DEVELOPMENT OF ANALYTICAL METHODOLOGIES FOR THE CHEMICAL INVESTIGATION OF INSECT ATTRACTANTS OR REPELLENTS.

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ABSTRACT

This study focuses on the design, development and testing of a portable olfactometer for the chemical investigation of volatile organic compounds (VOCs) with insect attractant and repellent activity. The olfactometer has a Y-shape design and comprises of four chambers: a) for hosting the under-investigation insect population, b) for hosting the chemical analytes of interest, c) a control chamber and d) a decision chamber. A compact portable membrane inlet quadrupole mass spectrometer (MI-QMS) has been integrated within the developed olfactometer and employed for the first time to detect and monitor continually, both qualitatively and quantitatively, VOCs with mosquito repellent activity. On-line monitoring of such compounds in field conditions (i.e. out of the laboratory) is of great importance and allows the chemical comprehension of the olfactory behaviour of insects with potential negative impact on public health. MI-QMS is a powerful, simple analytical technique ideal for in-situ applications that offers high sensitivity (low limits of detection), selectivity, fast and accurate analysis (within seconds), with no sample preparation requirements. Representative compounds examined include: (-)-a-pinene, γ-terpinene, (+/-)-linalool, (-)-b-pinene, p-cymene, R-(+)- limonene, b-Myrcene, (-)-linalool, butyl hexanoate, ethyl butyrate, o-xylene. Gas phase experiments were performed at concentration levels from low ppb to low ppm using two techniques for gaseous standards generation: a) static dilution bottles and b) a built-in-house vapour generator. Results obtained showed very good linearity within the examined concentration range, ppb limits of detection and fast response times (within sec).

INTRODUCTION

Olfaction is one of the most important senses and processes for chemical and biological communication and interaction between humans, animals and plants. Chemical signs of life and the plethora of their transported undercover information is an innovative and demanding research field, asking continuously for further investigation. Both human exhaled breath compounds and human body odour state an individual's characteristic odour or in other words a person's unique chemical fingerprint. The body odour is the result of the complex interaction of skin glands and secreting organic compounds with the colonised bacteria on the human skin which live by metabolising and transforming the odourless sweat to an odorous liquid. These end up in a human body odour profile of some hundreds of Volatile Organic Compounds that disperse in the surrounding environment and mediate the attraction of an undesired visitor like a vicious insect. Blood hosts' detectability is usually a matter of time, distance and is affected by the surrounding environmental conditions.

Insect pests such as mosquitoes consists a worldwide serious threat both for human public health as well as for the ravage of plant cultivations and industrial livestock production. This is caused because a bite from a mosquito may be responsible for a wide range of diseases in a human or in a crop or in an animal. A tiny and innocent bite is not finally as innocent as it seems to be initially. Malaria, yellow fever, leishmaniasis, plague, dengue fever and typhus are just some of the cases that mosquitoes are suspected and responsible for causing and spreading them. In Africa, the risk

for the public health because of these life-threatening diseases is nowadays more probable than ever, breaking the death records. Anthropophilic mosquitoes through physical and chemical cues are able to find their vulnerable hosts, sneak next to them and generously offer a bite.

In the current work, we examine a range of volatile organic compounds with potential insect repellent action. For this purpose, we developed a built-in-house olfactometer using 3D printing whereas odour generation at standard concentrations was achieved using a vapour generation based on controlled evaporation. Limits of detection and online monitoring was achieved using a portable MI-QMS system.

EXPERIMENTAL PART

OLFACTOMETER

The olfactometer developed in this study has a Y-shape design and comprises of four chambers: a) box A for hosting the under-investigation insect population, b) box C for hosting the chemical analytes of interest, c) box B that is a control chamber and d) box i that is a decision chamber. A compact portable membrane inlet quadrupole mass spectrometer (MI-QMS) has been integrated within the developed olfactometer and employed for the first time to detect and monitor continually, both qualitatively and quantitatively, VOCs with mosquito repellent activity. Vapours of interest were produced using a dynamic vapour generator.

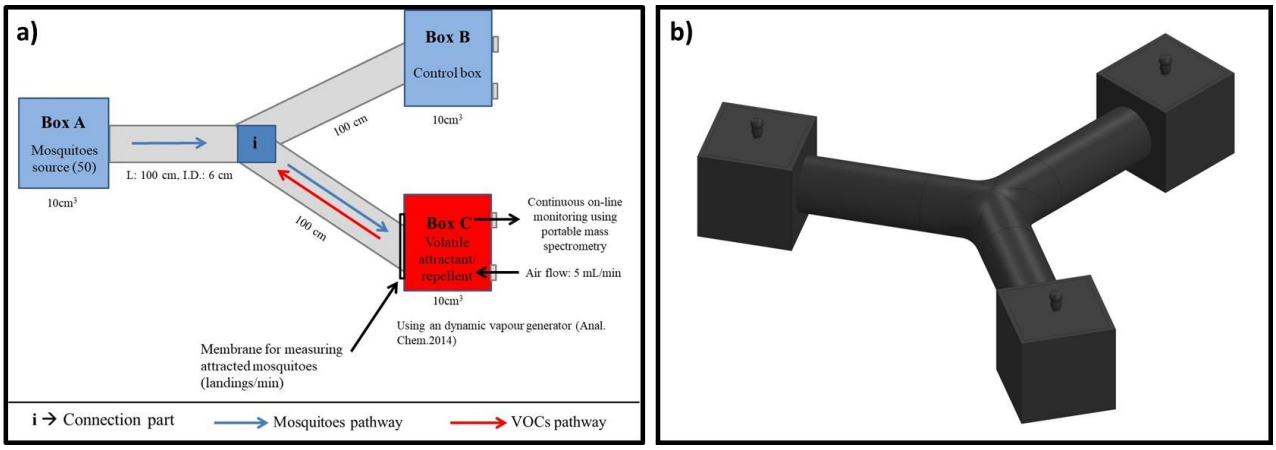

Figure 1. *a) Schematics of our experimental setup, b) 3D design of the tested olfactometer.*

DYNAMIC VAPOUR GENERATOR

The generation of the chemical signals was achieved using a dynamic vapour generator (DVG) described extensively elsewhere [1, 2]. The DVG operating principle is the controlled evaporation of a liquid chemical analyte and its diffusion in a carrier gas stream such as zero grade air. The main components of the DVG are: a) a mixing chamber (MC), b) an evaporation chamber (EC), c) two high accuracy programmable mass flow controllers (MFCs) for the accurate control of the flow rates within the MC and the EC, d) an automation platform for the simultaneous digital control of the MFCs e) a laptop PC and f) a digital controlled heating mantle for maintaining stable temperatures within the EC. The DVG was built-in house from stainless steel (SS) and standard Swagelok fitting unions. The MFCs (type GE50A) and the automation platform were purchased from MKS Instruments UK Ltd. and were digitally calibrated for zero air flow by the manufacturer. The MFC which controls the carrier gas flow passing through the MC had a flow range up to 10 L/min with 1 mL/min increments, whereas the MFC controlling the flow within the EC had a flow range between 0.1 mL/min and 10 mL/min with 0.1 mL/min increments. The automation platform was controlled via custom software in LabView 2015 32 bit for the synchronised operation of the two MFCs. The heating mantle (DM-Series, model number DM-602) was purchased from the Medline Scientific Ltd.,

with a volume capacity of 250 mL, providing controllably accurate and stable temperatures up to 400°C continuously be monitored using a high accuracy thermocouple. The original design of the DVG comprised of 3 ECs and the associated number of MFCs and heating mantles. The MC has three side inlets for connecting with the ECs. However, during our experimental work, only one EC was used, and the other two side inlets of the MC were sealed with 3.175 mm Swagelok SS plugs. The number of the ECs added on the OE can increase accordingly to the number of the different introduced chemical analytes or groups of analytes. This would enable the controlled generation of more complex vapour pulses at standard concentrations of mixtures of compounds with different physical and chemical properties.

MEMBRANE INLET QMS TECHNOLOGY

Experiments were performed using a portable membrane inlet quadrupole mass spectrometer (QMS) supplied by Q-Technologies Ltd., Liverpool, UK [3]. The MIMS system [4-8] consists of four components: a) a membrane inlet sampling probe that allows to the gas samples to penetrate through the membrane material into the vacuum chamber for ionisation and further mass spectrometric separation and analysis, b) a triple filter QMS, c) the vacuum system which maintains overall system's pressure stable in low levels and simultaneously offer a sufficient suction flow rate of the absorbed onto the membrane molecules and d) a laptop which is required for data acquisition and interpretation.

The triple filter QMS comprises of the following main parts: a) the electron impact (EI) ion source, b) the mass analyzer and c) the detector. The enclosed EI ion source has a twin Thoria filament assembly at about 1.68 mA electron emission current. The mass analyzer consists of a pre-filter (25mm length), a main filter (125mm length), and a post filter (25mm length) with rods of 6.3mm diameter. It has a mass range of *m/z* 1-200 with a unit resolution over the entire mass range. The mass analyzer is set up so that the ratio of peak height to valley with adjoining peaks is 10 %. The sensitivity of the quadrupole analyzer is 1×10^{-4} A/mbar. The detector contains both a Faraday cup for detecting usual ion currents and a Channeltron type electron multiplier for detecting very low signal currents like those produced from low concentration level VOCs. During data acquisition, 10 acquisition points were recorded per unit mass with average number of 20 scans per measurement throughout the whole mass range. Data were recorded on a laptop computer, plotted, and compared with reference mass spectra, using the NIST Chemistry WebBook [9] as reference database for spectral peaks of each compound.

The QMS was housed in a stainless-steel chamber pumped by a TURBOLAB 80 vacuum system (Oerlikon Leybold Vacuum Ltd., Chessington, UK) consisting of an Oerlikon dual-stage oil-free DIVAC 0.8 T diaphragm pump and a TURBOVAC SL 80 H turbomolecular pump. The diaphragm pump provides pressure down to 1×10^{-2} Torr, while the turbomolecular pump gives base pressure of 7.5×10^{-8} Torr. The system pressure was continuously being monitored by a highly accurate digital pressure gauge (model: MRT 100) supplied by Pfeiffer Vacuum Ltd., Newport Pagnell, UK that uses a Pirani/Cold cathode method of measurement. Operating pressure for mass analysis with the PDMS sheet membrane sampling probe attached and sample inlet valve fully open was varying between 2.5×10^{-6} Torr and 3.0×10^{-5} Torr depending on the concentration of the under analysis standard gas sample and on the nature (chemical structure, vapour pressure, etc.) of the under examination component that affect permeability through the PDMS membrane.

CONCLUSIONS

A portable membrane inlet mass spectrometer has been used to detect, analyse (qualitatively and quantitatively) and screen VOCs with potential insect repellent action. During measurements, minimum sample pre-treatment requirements was needed [10, 11] while fast detection response

times (some seconds) were observed. Table 1 summarizes the analytical characteristics of the portable QMS system examined during our tests.

Table 1. *Summary of the membrane rise times, R^2 values and limits of detection (LOD) for the target compounds that were examined using our M-QIMS system.*

No.	Compound	rise time (sec)	R^2	LOD (ppb)
1.	(-)-a-pinene	15	0.996	2.42
2.	γ-terpinene	18	0.9946	7.66
3.	(+/-)-linalool	25	0.9953	2.97
4.	(-)-b-pinene	32	0.999	1.34
5.	Geranyl acetate	21	0.9967	1.41
6.	Ocimene	22	0.9986	6.32
7.	p-cymene	25	0.9923	3.96
8.	1-S-(+)-3-carene	28	0.9991	8.34
9.	(-)-allaromadendrene	10	0.9965	1.46
10.	(+)-limonene oxide	6	0.9999	2.32
11.	Farnesene	7	0.9942	3.44
12.	a-pinene oxide	32	0.9988	10.34
13.	(+)-a-pinene	15	0.9965	1.11
14.	R-(+)-limonene	16	0.9946	5.31
15.	Farnesene	16	0.9956	5.97
16.	b-Myrcene	4	0.9987	0.34
17.	(-)-linalool	2	0.9961	0.61
18.	Linalyl acetate	7	0.9933	0.32
19.	S-(-)-limonene	11	0.9951	6.45
20.	Citral (62/35: neral/geranial)	20	0.9999	8.34
21.	Butyl hexanoate	13	0.996	3.41
22.	Hexyl hexanoate	11	0.996	2.55
23.	Ethyl carpoate	6	0.9998	1.69
24.	Ethyl butyrate	5	0.9955	2.78

25.	o-xylene	9	0.9990	6.54

As shown above – due to the fast response times and low limits of detection as well as the excellent R2 values obtained for the examined concentration are 100 -1000 ppb, the developed system has great potential for testing with insect population to determine their behaviour and response to the compounds tested.

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