiber was purchased from Supelco Canada and consisted of 7 μ m film of polydimethyl siloxane (PDMS) desorbed at 280 $^{\circ}$ C for 20 seconds in heated inlet of the IMS. The SPME fiber was inserted into a vial containing the pheromone and allowed to equilibrate before withdrawing the fiber and desorbing it in the IMS. Figure 1. shows the method of desorbing the SPME fiber into the heated inlet of the IonScan 400B.

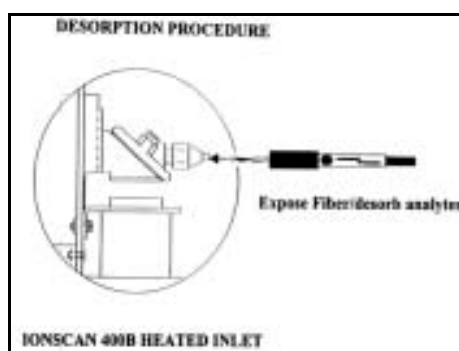


Figure 1. SPME desorption into heated inlet of IMS

The MESI interface to the GC-IMS system is shown in Figure 2. The operation of the MESI includes two steps in sequence, trapping and desorption, which correspond to sample preconcentration and injection. The membrane used was 25 μ m dimethylsilicone, held at constant temperature of 140 $^{\circ}$ C. Transmission through the membrane of the low volatile pheromones increased at elevated membrane's temperature.

The sorbent trap consisted of a 7 μ m thick film PDMS coated on a 0.53mm ID capillary column of 5cm length. The trap was resistively heated by a control circuitry to a constant temperature to desorb the trapped material onto the analytical capillary column.

The analytical column used was MXT-1, 15 meters length, 0.53mm ID coated with 1 μ m film thickness. The GC oven was temperature programmed at 120 $^{\circ}$ C, holding 30 seconds and ramping at 30 $^{\circ}$ C/min to 240 $^{\circ}$ C. Injector and transfer line temperature from the GC to the IMS were 250 $^{\circ}$ C and 200 $^{\circ}$ C respectively. Nitrogen gas carrier flow pressure was 10psi.

MESI operating conditions: Membrane interface temperature 140 $^{\circ}$ C, sample flow rate at

1 liter/min and desorption flow rate onto the GC column was 40cc/min. Cooling of the trap was carried out with a DC operated fan.

Desorption of the trap was carried out by applying approximately 9 W power for 7 seconds.

Vapor Generator. A dynamic gas blending system was used to generate known and controllable concentration of pheromones vapor in purified air. The single dilution vapor generator was reported in the literature (7-9). A small air stream of air passed through a thermostated flask containing the pheromone or mixture of pheromones; carrier gas stream was then diluted by a large air stream to produce low ppb to ppt concentrations of pheromones. The vapor source delivered 1 to 20 L/min. The actual pheromone concentration in the air stream was confirmed by GC-IMS analysis of air samples collected in heated gas tight syringe (100-1000 μ l) and based on calibration with standard solutions of pheromones. Reproducibility of pheromones concentrations from the vapor source was within +/-20%.

Estimation of equilibrium vapor pressure at 20°C was determined by analysis of head space vapor of each pheromones based on calibration data from standard solutions.



GC-IonScan 400B



MESI Assembly

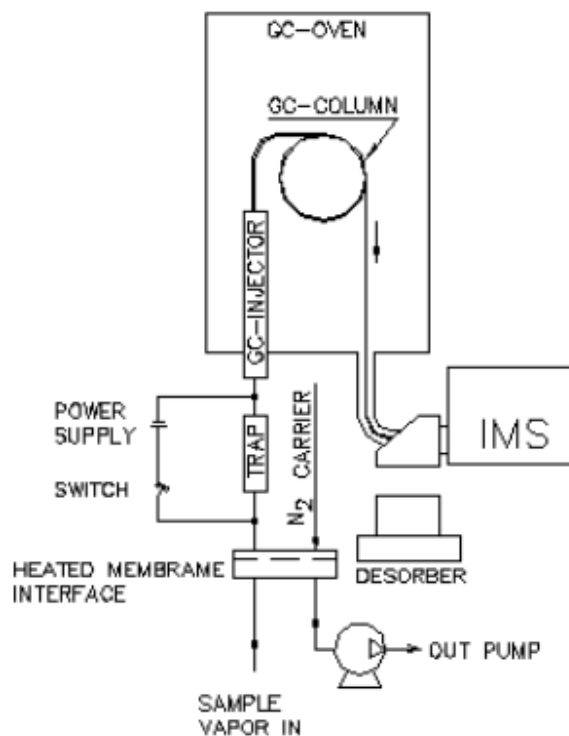


Figure 2. MESI-GC-IMS interface

Instrumentation. The IMS was operated in the positive ion mode with nicotinamide chemical ionization reagent (CIR) and water reagent chemistry. Pheromones are generally protonated from the chemical ionization reagent provided the proton affinity exceeds or equal the CIR. Water CIR produced better sensitivity for the acetate and alcohol pheromones than nicotinamide CIR.

Sample Introduction Techniques. Two sampling procedures were used in this study. The SPME fiber was exposed to the vapor generator for specific amount of time and desorbed directly into the heated inlet of the IMS as shown in Figure 1. The second procedure involved sampling directly the vapor generator through the heated membrane interface as shown in Figure 2. Different enrichment periods were used in the MESI procedure and the trap was resistively heated to desorb the sample onto the analytical GC column.

Chemicals. Pheromones samples were provided by 3M, Canada with highest purity and others were purchased from Sigma-Aldrich with minimum 99% purity.

3.0 Results And discussion

Comparison of detection limits for direct thermal desorption of standard solutions of pheromones with GC-IMS analysis are shown in Table 1. Generally, sensitivity is better with the front GC since it excludes solvent effect and possible contaminants in the sample, which compete for the available reagent charge.

Table 1. Detection Limits without Preconcentration

Substance	Retention time Seconds	Mobility K_0 $\text{cm}^2/\text{V}.\text{sec}$	IMS LOD (ng)	GC-IMS LOD (ng)
E8,E10-Dodecaden-1-ol	120	1.4628	1.4	0.7
Z8 Docdecenyl acetate	136	1.2160	0.6	0.3
Z11 Tetradecenyl acetate	181	1.1117	1.3	0.5
Z8 Dodecen-1-ol	103	1.4606	250	100
E4 Tridecenyl Acetate	156	1.1605	0.8	0.6
Z,Z7,11 Hexadecadienyl Acetate	216	1.2480,1.0770	3.0	10.0

Typical GC output of three pheromones is shown in Figure 3 and corresponding positive ion mobility spectrum in Figure 4.

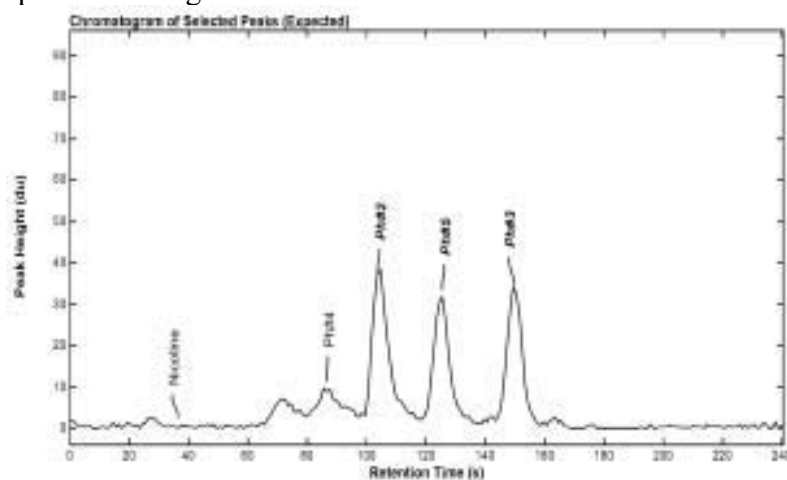


Figure 3. Chromatogram of three pheromones

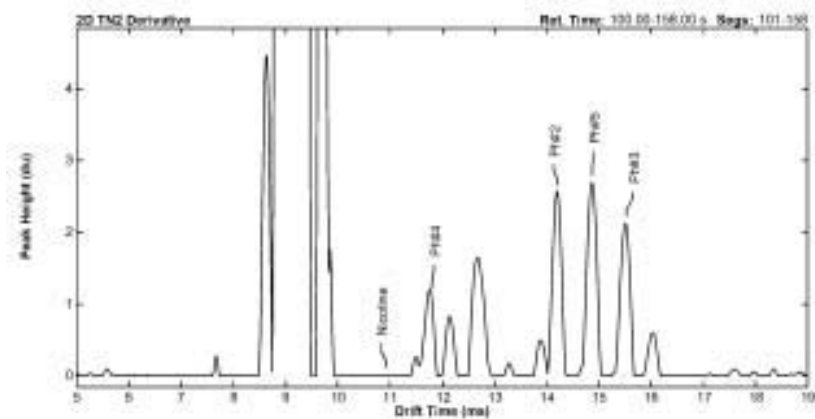


Figure 4. Corresponding Plasmagram of three Pheromones

GC-IMS calibration curve of pheromone 2,3 and 5 is shown in Figure 5. The method has a linear range from 0.2 to 10 ng with relative standard deviation of 0.99.

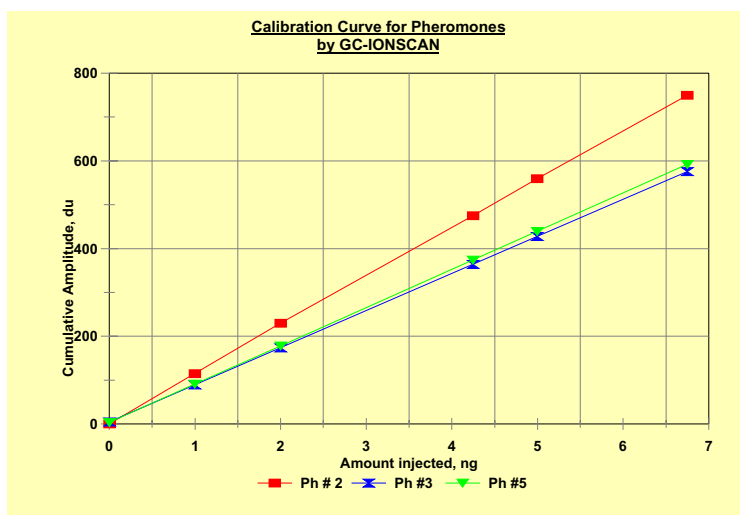


Figure 5

Equilibrium vapor concentrations of three acetate-based pheromones were determined based on headspace vapor GC-IMS analysis and calibration injection of standard solutions (Table 2). The vials containing the pheromones were kept at constant temperature of 20°C.

TABLE 2. Equilibrium Vapor Concentration of Acetate based Pheromones

#	Pheromone Type	Molecular Weight	Eq.Vap.Conc (ng/cc)	ppmv
2	Z8 Dodecenyl Acetate	226.4	91(±14%)	9.7
3	Z11 Dodecenyl Acetate	254.4	35(±10%)	3.4
5	E4 Tridecenyl Acetate	239.0	91(±8%)	9.2

3.1 SPME Sampling & Analysis

The SPME fiber was exposed for 5 and 60 minutes to 1 liter/min flow from the vapor generator at ambient temperature. The fiber was directly desorbed into the IMS heated inlet at 280°C. The minimum detection concentration with the SPME is shown in Table 3. For short sampling time, the lowest concentration of the acetate-based pheromones were 76ppbv and 99ppbv, whereas, for longer exposure time (1hour), the SPME method was approximately able to sample ten times lower concentration of the pheromones.

TABLE 3. Solid Phase Microextraction of Pheromones

#	Pheromone Type	Min.Conc.@ 5min	Min.Conc. @60 min
2	Z8 Dodecenyl Acetate	76ppbv	8ppbv
3	Z11 Dodecenyl Acetate	99ppbv	10ppbv
5	E4 Tridecenyl Acetate	98ppbv	10ppbv

3.2 MESI Sampling & Detection

The vapor generator was set at low ppbv concentration and sampling with the MESI-GC-IMS system was carried out for 1 hour. The concentrations were further reduced and sampling time was increased to allow detection above the LOD values for pheromones 2,3 and 5. Table 4 shows the minimum detection concentration achieved by the MESI method for sampling times of 1, 16 and 64 hours. The objective was to simulate long term monitoring of dispersed encapsulated pheromone concentration in the field. The method demonstrated the ability to concentrate pptv levels of pheromones over few days of continuous sampling.

The whole process can be automated and data evaluated to assess effectiveness of spraying procedure and the lifetime of the encapsulated pheromones.

TABLE 4. Effect of Sampling time on Minimum Detected Concentration of three pheromones using the MESI Technique

Pheromone#	1 hour	16 hours	64 hours
2	1.3ppbv	0.6ppbv	0.09ppbv
3	0.6ppbv	0.4ppbv	0.06ppbv
5	1.7ppbv	0.8ppbv	0.12ppbv

4.0 Conclusion

The MESI technique interfaced to a GC-IMS system offers a simple, flexible sampling time, low cost, freedom from solvent use, durability, good selectivity and sensitivity for continuous monitoring of organic compounds. Method capability was compared with the SPME approach and found to offers much better sensitivity, a factor of approximately 10 for 1hour exposure to constant concentration of pheromones. The membrane interface requires heating to increase the diffusion or permeability of relatively low boiling point substances like pheromones. The metallic capillary column with thick liquid phase coating used as the trap is quickly heated and cooled due to its low thermal mass. The system offers detection levels at the low parts per trillion concentrations for the investigated pheromones and can be used for continuous monitoring of a variety of organic compounds in air.

5.0 References

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