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LOCALISED EVOLVED GAS ANALYSIS BY MICRO-THERMAL ANALYSIS

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Abstract

Micro-thermal analysis employs a scanning probe microscope fitted with a miniature resistive heater/thermometer to obtain images of the surface of materials and then perform localised thermo-analytical measurements. We have demonstrated that it is possible to use the same configuration to pyrolyse selected areas of the specimen by rapidly heating the probe to 600–800°C. This generates a plume of evolved gases which can be trapped using a sampling tube containing a suitable sorbent placed close to the heated tip. Thermal desorption–gas chromatogaphy/mass spectrometry can then be used to separate and identify the evolved gases. This capability extends the normal visualisation and characterisation by micro-thermal analysis to include the possibility of localised chemical analysis of the sample (or a domain, feature or contaminant). The isolation and identification of natural products from a plant leaf are given as an example to illustrate this approach. Preliminary results from direct sampling of pyrolysis products by mass spectrometry are also presented.

Keywords: atomic force microscopy, evolved gas analysis, micro-thermal analysis, pyrolysis GC/MS, scanning thermal microscopy

Introduction

The herb feverfew (*Tanacetum parthenium* (L.) Schultz Bip.) has attracted considerable interest for the treatment of migraine and arthritis [1–3]. This has been attributed to the presence of the sesquiterpene lactone, parthenolide, which is reported to be the active principle [4, 5]. Smith and Burford reported studies on the selective extraction of this material from dried leaves of the plant using supercritical carbon dioxide [6]. Scanning electron microscopy of the leaf surface before and after extraction suggested that the parthenolide was present in oil cells which are ruptured during the extraction process [7]. Capillary gas chromatography of the extract also detected the monoterpene camphor as well as chrysanthenyl acetate and dihydroparthenolide.

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In order to confirm the location of the parthenolide and its associated essential oils, a study of feverfew leaves was undertaken using micro-thermal analysis. This is a technique which combines the imaging capabilities of atomic force microscopy (AFM) and scanning thermal microscopy (SThM) with the ability to use the same instrument to carry out physical characterisation by thermal analysis over a small region (<5 μm in diameter) [8, 9]. An ultra-miniature cantilevered resistive probe is used to provide and monitor the thermal flux to the specimen's surface as well as measuring the vertical and lateral forces between the tip and the sample. This provides images where different solid phases are distinguished by differences in thermal conductivity, and, when a temperature modulation is used, thermal diffusivity. The topography of the specimen is also determined using the force-feedback mechanism of the microscope. These images are used to select regions for subsequent physical characterisation by localised thermal analysis by placing the probe at a specified point on the sample's surface and using the tip to heat a small region of the specimen. The vertical deflection of the cantilever and the power required to make the tip follow the temperature programme are measured. These provide forms of thermomechanical analysis and calorimetry respectively. Both measurements are obtained simultaneously and, because of the small size of the probe (a metal filament 5 μm in diameter with a contact area of the order of 1 μm^2) and volume of material affected, heating rates of the order of 10°C s^{-1} may be employed. In this way, events such as glass transitions, crystallisation, melting and decomposition can be detected. This capability transforms AFM and SThM into a powerful new form of analytical microscopy.

Localised calorimetry and localised thermomechanical measurements have been carried out for the identification of phases, inclusions, contaminants etc. or for mapping of changes in materials properties (crystallinity, cross-link density, etc.) across a specimen [10–14]. Measurements of the melting of waxy coatings on the surface of plant leaves have been reported elsewhere [15]. We have also developed a means of using the same instrument to pyrolyse specific regions of the sample, trapping evolved gases and subsequently analysing them by thermal desorption–gas chromatography/mass spectrometry (GC/MS) [16, 17]. This affords a means of localised chemical analysis, which complements the imaging and physical testing described above. Preliminary measurements using direct sampling of pyrolysis products from a coated polymer film into a mass spectrometer are also described here.

Experimental

Samples of feverfew were obtained from plants grown locally from seed. The fresh leaves were mounted on metal stubs using double-sided adhesive tape and allowed to dry overnight before analysis. Polyethylene coated synthetic rubber film was supplied by the Procter and Gamble company, Ohio, USA and mounted in the same way.

Measurements were carried out on a TA Instruments 2990 Micro-Thermal Analyzer using an ultra-miniature cantilevered resistive probe of the type described by Dinwiddie *et al.* [18]. The probe was calibrated for temperature using the melting points of zone-refined benzoic acid and caffeine according to the procedures de-

scribed by Blaine *et al.* [19]. Contact mode AFM topographic images of the sample's surface were obtained under sufficient load (50–100 nN) so as to obtain good quality images the surface without causing damage to the specimen. These images were then used to select regions for subsequent pyrolysis measurements.

For pyrolysis experiments the probe tip was placed in contact with the sample and rapidly heated to 800°C. The evolved gases were trapped in a specially designed tube packed with a mixture of Tenax and Carbopak. The tube ended in a short section of hypodermic tubing (0.8 mm o.d., 0.4 mm i.d.), the open end of which was placed immediately adjacent to the heated thermal probe using a micro-manipulator. As the tip was heated, a pump was used to draw gas through the tube which was then placed in a thermal desorption unit (TA Instruments Evolved Gas Collector) for analysis of the trapped volatiles by GC/MS (Hewlett–Packard 6890 Gas Chromatograph with HP5973 Mass Selective Detector). The GC was fitted with a HP-5 MS capillary column (30 m×0.25 mm i.d.×1.0 μm d.f.). The oven program consisted of a 5 min hold at 40°C following desorption and then a ramp to 250°C at 15°C min^{-1} followed by a 10 min hold at this temperature. Mass spectra (*m*/*z* 45–350) were acquired every 0.5 s. A blank desorption run was carried out before and after each experiment to confirm the cleanliness of the detection system.

Direct sampling of the gases from the environment around the thermal probe was carried out using a 200 mm length of 25 μm i.d. capillary tubing as a transfer line to the ion source of a ProLab 300 a.m.u. quadrupole mass spectrometer (Onix Process Analysis, Winsford, Cheshire, UK). The capillary also acted as a restrictor in order to bring the operating pressure for gas sampling down to that which the detector could accommodate. Perfluorotributylamine was used to adjust the mass spectrometers for *m/z* peak position according to the manufacturers' recommendations.

Results and discussion

Contact mode AFM images showing the topography of a 100×100 μm area of the leaf surface before and after the pyrolysis experiment are presented in Figs 1a and 1b. An oil cell can be observed in the centre of Fig. 1a. The thermal probe was placed on this

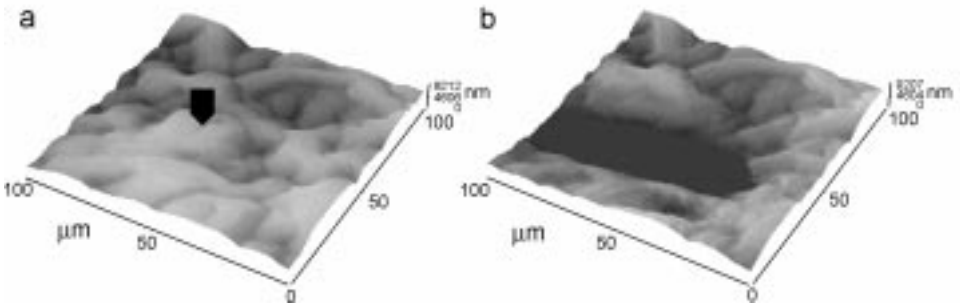

Fig. 1 Topography (100×100 μm) of feverfew leaf before (a) and after (b) localised pyrolysis. Marker denotes position of oil cell in (a)

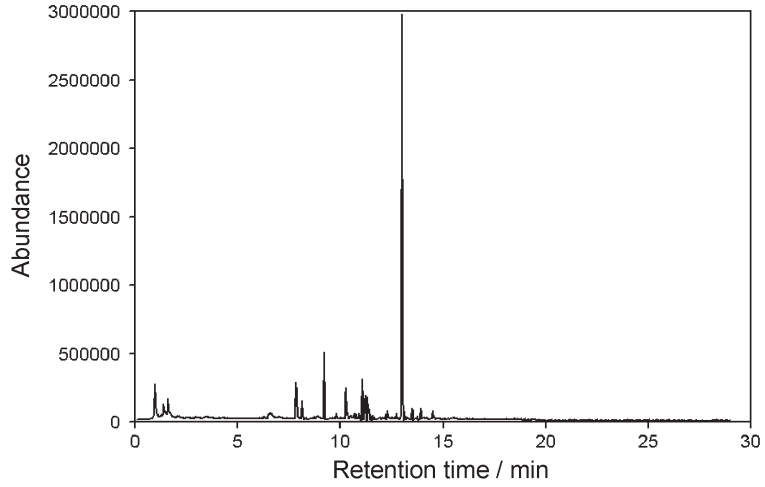

Fig. 2 GC/MS total ion chromatogram of gases from the pyrolysis of an oil cell

region and a pyrolysis–GC/MS experiment carried out. The resulting 'crater' in the sample is visible in Fig. 1b. The disruption caused by the pyrolysis measurement is quite large and can be controlled by the proximity of the probe to the surface and the time and temperature of the heat pulse. In this case the leaf surface was quite delicate and a large area was affected even under the mildest conditions required to obtain a sufficient yield of evolved gases. However, higher resolution probes are under development which will be able to pyrolyse smaller areas and thus minimise sample damage [20].

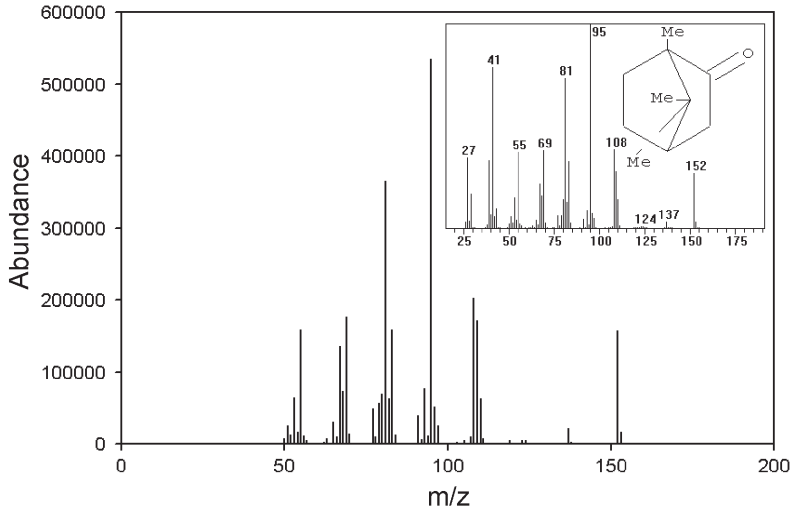

Fig. 3 Mass spectrum of peak at retention time of 13 min from Fig. 2. Inset shows library spectrum and structure of camphor

The GC/MS chromatogram showing total ion count *vs.* retention time is shown in Fig. 2. The mass spectrum of the peak at a retention time of 13 min is shown in Fig. 3. A search of the library of mass spectra identified camphor as the most likely (>95%) match for this peak (spectrum shown inset into Fig. 3). Parthenolide was not detected under the chromatographic conditions used (it would probably elute beyond 30 min). Furthermore, parthenolide is thermally unstable [4] and it is probable that any material present at this location would be degraded during the pyrolysis experiment. Measurements on other areas of the leaf did not find camphor; thus it is probably concentrated in the oil cells.

We have recently added the capability to perform direct gas sampling of the area surrounding the thermal probe in order to allow rapid point measurements of selected evolved species. This is achieved by bringing the open end of a small-bore capillary transfer line to the mass spectrometer close to the probe during pyrolysis experiments. An example of this type of measurement is shown in Fig. 4 where the heated tip (at 700°C) was gradually pushed into the surface of a polyethylene coated synthetic rubber film used for medical applications. Ion masses of *m/z*=57 and 71 corresponding to $C_4H_9^+$ and $C_5H_{11}^+$ ions expected from the polyethylene degradation and ion masses of *m*/*z*=78 (benzene) and 104 (styrene) for the styrene–butadiene rubber were monitored. The results indicate that the top polyethylene surface is degraded before the rubber substrate is encountered as might be expected from the construction of the sample. Ancillary experiments by sorbent trapping and thermal desorption–GC/MS confirmed the composition of the film which was also examined in cross-section by AFM to determine the thickness (5 μm) of the polyethylene coating.

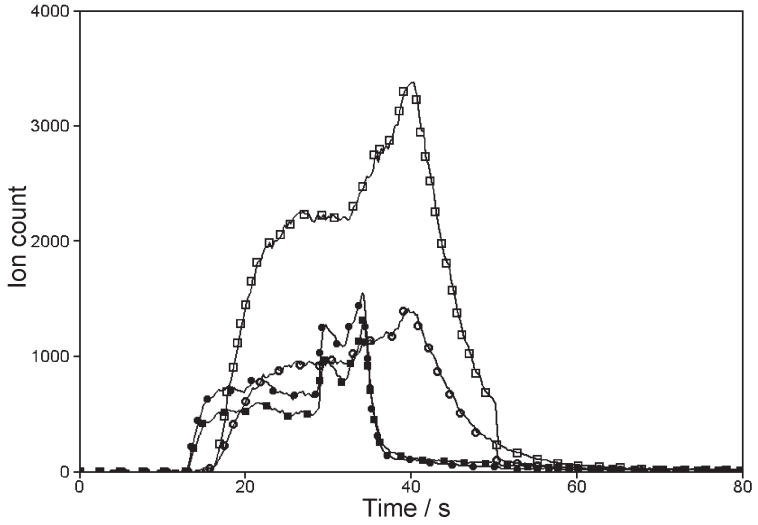

Fig. 4 Single ion counts for a polyethylene coated synthetic rubber film as a heated thermal probe is brought into contact with its the surface (ion mass: ● – 57, ■ – 71, o – 78, □ – 104)

Conclusions

These examples illustrate the use of micro-thermal analysis to carry out localised chemical analysis of biological specimens in order to determine the distribution of natural products in plant leaves and to confirm the construction of a coated polymer film. In the case of the plant leaf, the target material (parthenolide) was not found – probably due to its thermal instability and the choice of experimental conditions. Camphor was detected however, and from this work we were able to conclude that it is located within oil cells on the leaf surface.

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